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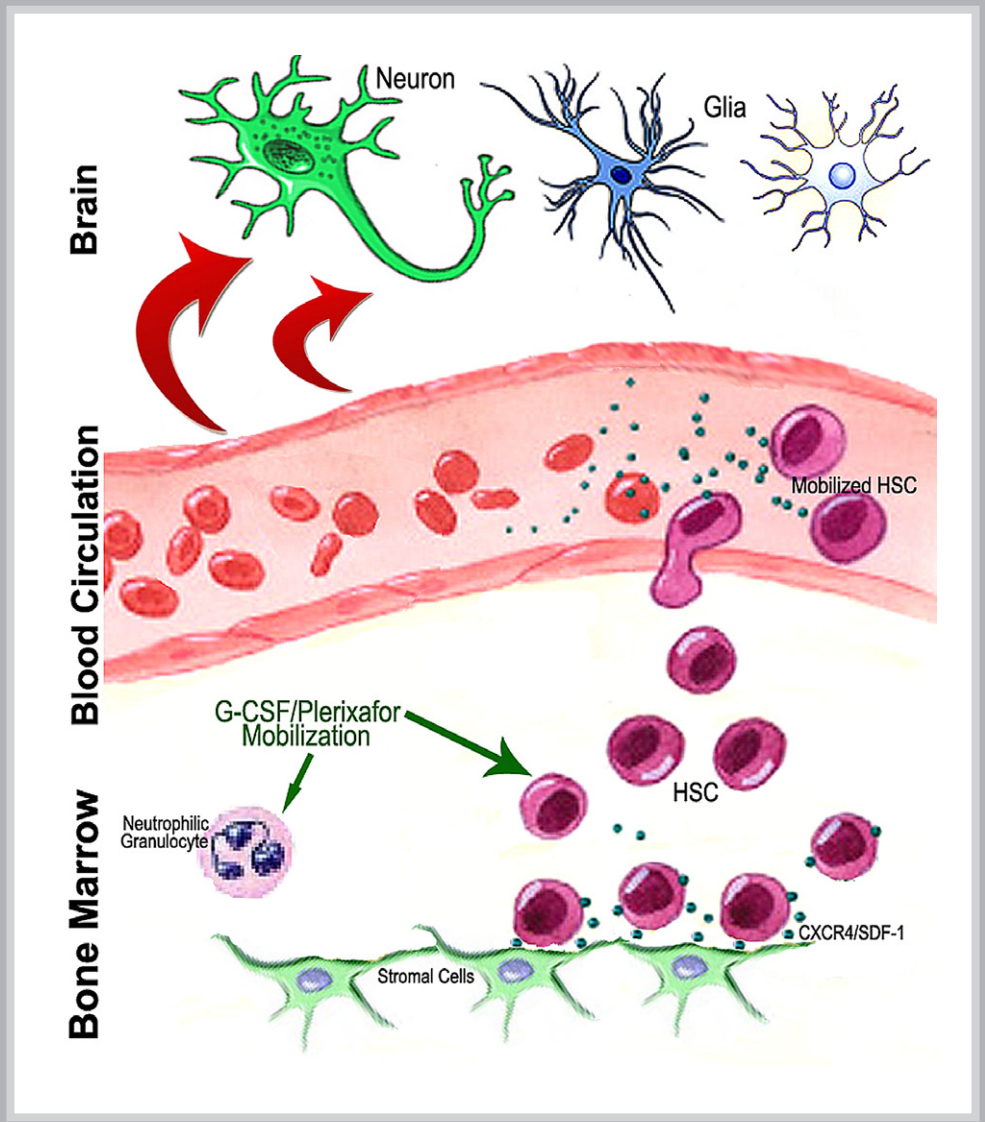

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BIOMEDICAL REVIEWS

An International Journal of Cell Biology of Disease

Volume 22, 2011

Scope and Purpose

Biomedical Reviews (ISSN 1314-1929) is an official journal of the Bulgarian Society for Cell Biology. The Journal is published annually, and includes state-of-the-science Reviews and Dance Round articles (a form of short, position papers) focused on disease-oriented molecular cell biology, presented in concise form.

Editorial Policy

Contributors to **Reviews** as well as **Dance Rounds** and **State-of-the-molecules** are, in general, invited by the Editors and the Editorial Board, but idea proposals for Reviews and Dance Rounds are welcome. Prospective authors should send a brief summary, citing key references, including their own, to the Editors or a member of the Editorial Board. Submission of full-length articles without prior consultation is not prefered. Manuscripts are peer-reviewed by the Editors, Editorial Board members, and/or external experts before final decisions regarding publication are made. All material in *Biomedical Reviews* represents the opinions of the authors and does not reflect opinions of the Bulgarian Society for Cell Biology, the Editors, the Editorial Board, or the institutions with which the authors are affiliated.

Publication of *Biomedical Reviews* is truly a collaborative process. We appreciate the brain-and-heart partnership having with our authors and are committed to further maintaining the excellence of the Journal.

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Contents

Front cover: *Illustration of the bone marrow, blood, and brain tissues. CXCR4+ HSC are anchored to SDF-1+ stromal cells of the bone marrow. The administration of G-CSF combined with CXCR4-antagonist plerixafor leads to hypermobilization of endogenous HSC into the blood circulation along with stimulation of neutrophilic granulocytes. The blood-borne HSC are recruited to the injured brain by chemotaxis. Inside CNS, bone marrow cells improve neuroprotection, plasticity, and brain repair.* From Milena Penkowa's review, pages 1-6.

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Editor's Foreword

A photograph by Nick Chaldakov (www.chaldakov.com)

Sometimes our best efforts do not go
amiss; sometimes we do as we meant to.
The sun will sometimes melt a field of sorrow
that seemed hard frozen: may it happen for you.
Sheenagh Pugh, From *Sometimes* (1)

Dear Colleagues,

In 1992, Editor's Foreword of *Biomedical Reviews* (*BMR*), volume 1, started with the above cited poetry of Sheenagh Pugh, a British poet, novelist and translator, who taught creative writing at the University of Glamorgan in Wales until retiring in 2008. Then, from London, I wrote: "After 45 years of sorrow, the "sometimes" melting point reached Bulgaria too. The Journal is a part of such a sometime-ness that has become possible through collaborative work with my friends, Dr P. Ghenev, Dr K. Dikranian and Mr K. Kralev – executive director of the Bulgarian-American Center in Varna."

The first volume of *BMR* was published on behalf of the Bulgarian-American Center (2). Its publication was materialized with the cheque of Mrs Ruth Rudovsky "pay to the order of Bulgarian-American Center" (the copy of the cheque is shown below). In 1990 in Varna, Bulgaria, we personally met Mrs Rudovsky, a retired teacher from USA, on her tour in East European countries welcoming the democracy since 1989.

In 2011, we, Bulgarians, celebrate 22 years of democratic changes in our country. Coincidentally, we are releasing *BMR*, volume 22. Now we wish to express our cordial appreciation to the collaborative spirit and action of generous Mrs Ruth Rudovsky! Reminding us of a part of Robert Frost's *Mowing:*

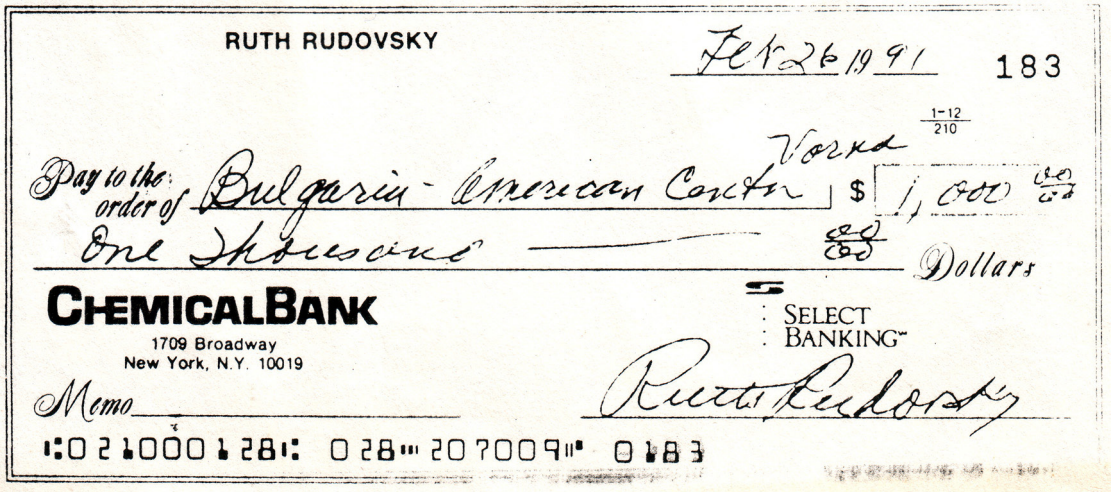
The fact is the sweetest dream that labour knows.
My long scythe whispered and left the hay to make.

George N. Chaldakov
Editor-in-Chief, *BMR*

1. In: G. Benson, J. Chernaik, C. Herbert, editors. *100 Poems on the Underground.* Cassel Publishers Ltd, London, UK. 1991; p 124.
2. In 2006 the Bulgarian Society for Cell Biology (BGSCB), a professional society serving the cell biology community of collegues devoted to the excellence of research, education, and friendship, was launched.

On behalf of the Society the following main activities are developing:
(i) Publication of two international journals
(a) *Biomedical Reviews*, and
(b) *Adipobiology*, since 2009 presenting state-of-the-science reviews and research articles on adipose tissue in health and disease
(ii) Organization of Biomedical Forum, since 1990 an university program of CME (continuing medical education)
(iii) Organization of International Symposia on Adipobiology and Adipopharmacology (ISAA).

NOTE: The 3rd ISAA will be held 25-27 October 2012 in Burgas, Bulgaria.
Welcome message, Preliminary Program and related information are published on pages 91-94.


RUTH RUDOVSKY

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Memo

Ruth Rudovsky

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New members of the Editorial Board

We are pleased to welcome Professor John Heuser, Director, Electron Microscopy Center, WPI Institute for Cell and Material Sciences, Kyoto University, Kyoto, Japan who was elected Editor of *Biomedical Reviews.* We are also pleased to welcome Professor Bhanu Jena, Director, Institute of NanoCell Biology, Department of Physiology, Wayne State University School of Medicine, Detroit, MI, USA, and Dr Stephen Manning at Medical Education Centre, Sandwell General Hospital, Lyndon, West Bromwich, UK who were elected members of the Editorial Board of the Journal.

Biomedical Reviews 2011; 22: 1-6.

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FROM BONE MARROW TO BRAIN: STEM CELLS IN NEUROPROTECTION, PLASTICITY, AND NEUROREGENERATION

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At present, curative therapies for neurological diseases are limited, even though they are prevalent worldwide. So far, molecular strategies developed for brain disorders act through one single molecular mechanism, yet, these diseases are multifactorial and highly complex, as to why a successful therapy likely calls for a more multifaceted and cell-based approach. The bone marrow contains a mixed stem and progenitor cell population including hematopoietic stem cells (HSC) and mesenchymal stromal stem cells (MSC), which are potential endogenous candidates for cell-based therapy in various brain disorders like stroke, trauma, and neurodegeneration. Unlike the neural stem cells (NSC), bone marrow HSC are readily isolated, mobilized and expanded by means of treatment with granulocyte-colony stimulating factor (G-CSF) and CXCR4-antagonist plerixafor. Once in the blood circulation, the cells preferentially home to injured tissues including the brain. Bone marrow cells may convey neuroprotection, plasticity, and neuroregeneration by different mechanisms of action, which include either transdifferentiation or cell-cell fusion with resident brain cells. Bone marrow cells also benefit the injured brain by secreting bioactive factors, which in a paracrine manner convey intrinsic repair and enhance neurogenesis. Furthermore, transplanted MSC may activate the astrocytes leading to increased glial secretion of neurotrophic growth factors and enhanced proliferation and migration of the resident NSC. These neuroregenerative mechanisms of action are not mutually exclusive, in fact they may provide a multifaceted therapeutic approach, which is requested in order to move neurorestorative and protective strategies into the clinic. ***Biomed Rev*** **2011; 22: 1-6.**

Key words: bioactive factors, cell renewal, neuropathology, neurotherapy

INTRODUCTION: STEM CELLS IN BRAIN

In animal and human central nervous system (CNS), neurons are generated in intracerebral germinal zones such as the subventricular zone (SVZ) and the dentate gyrus (DG) of hippocampus (1-3). Inside the germinal zones, neural stem cells (NSC) proliferate and migrate in response to brain injury and various pathological conditions including: inflammation, degeneration and ischemia (2,4-6). However, in the adult human brain, significant neurogenic activity and NSC recruitment from the germinal zones to an injured area remain controversial (1,3). While some groups report NSC and their proliferative capacity

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intact in the human parkinsonian brain as compared to those of human adult controls (3), others suggest that proliferating and migrating NSC are abundant in the human brain only before 18 months of age, after which the neurogenic capacity subsides and by adulthood, it is almost extinct (1). Other limitations in neurogenesis include that the differentiation of endogenous NSC might be restricted to glial cell types (2,7).

To address some of these hurdles, NSC activation in SVZ and DG as well as their regulation and recruitment have been extensively studied in experimental studies (2,5-7). Even if adult neurogenesis can provide neuroregeneration after some types of acquired brain injuries (5,6), this approach remains controversial, since the NSC found in brains from e.g. patients suffering Alzheimer's Disease (AD) were demonstrated to be implicated in AD pathogenesis (8). Particularly, NSC in the adult human brain were reported to generate aneuploid cells, characteristic for histopathology of neurodegenerative disorders (8,9). Accordingly, the therapeutic use of human endogenous NSC for neurodegenerative diseases, in particular AD remains contentious and not yet realistic. It is needed to reconsider the current stem cell-based concepts and their therapeutic application in neuroregenerative medicine as well as to investigate non-NSC-derived stem cell sources. For this purpose, stem and/or progenitor cells derived from other tissues than the brain, such as the bone marrow, could prove suitable. Bone marrow-derived stem and progenitor cells offer significant advantages over resident NSC in the diseased brain, since they are: 1. devoid of intrinsic cellular dysfunctions relating to neurodegenerative disorders; 2. easily obtained, expanded, and mobilized from host bone marrow into blood; 3. show preferential homing to the inflamed brain; 4. able to cross the blood-brain barrier due to chemotactic migration; and 5. interact with resident neuroglia and the neural micromilieu leading to improved neuroplasticity, repair and functional recovery (8-13). Significant crosstalks between the nervous and hematopoietic systems might involve the CNS' neuroendocrine regulation of host immune responses (13), although the possible neurotherapeutic use of bone marrow in CNS pathologies is still not completely elucidated.

This review highlights the recent and major findings in the field of neuroregeneration by means of recruitment of non-neural cells from the bone marrow. It summarizes the putative mechanisms of actions comprising 1. cellular replacement by putative transdifferentiation, 2. cell-cell fusion leading to hybrid brain cells, 3. indirect signalling through activated astroglia, and 4. paracrine secretion of neurotrophic growth factors supporting neuronal survival and brain repair.

STEM CELLS FROM BONE MARROW TO BRAIN

In the adult bone marrow reside multipotent and easily mobilized stem cells. Accumulating data support the observation that bone marrow-derived hematopoietic and non-hematopoietic stem cells transdifferentiate into cell types of different tissues including glia and neurons (2,10-15). This degree of plasticity, self-renewal, and pluripotency with the ability to undergo neurodifferentiation is a hallmark of bone marrow-derived stem cells, although the same wide-ranging neuroplasticity can be found in a range of other stem cell lineages such as adipose-derived stem cells (16). The 'cell replacement' concept is likely not the only therapeutic mechanism provided by stem cells. Accumulating data have shown that expression and paracrine secretion of neurotrophic factors from bone marrow-derived stem cells also provide support for neuronal survival, intrinsic brain repair, and neuroregenerative processes (10,17,18). These mechanisms open up new possibilities regarding the neurotherapeutic use of mobilized stem cells from the bone marrow.

Advantage of the bone marrow stem cells

As compared to other stem cell lineages, the bone marrow-derived CD34+ hematopoietic stem cells (HSC) possess a major advantage as compared to other stem cell types, as the HSC are easily expanded and mobilized endogenously leading to their blood-borne circulation, from which these cells are particularly able to home to injured or inflamed tissues in any compartment of the human body including the CNS (17-22). The damaged brain displays high levels of chemokines like stromal cell-derived factor-1 (SDF-1), which attract circulating HSC, due to their surface expression of CXC-chemokine receptor-4 (CXCR4; also known as fusin or CD184) (10,14,15,17-21). In the bone marrow, stromal expression of SDF-1 that binds CXCR4 on HSC is a key factor in HSC residency (2,14,15,18-20). However, by means of granulocyte colony-stimulating factor (G-CSF) combined with synergistic agent plerixafor (a rapidly degraded, reversible CXCR4 antagonist), hypermobilization of endogenous CD34+ HSC into the circulation is obtained (13-15,17-20,23). After HSC egress to the circulation, the HSC home to the injured brain by means of increased neuroglial expression of SDF-1 (14,15,18-23). Accordingly, CD34+ cells are recruited in significant numbers to the injured brain (17,22). Both recruitment to injured or inflamed brain as well as their homing to a depleted bone marrow are characteristic for HSC and combined with their feasible G-CSF/plerixafor-mobilization, these cells are highly

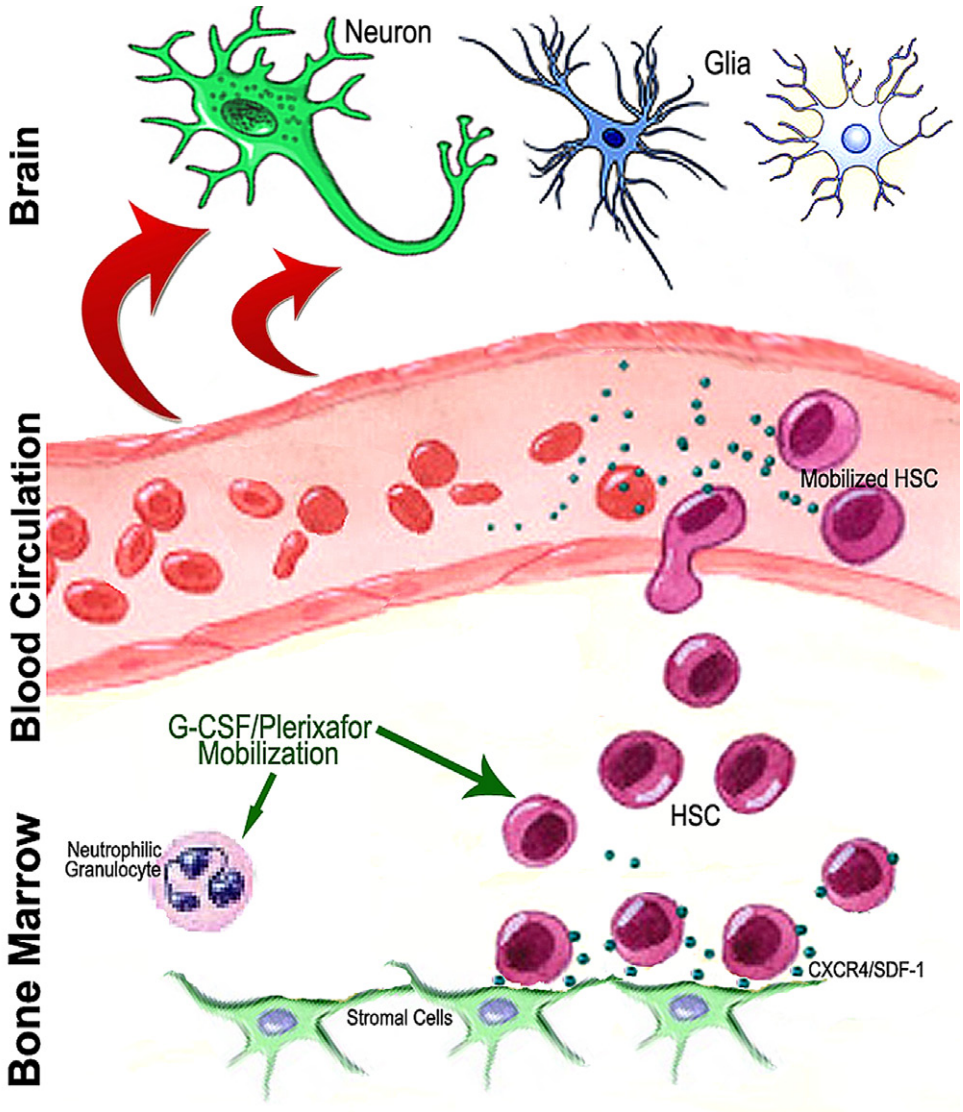

Figure 1. *Illustration of the bone marrow, blood, and brain tissues. CXCR4+ HSC are anchored to SDF-1+ stromal cells of the bone marrow. The administration of G-CSF combined with CXCR4-antagonist plerixafor leads to hypermobilization of endogenous HSC into the blood circulation along with stimulation of neutrophilic granulocytes. The blood-borne HSC are recruited to the injured brain by chemotaxis. Inside CNS, bone marrow cells improve neuroprotection, plasticity, and brain repair.*

suitable for regenerative medicine. Moreover, since this approach allows a strictly endogenous strategy, the well-known ethical, technical and biological problems relating to allogenic and donor-based approaches are bypassed.

Neuroregenerative mechanisms of action

As reviewed here, various independent researchers have reported that bone marrow-derived subpopulations of stem cells can generate neural cell types both *in vitro* and *in vivo* (2,25-29).

Hence, in mice without the capacity to generate myeloid and lymphoid cell lineages, administration of adult bone marrow resulted in transformation of the transplanted cells into neuronal phenotypes expressing neuron-specific antigens (25,27). Thus, by receiving male bone marrow-derived cells, female recipient mice displayed Y chromosome in neurons dispersed throughout their female brain (27). Likewise, olfactory bulb neurons contained bone marrow-derived graft cells as dem-

onstrated due to the genetic alteration of the transplants (25).

Moreover, bone marrow-derived MSC may *in vivo* adopt both neuronal phenotype and functions as shown in a study of transplanted bone marrow cells including their migration, phenotypic expression, and long-term survival in rats (2,28). Consequently, bone marrow-derived cells contain the capability to transform into CNS cell types that are viable within the microenvironment. Also, the cell grafts are likely differentiated into neural phenotypes as they express both markers of neural progenitors as well as mature neuronal markers such as e.g. neuronal-specific nuclear (NeuN) protein (2,28). In an interesting study, adult human bone marrow cells were demonstrated to give rise to adult human brain cells, as shown in cerebellum from patients, who suffered from malignant hematologic neoplasms for which they received radiation, chemotherapy, and bone marrow transplantation (29). In the Purkinje neurons of female patients receiving male bone marrow transplants, both an X and a Y chromosome were detected in the nuclei, which indicates that male donor cells had infiltrated the CNS, in which they either transdifferentiated into Purkinje neurons or fused with the host cerebellar cells (29).

In favor of the cell fusion concept are studies that have verified how co-culturing of embryonic stem cells with bone marrow-derived stem cells results in cellular fusion (30,31). Likewise, co-culturing of murine brain cells with embryonic pluripotent cells led to a spontaneous formation of hybrid cells due to cellular fusion (32). Accordingly, spontaneous fusion of cells may likely provide an explanation for the reported 'cell fate-switches' or the apparent "transdifferentiation" observations, which otherwise have been attributed to pluripotency and plasticity of stem cells (30,32). In line with this, it was shown that cell-cell fusion of donor and recipient cells is responsible for the regeneration of normal hepatic function and structure after bone marrow transplants in mice (33-35).

However, it is unlikely that this mechanism of cell-cell fusion is going to reduce the excitement relating to stem cell pluripotential properties and cell-based treatment strategies. Hence, formation of hybrid cells due to spontaneously fused bone marrow and resident cells is a process considered important within the field of stem cell therapy and regenerative medicine (33-35).The fusion of cells as a mechanism of cell replacement may however seem discouraging compared to the concept of transdifferentiation, where bone marrow stem cells are considered to undergo steps of de- and transdifferentiation into functionally complete neurons. However, bone marrow cell fusion in the CNS may in fact provide unpredicted advantages by endowing damaged or pre-apoptotic neurons with intact genes and gene regulatory machinery. Since it is not easy to reproduce *de novo* and appropriately replace the adult brain's highly specialized neurons and their neural networks, the fusion-mediated cell resuscitation could provide a quite compliant practice. Nevertheless, *in vitro* studies have demonstrated that bone marrow stem cells are capable of undergoing complete and functional transdifferentiation into neurons, and in these cell culture experiments, fusion mechanisms can be ruled out, since no co-culturing with other neuronal cell types was applied (2,10-15,25-29). In addition to the cell replacement mechanisms, the neurotrophic growth support mediated by bioactive factors secreted in a paracrine manner by stem cells may cause more significant and immediate treatment effects in the injured CNS (18). In fact, transplantation of bone marrow stem cells results in surprisingly low survival rates of the engrafted cells inside the brain tissue (10,17).

Among the bioactive factors secreted by bone marrow cells are brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF, FGF-2), nerve growth factor (NGF), and insulin-like growth factor-1 (IGF-1), which all are demonstrated to exert profound neuroprotective and neuroregenerative actions including the stimulation of both intrinsic neurogenesis and angiogenesis in the CNS (10,17,18,36,37). Another indirect molecular mechanism of action might convey the neuroprotective and -regenerative effects in the CNS of administered bone marrow. Hence, in a recent study it was shown that transplanted bone marrow MSC can activate resident astroglia in the ischemic brain leading to increased astrocytic secretion of glial cell derived neurotrophic factor (GDNF), which in the CNS promotes plasticity and recovery (36,37). Hence, the increased astroglial GDNF levels led to enhanced proliferation and migration of the resident NSC in the ischemic brain (37).

Reactive astroglia have several neuroprotective and regenerative actions in the brain and are the main source of neurotrophic growth factors, antioxidants, and neuronal survival signals as well as an astrocytic phenotype provides the endogenous source of NSC within the brain (36,38). Consequently, the study of transplanted MSC effects upon astroglia during brain ischemia (37) is fundamental, as it reveals how stem cell-based therapy may not only work through cellular replacement and/or cellular fusion. Hence, a range of molecular and/or paracrine mechanisms are likely contributing to the neurotherapeutic effects of bone marrow cells and

these involve secretion of bioactive factors, signal transduction mechanisms, extracellular cross-talking, and repair through activation of astroglia.

CONCLUSION

As reviewed here, the neurotherapeutic use of bone marrow-derived cells in brain injury and diseases has opened up new avenues of stem cell-based insight and treatment. Moreover, we will likely see in the near future that there are more options in terms of the different possibilities regarding how to apply which types of stem cells and/or their molecular repertoire in terms of secreted bioactive factors. Further elucidation of the diverse biological mechanisms by which various stem cells may exert neuroprotective, restorative and neuroregenerative functions will most likely result in the discovery of new and safer neurotherapies that can be applied in the clinic.

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PATHOGENESIS OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis is a devastating neurodegenerative disease affecting both upper and lower motor neuron. Despite extensive research the primary cause of the disease has not been indentified and the causative treatment is lacking. The present article describes mechanisms involved in the disease development and progression, including oxidative stress, excitotoxicity, mitochondrial dysfunction, protein aggregation, RNA processing, alterations of cytoskeleton functions and axonal transport, glial cell involvement and programmed cell death. ***Biomed Rev* 2011; 22: 7-14.**

Key words: apoptosis, axonal transport, glial cells, glutamate excitoxicity, mitochondrial dysfunction, oxidative stress, superoxide dismutase

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting up to 500 000 people worldwide. It is caused by a selective and progressive loss of upper motor neurons of the corticospinal and corticobulbar tracts and lower motor neurons localized in the brain stem and anterior horns of the spinal cord. At present ALS is an incurable disease leading to respiratory insufficiency and death within three years from onset. The symptoms usually start in the fifth decade but they may become apparent before the age of thirty or over the age of 70. The onset is either bulbar or spinal. In the bulbar form, the main symptoms include dysarthria and dysphagia. The limb symptoms start with asymmetric muscle paresis and wasting either in distal or, less frequently, in proximal muscles. Both forms end up involving all voluntary muscles with the exception of sphincters. There is no sensory involvement. The disease progression varies between individuals. In approximately 20% of patients the survival exceeds 5 years and only in about 10%, more than 10 years (1). There is no causative treatment. A vast majority of ALS cases are sporadic (sporadic ALS, SALS). Only approximately 10% of cases are inherited, mainly as an autosomal dominant trait (familial ALS, FALS). Up to 23% of FALS and 7% of SALS cases are due to mutations in the *SOD1* gene (2). Eight percent of FALS and over 1% of SALS cases are caused by mutations in *TARDBP* and *FUS/TLS,* two recently discovered genes encoding for proteins involved in RNA processing (3). Other genetic factors among which the proteins alsin, progranulin, angiogenin, vascular endothelial cell growth factor, vesicle-associated membrane protein and

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senataxin are less frequent.

The present article highlights recent advances in our understanding of molecular mechanisms responsible for ALS.

OXIDATIVE STRESS

Radical oxygen species (ROS) are reactive forms of oxygen containing one unpaired electron. They can easily peroxidize organic and inorganic compounds changing their structural and functional properties. Lipid peroxidation may influence cell membrane permeability. Oxidation of proteins leads to alteration of their enzymatic activity and/or conformation. When reacting with nucleic acids, ROS may lead to mutagenesis. Radical oxygen species are synthesized in reactions of respiratory chain or beta-oxidation. In physiological conditions they are efficiently neutralized by a number of enzymatic and non-enzymatic cell defense mechanisms. However, the imbalance between their production and removal leads to oxidative stress. CuZn superoxide dismutase (SOD1) is a free radical scavenging enzyme, catalyzing the reaction of peroxidation of oxygen peroxide ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) and oxygen: $O_2^{\bullet-} + O_2^{\bullet-} + H_2 \rightarrow H_2O_2 + O_2$.The discovery of mutation in the *SOD1* gene in FALS in 1993 directed the studies on ALS pathogenesis toward the role of oxidative stress (4).

It was primarily suggested that mutations in *SOD1* decreased cell capability of neutralizing ROS. However, in many cases of *SOD1* mutations there were no changes in SOD1 enzymatic activity. Transgenic mice harboring FALS-linked *SOD1* mutations demonstrate normal or enhanced enzymatic activity (5). Moreover, it was proved that transgenic mice either knocked-out for *SOD1* or over-expressing the wild type SOD1 (wtSOD1) did not develop motor neuron disease (MND) (6). Interestingly enough, transgenic mice harboring human mutated SOD1 develop MND despite normal expression of wtSOD1 (7). It was therefore postulated that mutations in *SOD1* gene induce a toxic gain of function of the encoded protein (8). Hundred fifty seven ALS-linked mutations in the *SOD1* have been reported to date (for complete list please refer to http://www.alsod.org, http:// alsod1.iop.kcl.ac.uk/reports/mutations). Currently proposed mechanism of mutated SOD1-induced neurodegeneration include alteration of substrate specificity and instability of mutated protein (9). Nevertheless, nearly 20 years after the discovery, the exact mechanism in which the mutations lead to selective death of motor neurons remains unknown.

Despite the lack of influence of decreased SOD1 activity on the clinical course of ALS, several studies have shown signs of alteration of ROS scavenging mechanisms in MND (reviewed in 10). Increased concentration of carbonyl groups (products of protein peroxidation), malone dialdehyde (products of lipids peroxidation), and 8-OHdG (nucleic acids) was found in cortex and/or spinal cord of patients who died in course of SALS (10,11). There was an increase of 3-nitrotyrozine (products of tyrosine peroxidation) concentration in anterior horns of the spinal cord in both SALS and FALS patients. High level of 8-OHdG and hydroxynonenal (lipid peroxidation product) was observed in CSF of patients suffering from ALS. The presence of peroxidation products of proteins, lipids and nucleic acids was also shown in mouse transgenic models of MND both at pre symptomatic and symptomatic stage (10). Antioxidant treatment was able to delay disease onset and progression in SOD1-transgenic mice, but all completed clinical trials in ALS patients showed no clinical efficacy of antioxidants (1).

EXCITOTOXICITY

A report on increased glutamate concentration in CSF of ALS patients directed the pathogenetic studies toward excitatory amino acids (12). Exposition of cultured motor neurons to glutamate or aspartate was shown to induce cell death by apoptosis or necrosis, dependent on the amino acid concentration (13,14). In physiological conditions, activation of glutaminergic receptors, mainly N-methyl-D-aspartate (NMDA) receptors, leads to opening of post-synaptic calcium channels and the intracellular influx of Ca^{+2}. Given the role of calcium ions in regulation of cellular growth, differentiation and synaptic activity, maintaining the calcium homeostasis is vital. For this reason, an excessive activation of glutaminergic receptors due to pathologically increased glutamate concentration may alter calcium homeostasis and lead to cell death. The main protein responsible for a reduction of glutamate concentration in the synaptic cleft is a glial transporter of excitatory amino acids (EAAT2). It is a Na^{+}-dependent protein localized on the surface of glial cells, involved in presynaptic uptake of the glutamate. EAAT2 is particularly vulnerable to oxidative stress (15). Excessive influx of calcium ions due to activation of glutaminergic receptors leads to increased mitochondrial production of ROS, which alter EAAT2 function and increase excitotoxicity. Defects in EAAT2 have been found in 80% of SALS patients (16). Decrease of EAAT2 concentration was observed in brains of patients who died in course of ALS. In transgenic models of the disease, reduced EAAT2 expression correlated with increased concentration of glutamate in the nervous system (17). Riluzole, an inhibitor of NMDA receptors, is at present

the only drug for ALS approved by Food and Drug Administration in USA and by European Medicines Agency. Although its accurate mechanism of action remains unclear, it inhibits glutamate release from presynaptic membrane, increases its extracellular uptake and stabilizes voltage-gated sodium channels in inactive state (18). An 18-month treatment with riluzole prolongs patients' survival by approximately 7% (19).

Until 20 years ago, the NMDA receptor was the only glutamate receptor known to be Ca^{2+}-permeable. It is now well established that the ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor of glutamate is densely distributed in the mammalian brain and is involved in mediating fast excitatory synaptic transmission, motor neurons showing selective vulnerability to activation of AMPA receptors; expression of modified human AMPA receptor in transgenic animals induces MND (20). The permeability of AMPA receptors for calcium ions depends on their subunit structure (21). Increased expression of GluR3, one of the AMPA receptor subunits, in motor neurons carrying *SOD1* mutations increases their susceptibility to kainate-induced excitotoxicity. The toxicity results from facilitation of the Ca2+ influx (22). Antisense oligonucleotides therapy against *GluR3* was able to delay motor impairment and extend survival of transgenic mice with *SOD1* mutation (23). Human trials have not been performed.

Noteworthy, recent data suggests a crosstalk between glutamate receptors and brain-derived neurotrophic factor (BDNF) in modulating synaptic functions (24,25, also see 20-22). Such a BDNF-NMDA/ AMPA receptor signaling might be pursued in the pathogenesis and therapy of ALS.

MITOCHONDRIAL DYSFUNCTION

Post mortem studies in ALS revealed the presence of abnormal mitochondria localized under the sarcolemma, in synaptic terminals and in anterior horn cells (26). Muscle biopsies performed in ALS showed big mitochondria with increased calcium concentration (26). There were also reports on impaired activity of complex I and IV of the respiratory chain (encoded by mitochondrial DNA) in skeletal muscles and spinal cord of ALS patients (27). In transgenic models of ALS with human *SOD1* mutation, aggregates of abnormal mitochondria with dilated external membrane were found within the motor neurons. They resulted from membrane detachment, which occurred after aggregation of mutated SOD1 in the intermembrane space of mitochondria. The presence of these structures early in course of the disease, when the loss of motor neurons is not yet accompanied by clinical symptoms, points at an active role of mitochondria in ALS pathogenesis (28). Currently, there are 2 clinical studies with the use of mitochondrial protection agents, which may show some promise in the treatment of ALS. Among them there are tamoxifen, which binds to the mitochondrial permeability transition pore, and dexpramipexol, which reduces the ROS production and improves mitochondrial function (1).

PROTEIN AGGREGATES

Although protein aggregates are a hallmark of neurodegeneration, there is still no agreement on their exact role. By binding proteins necessary for cell survival, the protein aggregates may lead to cell degeneration. On the other hand, they may protect the cell from toxic products by their direct binding. And finally, as by-products of pathological processes they can simply be markers of degeneration. There are three types of cellular inclusions typically found in ALS. They are hyaline conglomerate inclusions (HCI), Bunina bodies and ubiquinated inclusions (UBI) (29). Hyaline conglomerate inclusions are big inclusions of phosphorylated and non-phosphorylated neurofilaments and random proteins or cellular organelles. Since they were found in a number of neurodegenerative diseases and control tissue, they are considered unspecific derivatives of various pathological processes. Bunina bodies are small eosinophilic inclusions found in cell bodies of motor neurons localized in the spinal cord of 80–100% SALS cases. They are positive for cystatin C. Similar structures were found in physiological aging but not in other neurodegenerative diseases. Most characteristic aggregates present nearly in all cases of SALS are ubiquinated inclusions. They were found in motor neurons of brain stem and anterior horns of the spinal cord. They present a variety of shapes from fibrillary, through skein-like to compact ('Lewy-like') inclusions. Until 2007 the main protein compound of UBI was unknown. They were not reactive to antibodies against tau protein, neurofilaments, alpha-synuclein or cystatin C, the elements characteristic for neurodegenerative precesses (30). In 2007 Mackenzie and coworkers (31) reported that UBI were positive for transactive response DNA-binding protein (TDP-43), previously described in frontotemporal lobe degeneration (FTLD) (32). Most interestingly, TDP-43 was found in cases of SALS or FALS but not in FALS with mutation in *SOD1* gene indicating a different pathological entity of these two conditions (33). It was further identified in some cases of Alzheimer's and Parkinson disease, inclusion

body miositis and myopathy with rimmed vacuoles (34). In neurodegenerative diseases it did not however colocalize with tau and synuclein inclusions. Within the group of FALS, TDP-43 inclusions were also found in cases with *ANG* and *TARDBP* but not *FUS/TLS* mutations. TDP-43 is a 414-aminoacid protein encoded by *TARDBP* gen. In physiological conditions it is localized in the nucleus. However, no wt TDP-43 protein was found within the nuclei of the neuronal cells from patients affected by FTLD or ALS (35). In these cases all the pool of intracellular TDP-43 accumulated in the cytoplasm. It was subjected to hyperphosphorylation, ubiquitination and cleavage to generate C-terminal fragments. The cytoplasmic redistribution of TDP-43 was found to be an early event (36). Following this discovery, mutations in *TARDBP* were found responsible for 4% of FALS and1% of SALS cases (33). Till now 38 disease-causing mutations were identified.

RNA PROCESSING

Since *TARDBP* contains two RNA-recognition motifs, TDP-43 is a RNA- and, to a lesser extent, a DNA-binding protein. It attaches to a TG-rich fragment of RNA within the promoter of HIV-1 gene to stop its transcription (37). By binding to a 3'-UTR sequence encoding for human neurofilament light chain (hNFL1), it stabilizes the transcript and helps maintain the correct neurofilament stoichiometry (34). The plausible role of RNA processing in pathogenesis of ALS was reinforced by identification of disease causing mutations in *FUS/TLS* gene (38). It encodes for another RNA-binding protein called fused in sarcoma/translated in liposarcoma (FUS/TLS). FUS/TLS is a 526-aminoacid protein encoded by 15 exons. It was shown to bind both RNA and DNA, and, like TDP-43, function in diverse processes including transcription, alternative splicing and microRNA processing (33). The mutant form of FUS/TLS accumulates in cytoplasm of neurons and glial cells although the nuclear cleavage of FUS/TLS in ALS is less spectacular. No ubiquitination and phosphorilation of the protein was ever reported. Beside ALS, FUS/TLS forms neuronal intranuclear inclusions in poliglutamine diseases such as Huntington disease and spinocerebellar ataxias (39). It does not however form aggregates in FALS with *TARDBP* mutations.

Thirty mutations of *FUS/TLS* were found in ALS, being responsible for 4% of FALS and rare cases of SALS. Several *in vitro* studies performed recently shaded more light on the role of TDP-43 and FUS/TLS in RNA processing (33).

ALTERATIONS OF CYTOSKELETON FUNCTIONS AND AXONAL TRANSPORT

Among other factors, neural cell homeostasis strongly depends on axonal transport. It provides cell with neurotrophic factors, carries signal proteins, cell organelles, cleaved proteins and membrane fragments. Human motor neurons may be 5000 fold larger than average cells and their axon length may exceed 1 meter (40). For this reason, the efficient axonal transport within motor neurons is particularly important. It depends on interactions between cytoskeleton and motor proteins. The cytoskeleton is composed of microtubules (MT) and MT-associated proteins, actin filaments/actin-associated proteins, and intermediate filaments, neurofilaments being a subtype of the latter. Microtubules are composed of assembled (polymerized) tubulin heterodimers. In course of their formation, the tubulin dimers are preferentially added to the plus end of the MT assuring its growth from the cell body towards the periphery (41). The transport of cargoes by MT-dependent ATP-associated motor proteins, kinesin and dynein, is baseds on MT's structural polarity. Actin filaments are localized mainly in the cell cortex thus not directly involved in neuronal transport. Neurofilaments, run along the axons and their main role is to control axonal caliber. Since the speed of signal conduction depends on axonal diameter, the neurofilaments are particularly abundant in large-diameter axons (42). Kinesins transport synaptic vesicles, membrane constituents and mitochondria from the cell body towards the plus end of the microtubules localized in the cell periphery. On the contrary, neurotrophic factors, exogenous substances and waste membrane fragments use the retrograde transport mediated by the denein/dynactin complex (43).

Several mutations of genes encoding for motor proteins have been found in motor neuron diseases. Hereditary spastic paraplegia is caused by mutations of kinesin heavy chain (*KIF3A*) (44). ALS with vocal cord paralysis was linked to mutation in the p150 subunit of the dynactin (45) Transgenic mice harbouring this mutation develop progressing loss of motor neurons (46). Mice overexpressing dynamitin, a dynactin inhibiting protein also develop MND (47). Mutation of the dynein heavy chain produces large fibers sensory neuropathy leading to secondary motor neuron loss in transgenic animals (48,49). The impairment of axonal transport was observed in preclinical stage of MND in transgenic mice with human *SOD1* mutation. It was postulated that accumulation of SOD1 aggregates in cells with already impaired transport might enhance motor neuron distress causing cell death in the mechanism of axonal strangulation (50).

GLIAL CELLS INVOLVEMENT

Expression of mutated SOD1 exclusively in motor neurons or in glial cells does not induce MND in transgenic models of the disease (51). Studies with the use of transgenic chimers expressing mutated human SOD1 or wtSOD1 in different population of cells within the same organism provided more evidence on the involvement of glial cells in the pathogenesis of ALS. It was found that expression of mutated SOD1 in some cells of the CNS induced the animals death in time inversely proportional to the number of cells harboring the mutation. Moreover, not all motor neurons harboring the mutation underwent degeneration. When mutated SOD1 was expressed in all motor neurons and only in a proportion of glial cells, the neuronal death would depend on the number of non-neuronal cells harboring the mutation (52).

The results of these studies show that the loss of motor neurons depends on the expression of mutated SOD1 in non-neuronal cells and the microenvironment they create. The expression of mutated SOD1 in glial cells is thus indispensible but still insufficient to induce MND.

Another issue is the plausible toxicity of the microglia in MND. Microglial cells expressing mutant human SOD1 reduce survival of motor neurons derived from human neural stem cells (53). The toxicity is alleviated in the presence of stem cell-derived astrocytes. On the other hand, IgG immune complexes or proinflammatory lipopolisacharides isolated from ALS patients are capable of inducing microglia activation, generation of ROS and release of glutamate, what induces toxicity towards primary motor neurons (54,55). The over-activated microglia is even able to render otherwise neuroprotective astrocytes dysfunctional and toxic to motor neurons (56). Anti-inflammatory treatment provides neuroprotection in the culture (57). The toxicity of spinal cord microenvironment may therefore be an important obstacle in implementation of stem cell-bases treatment strategies in ALS (1).

PROGRAMMED CELL DEATH

Although the morphological features of programmed cell death (apoptosis) were observed in some motor neurons of transgenic ALS model with *SOD1* mutation, the involvement of apoptosis in ALS is controversial (58,59). In humans, the *post mortem* studies in the spinal cord allowed to identify three stages of motor neurons death. In the first stage, called chromatolisis, there was cell edema, loss of Nissl substance and a translocation of the nucleus to the cell periphery. The second stage started with the loss of cell processes followed by cytoplasm homogenization and chromatin shrinkage. The last, apoptotic, step ended up with reduction of cell volume with round or fusiform shape formation. There was however no nucleus fragmentation typical for apoptotic cell death (60).

Even if the cell death in ALS does not occur in a way typical for classic apoptosis, several intracellular changes consistent with those observed in apoptosis have been found in motor neurons cell line NSC34, transgenic models of ALS/MND and ALS in humans (reviewed in 58). The up-regulation of caspase 9 expression was found in NSC34 cells cultured in the presence of mutated SOD1. Trophic deprivation resulted in additional activation of effector caspases 3 and 6 in the same model. Activation of caspase 1 and 3 was also observed in the spinal cord of transgenic mice in course of the disease. In another study, the activation of caspase 9 and 7 was accompanied by translocation of proapoptotic Bax protein from cytoplasm to mitochondria with cytochrom c release. Also in human *post mortem* studies there was an increase of caspase 1 and 9 expression in the spinal cord as well as increased activity of caspase 3 in anterior horns of the spinal cord and motor but not sensory cortex in ALS (61). In transgenic ALS model with *SOD1* mutation, there a decreased concentration of antiapoptotic Bcl-e and Bcl-xL proteins and increased expression of proapoptotic Bad and Bax proteins was observed in the spinal cord of symptomatic animals (59). The changes in human tissues are less spectacular. Immunohistochemical studies did not show differences between the reactivity of Bcl-2 and Bax proteins in ALS motor cortex and spinal cord compared to control. However in ALS, the proapoptotic Bax proteins was enriched in mitochondria compared to cytosol in anterior horn cells and motor cortex compared to the sensory cortex of the same patients (60).

CONCLUSION

Amyotrophic lateral sclerosis is a disorder of complex pathogenesis. The role of oxidative stress and mitochondrial dysfunction is supported by the usual middle age symptom onset. Like in physiological aging, at this time-point the antioxidant mechanisms are no longer thoroughly efficient. Since a high percentage of oxygen is used in mitochondria, these organelles are also the main source of ROS. These can increase the glutamate release or decrease its retrograde uptake by EAAT2. The NMDA-dependent influx of calcium ions induces the synthesis of nitric oxide (NO) within the cell, what may in turn lead to nitration-dependent impairment of neurofilaments

phosphorylation. The protein aggregates formed in this process lead to impairment of axonal transport and motor neuron death in the mechanism of axonal strangulation. The imbalanced calcium homeostasis may activate the mitochondrial pathway of apoptosis. The toxic environment created by the glial cells might further decrease the efficacy of reparatory mechanisms. Although the recent discovery of the involvement of DNA/RNA binding proteins in pathogenesis of ALS shaded more light on the pathogenesis of ALS without *SOD1* mutations, it did not yet allow identifying the trigger point for the motor neuron death in this lethal disease.

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G PROTEIN-COUPLED SPHINGOSINE-1-PHOSPHATE RECEPTORS: POTENTIAL MOLECULAR TARGETS FOR ANGIOGENIC AND ANTI-ANGIOGENIC THERAPIES

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Sphingosine-1-phosphate (S1P) is a plasma lipid mediator with pleiotropic activities; it is constitutively produced in red blood cells and vascular endothelial cells through phosphorylation of sphingosine by one of two S1P synthesizing enzymes, sphingosine kinase 1 and 2 (SphK 1, 2), and exported into plasma to bind to high density lipoprotein and albumin. Sphingosine-1-phosphate acts through five members of the G protein-coupled S1P receptors (S1PR1-S1PR5) to exert diverse actions, which include vascular maturation in embryonic stage and postnatal angiogenesis, maintenance of functional integrity of vascular endothelium, regulation of vascular tonus, and lymphocyte trafficking. Sphingosine-1-phosphate is unique in its ability to regulate cell migration either positively or negatively by acting through different receptor subtypes. S1PR1 and S1PR3 mediate chemotactic cell migration toward S1P via G_i/Rac pathway, whereas S1PR2 mediates S1P inhibition of chemotaxis via $G_{12/13}$/Rho-dependent inhibition of Rac. Sphingosine-1-phosphate positively or negatively regulates tumor cell migration, invasion in Matrigel, and hematogenous metastasis in manners strictly dependent on S1P receptor subtypes expressed in tumor cells. S1PR1 (and S1PR3) also mediates activation of G_i/phosphatidylinositol 3-kinase (PI3K)/Akt and stimulation of cell proliferation/survival, whereas S1PR2 could mediate suppression of cell proliferation/survival through $G_{12/13}$/Rho/Rho kinase/PTEN-dependent Akt inhibition. S1PR1 (and S1PR3) expressed in endothelial cells mediates angiogenic action of S1P by stimulating endothelial cell migration, proliferation and tube formation. In a mouse model of hindlimb ischemia after femoral artery resection, repeated local administration or sustained delivery of S1P, or transgenic overexpression of SphK1, accelerates post-ischemic angiogenesis, through the S1P actions on both endothelial cells and bone marrow-derived myeloid cells (BMDCs). In tumor cells, SphK1 is upregulated especially in advanced stages, through mechanisms involving both activating Ras mutation and hypoxia, which leads to increased S1P production and also decreased cellular content of pro-apoptotic sphingolipid ceramide, a metabolic precursor of S1P. Apoptotic tumor cells also produce S1P through SphK2 activation, thus implicated in tumor angiogenesis by acting on endothelial cells through S1PR1/S1PR3, as well as tumor-infiltrating macrophages and BMDCs. Inhibition of S1PR1 function by either an anti-

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S1P antibody or FTY720 inhibits tumor angiogenesis and tumor growth. Differently from S1PR1, S1PR2 expressed in host cells mediates inhibition of tumor angiogenesis and tumor growth, through mechanisms involving the suppression of endothelial cell migration, proliferation and tube formation, and inhibition of BMDC recruitment to tumor stroma with suppressed expression of pro-angiogenic factor and matrix metalloprotease 9. These findings provide the molecular basis for S1P receptor subtype-selective targeting strategies aiming at angiogenic therapy for occlusive peripheral arterial diseases, and anti-angiogenic and anti-tumor therapies against cancer. ***Biomed Rev*** **2011; 22: 15-29.**

Key words: sphingosine-1-phosphate, S1P receptors, peripheral arterial disease, tumor angiogenesis

INTRODUCTION

During the past two decades the existence of the novel intercellular signaling system has been elucidated, which comprises a plasma lysophospholipid mediator sphingosine-1-phosphate (S1P) (1), its synthesizing/degrading enzymes (2-6), membrane S1P transporters (7,8), S1P carrier proteins in the plasma (9), and five members of the G protein-coupled S1P-specific receptor subtypes, S1PR1~S1PR5 (10-22). The S1P signaling system plays crucial roles in mammalian embryonic development (24-26) and post-natal homeostasis in the cardiovascular (27-36), immune (37-47) and nervous systems (26,48). The S1P signaling system is also implicated as the target of therapeutic intervention in a variety of human diseases. Multiple sclerosis, a debilitating autoimmune disease, is now treated with the S1P receptor agonist prodrug FTY720 (49), whose phosphorylation product downregulates S1PR1 in lymphocytes to inhibit their recirculation, thus resulting in lymphopenia (38,50). In addition, accumulated evidence in experimental animal disease models indicates that targeting the S1P signaling system is a promising tactic for both angiogenic and anti-angiogenic therapies, for treating obliterative peripheral arterial diseases and malignant tumors, respectively (51-57). Before addressing this point, we will overview how blood vessels are generated during embryonic phase and after birth, and how the S1P signaling system is involved in blood vessel formation in physiological and pathological conditions.

VASCULOGENESIS, ANGIOGENESIS, AND VASCULAR MATURATION IN DEVELOPMENT

Mammalian blood vessels are generated by two different mechanisms, i.e. vasculogenesis and angiogenesis, in which endothelial cells derived from distinct sources are involved (58-61). In vasculogenesis, which is a process of *de novo* blood vessel formation in the early embryonic phase, endothelial progenitor cells (EPCs) or angioblasts differentiate from mesodermal cells, which proliferate, migrate and differentiate to become endothelial cells that are endowed with cell surface adhesion molecules including PECAM (platelet endothelial cell adhesion molecule) and vascular endothelial (VE)-cadherin. Endothelial cells then form aggregates through the homologous cell-cell adhesion, followed by dynamic morphogenesis and remodeling to form a primordial tubular network. The endothelium in the early embryo is then destined to either arterial or venous differentiation, leading to the formation of the dorsal aorta and cardinal vein. A part of arterial endothelial cells give rise to hematopoietic stem cells to initiate definitive hematopoiesis, whereas a part of venous endothelium differentiate to become lymphatic endothelial cells to form blind-ended lymphatic vessels (61).

Vasculogenesis is followed by angiogenesis, which takes place not only in embryonic period but also after birth. In angiogenesis, new vessels are created by sprouting of pre-existing capillaries (61,62). In response to pro-angiogenic chemoattractant signals in the microenvironment, capillary endothelial cells are destabilized, stimulated to migrate, invade through extracellular matrix (ECM) with its proteolytic degradation, and proliferate to form a cylindrical sprout, in which tip cells, a minor population of leading cells that sense and respond to pro-angiogenic cues, and stalk cells that trail the tip cells and form a cylinder structure, are distinguished. Sprouting new vessels get together to coalesce and undergo remodeling to build up microvessel networks in previously avascular areas (61,62).

Newly formed blood vessels then undergo the process of maturation, in which endothelial tubes are covered with mural cells (pericytes and vascular smooth muscle cells), which stabilize and strengthen the vessels, resulting in the formation

of a mature vascular bed (61-64). This process depends upon the recruitment of mural cells and heterologous endothelial-mural cell adhesion.

Angiogenic expansion, remodeling and vascular maturation leads to the formation of a fully functional embryonic vascular system. In addition, angiogenesis takes place after birth under both physiological and pathological conditions, which include wound healing, post-menstrual endometrial regeneration, inflammation-associated angiogenesis, post-ischemic angiogenesis and tumor angiogenesis (61,62,65). Importantly, bone marrow-derived myeloid cells and macrophages play a crucial role in preparing angiogenic microenvironment, through the production of pro-angiogenic growth factors, chemokines and cytokines, and proteases that degrade ECM and release ECM-bound growth factors, and by physically interacting with and thereby supporting vascular sprouting. In addition, under certain conditions circulating EPCs derived from bone marrow could be incorporated into angiogenic neovessels (65-67).

Vasculogenesis and angiogenesis are controlled by multiple arrays of pro-angiogenic growth factors, chemokines, cytokines, guidance molecules and their receptors, ECM components and their receptor/adhesion molecules, in a complex, spacio-temporally organized manner. Vascular endothelial growth factors (VEGFs) are the most critical driver of vasculogenesis and angiogenesis. Angiopoietins, ephrins and platelet-derived growth factors (PDGFs), and transforming growth factor-β (TGF-β) are required for vascular remodeling and maturation (58-67). In addition to these angiogenic factors, S1P is attracting increasing attention as a regulator of vascular formation.

TUMOR ANGIOGENESIS

Tumor angiogenesis plays a crucial role in tumor progression (68). As soon as a tumoral micronodule becomes hypoxic especially in the core region, the cellular level of hypoxia inducible factor1a (HIF1a) is upregulated, through the escape from von Hippel-Lindau protein-mediated proteasomal degradation (69). The formation of a HIF1a-containing transcription factor leads to stimulation in the expression of multiple genes that are involved in tumor progression, which include glucose transporters GLUT1/3, glycolytic enzymes, VEGF and other pro-angiogenic factors and SphK1 (69-72). These events switch on tumor angiogenesis, a critical event that allows abundant oxygen and nutrient supply to tumor cells to let the previously minute tumor proceed through the next stage, i.e. tumor progression with a rapid growth, invasion and eventual metastasis, processes dependent on newly constructed tumor vessels (68).

In tumor angiogenesis, endothelial cells in preexisting blood vessels located in the vicinity of tumors are induced to destabilize, migrate toward a tumor, proliferate and undergo morphogenesis to form networks of microvessels (65). These processes are reminiscent of developmental angiogenesis and regulated by multiple pro-angiogenic growth factors including VEGFs, angiopoietin-1, fibroblast growth factors (FGFs) and PDGFs, which are produced by both tumor cells and host stromal cells, among which are tumor-associated macrophages (TAMs) and other bone marrow-derived cells (BMDCs), which include $CD11b^+$ myeloid cells (58,59,65-69). TAMs are macrophages that have undergone phenotypic change in tumor microenvironment from a classically activated phenotype of proinflammatory anti-tumor immunocompetent M1 state to an alternatively activated, anti-inflammatory and angiogenic M2 state that favors tumor growth. Tumor-associated macrophages and BMDCs not only produce pro-angiogenic growth factors, tumor promoting growth factors and chemotactic factors that mobilizes BMDCs to tumor microenvironment, but also activate matrix metalloproteases (MMPs), which degrade ECM and liberate matrix-bound proangiogenic factors, thus establishing an angiogenic tumor microenvironment (69). The recruitment of pericytes and SMCs to the newly formed microvessels stabilizes them, establishing stable and abundant blood supply (60-64). Accumulated evidence now indicates that S1P is involved in tumor angiogenesis and tumor progression.

PRODUCTION AND DELIVERY OF S1P

S1P is present in the plasma at the order of 10^{-7} mol/L, which is for the most part bound to HDL and albumin (19,35,36,42,70). The major source of plasma S1P is red blood cells (43,71) and vascular endothelial cells (72), in which S1P is constitutively produced by SphK1 through phosphorylation of sphingosine, and exported by plasma membrane S1P transporters (7,8), followed by specific binding to HDL-associated apolipoprotein M (and non-specific binding to albumin) in the plasma (73). SphK1 and the other S1P synthesizing enzyme SphK2 share a conserved catalytic domain but are expressed in spacio-temporally distinct manners (74). Deletion of either SphK1 or SphK2 is functionally fully compensated by each other, whereas SphK1/SphK2 double knockout (KO) mice are embryonic lethal with an undetectable tissue level of S1P, indicating that S1P is produced exclusively by SphK1 and SphK2 in

vivo (26,75,76). In addition to erythrocytes and endothelial cells, activated platelets, mast cells and other types of cells release S1P (77). S1P thus released by blood cells and vascular endothelial cells activates endothelial S1PR1, the principal S1P receptor subtype expressed in this cell type, which mediates mitogenic, anti-apoptotic and chemotactic effects of S1P, as well as S1P-dependent suppression of vascular permeability, implicating critical role of S1P in maintenance of vascular integrity and wound healing (35,36). In addition, in various types of cells either of the SphKs could be stimulated to produce S1P in response to growth factors and cytokines (74).

It is of note that in tumor cells, especially in advanced stages, SphK1 is upregulated through multiple mechanisms, which include Ras activation, deletion of p53, and hypoxia (69-72, 78-89). SphK1 upregulation leads to not only increased production of S1P, but also a reduction in cellular levels of pro-apoptotic ceramide and sphingosine, which are metabolic precursors of S1P (90-94). Upregulation of SphK1 and a reduction in ceramide in tumor cells are closely associated with their acquisition of resistance against chemotherapeutic agents (78,80,81,84,95). On the other hand, SphK1 is implicated in upregulation of HIF1a (71,72). Thus, SphK1 and HIF1a may constitute a feed-forward amplification loop favoring tumor progression. In addition to S1P derived from hypoxic tumor cells through the action of SphK1, S1P is also released from apoptotic tumor cells, in which SphK2 is responsible for S1P production (96). S1P thus released from tumor cells is implicated in tumor angiogenesis, in which endothelial cells and tumor-infiltrating BMDCs and macrophages are targets of S1P actions (94,96-98). Indeed, S1P plays an essential role in inducing M2 phenotype in macrophages to render them to behave as TAMs, in which transcriptional upregulation of HIF1a under normoxia is implicated (98).

G_i-COUPLED CHEMOTACTIC S1PR1/S1PR3 MEDIATE A PRO-ANGIOGENIC ACTION OF S1P

Ubiquitously expressed S1P receptor subtypes, S1PR1, S1PR2 and S1PR3 have been studied extensively. They play crucial roles in mediating diverse actions of S1P, which reflect receptor subtype-dependent distinctive intracellular signaling and their cell type-specific expression patterns (Fig. 1) (12-19, 20-23,40,99-104). Vascular endothelial and smooth muscle cells, as well as leukocytes of both myelocytic and lymphocytic lineages, are the major targets of S1P action through either of the three major S1P receptor subtypes. The other members, S1PR4 and S1PR5, are relatively restricted in their expression to the immune and the nervous system, respectively (22).

S1PR1 couples exclusively to G_i and activates well known G_i-dependent pathways, including Ras-ERK and PI3K-Akt pathways, which lead to stimulation of mitogenesis and survival (Fig. 1). S1PR1- G_i-PI3K axis also activates Rho family small GTPase Rac, which is essential for regulating actin cytoskeleton and cell migration. In addition to Ras-ERK, PI3K-Akt and -Rac activation, S1PR1 mediates activation of phospholipase C (PLC) with consequent Ca^{2+} mobilization *via* G_i (13-15,19-23,40,99,102-104).

Rac activation leads to subcellular cortical actin assembly and focal contact to ECM, giving rise to lamellipodia or membrane ruffling (105) that features the leading edge of migrating cells toward chemotactic ligands and growth factors for G_i-coupled receptors and receptor tyrosine kinases. Rac is required for cell migration in vitro and in vivo for vascular formation in embryo (99,106).

S1PR1 is expressed in a variety of cell types including vascular endothelial cells, leukocytes of both lymphoid and myeloid origins, fibroblasts and tumor cells of epithelial and non-epithelial origins. S1PR1 expressed in these cell types, if not counteracted by an opposing function mediated by S1PR2, mediates chemotaxis toward S1P in pertussis toxin-sensitive, Rac-dependent manners (24,28-31,47).

S1PR1 expressed in lymphocytes is responsible for their egress from secondary lymphoid organs to lymph and then blood according to an increasing S1P concentration gradient imposed among these compartments, ensuring lymphocyte recirculation and immune surveillance. A Chinese medicine-derived sphingosine mimetic FTY720 (Fingolimod^R) is a potent S1P receptor modulator prodrug with multiple actions, which after phosphorylation by SphK2 *in vivo*, acts as an agonist for S1PR1, S1PR3, S1PR4 and S1PR5 but not S1PR2, and also as a functional antagonist for S1PR1 because of inducing its downregulation (38,39,44,45,107). S1PR1 downregulation in lymphocytes results in the failure of their egress from secondary lymphoid organs and, thereby, peripheral blood lymphopenia. FTY720 is now clinically employed as an immunosuppressive drug for the treatment of multiple sclerosis (49).

S1PR1 expressed in vascular endothelial cells, together with S1PR3, mediates angiogenic action of S1P, which include stimulation of endothelial cell migration, proliferation, survival and morphogenesis of tubular structures, which depends upon reinforcement of VE-cadherin mediated adhesion between endothelial cells (27,32-35,104,109). Endothelial

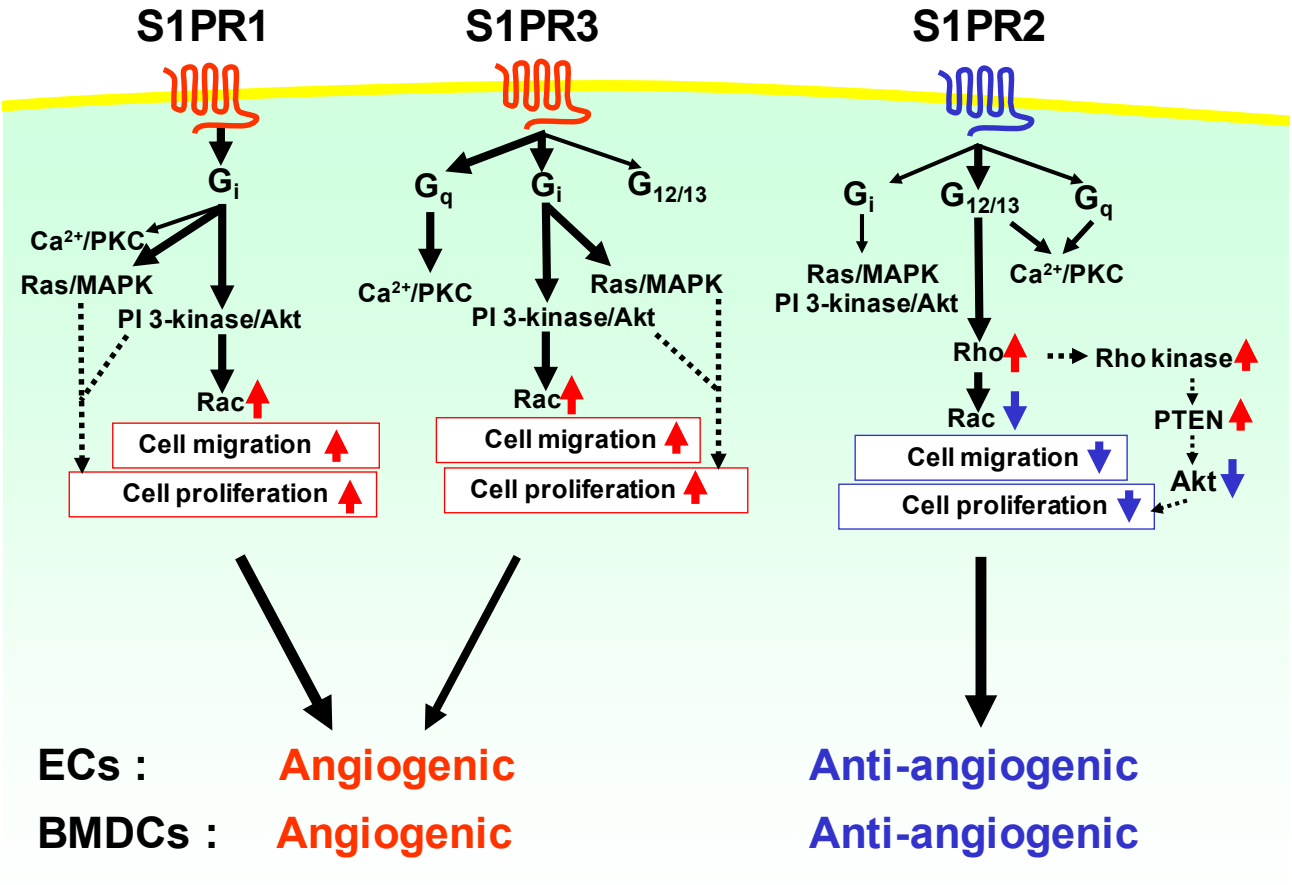

Figure 1. S1P receptor subtype-specific coupling to heterotrimeric G proteins and intracellular signaling pathways.
S1PR1 couples exclusively to G_i to activate Ras-ERK and PI 3-kinase (PI3K)-Akt/Rac pathways, leading to stimulation of chemotaxis and cell proliferation. S1PR3 activates G_q-PLC-Ca^{2+} pathway, and G_i-Ras-ERK and -PI 3-kinase-Akt/Rac pathways. S1PR3- $G_{12/13}$ –Rho pathway is masked and not evident unless G_i is inhibited by pertussis toxin. S1PR2 couples to $G_{12/13}$ to induce potent Rho activation, leading to inhibition of Rac and cell migration, and also inhibition of cell proliferation via inhibition of Akt. S1PR1/S1PR3 expressed in endothelial cells (ECs) and bone marrow-derived myeloid cells (BMDCs) mediate angiogenic effects of S1P. In sharp contrast, S1PR2 expressed in these cells mediates anti-angiogenic effects of S1P.

S1PR1 is also required for maturation and stabilization of nascent microvessels, which includes recruitment of mural cells and their tight coverage around naked endothelial tubes, in which heterologous endothelial-mural cell adhesion should take place. Indeed, in S1PR1-null mice vasculogenesis takes place normally but mural cell coverage is defective, leading to massive hemorrhage and embryonic death at mid-gestation (24). This phenotype is reproduced in endothelial cell-specific conditional S1PR1 knockout (KO) mice, and also in SphK1/2-double KO mice. PDGF-B null mice also show defective mural cell coverage and vascular maturation among other abnormalities. In endothelial-mural cell adhesion, endothelial S1PR1-G_i-PI3K-Rac signaling is required for phosphorylation of N-cadherin and N-cadherin/β-catenin/actin complex formation (110).

Endothelial S1PR1-dependent cell-cell adhesion is essential not only for angiogenesis and vascular maturation, but also for maintenance of vascular integrity through suppression of transmural permeability and leakiness. In this procss S1PR1-G_i-PI 3-kinase-Rac-dependent signalings are also operating, which include enhancement in formation of VE-cadherin-based formation of adherens junctions (111) and also that of tight junctions, in which junctional adhesion molecules such as zona occludens (ZO) proteins are involved (27,112).

It is reported recently that S1PR1/3 mediate macrophage phenotype switching from proinflammatory (classic) to anti-inflammatory (alternative) phenotype, leading to suppression of proinflammatory cytokine secretion. It is also reported that S1PR1/3 signaling contributes to induction of TAM phenotype (96).

S1PR3 resembles S1PR1 in that it activates Ras-ERK, PI 3-kinase-Akt and -Rac via G_i, mediating mitogenic/prosurvival and chemotactic effects of S1P (17-23,40,99-104,113). In addition, S1PR3 couples to Gq to potently activate PLC-Ca^{2+} signaling, and to $G_{12/13}$-Rho, the latter being a relatively minor pathway that becomes evident in pertussis toxin-treated, G_i-inactivated condition (99,113).

S1PR3 (and S1PR1) plays a pivotal role in S1P activation of endothelial nitric oxide synthase (eNOS), in which Ca^{2+}-dependent, Akt-dependent and ERK1/2-dependent mechanisms are operating (103,114-117). Thus, S1P induces NO-dependent vascular relaxation via S1PR3 (and S1PR1) activation in endothelial cells. In addition, NO plays crucial roles in protection of endothelial function and prevention of atherogenesis. S1P specifically binds to M apolipoprotein in HDL. HDL exerts its anti-atherogenic effects not only by subtracting excess cholesterol from vascular endothelium but also by providing endothelial S1PR1 and S1PR3 with S1P (114).

S1PR3 expressed in vascular smooth muscle cells, on the other hand, mediates vascular contraction via G_q-coupled Ca^{2+} mobilization and consequent activation of myosin light chain kinase and protein kinase C. Relative contributions of S1PR3 expressed in vascular endothelial vs. smooth muscle cells on vascular tone could depend upon vascular beds. In addition to mediating S1P actions on vasoconstriction or relaxation, S1PR3 expressed in the heart mediates negative chronotropic effect in a G_i-dependent manner, which is reminiscent of muscarinic acetylcholine receptor. S1PR3 KO mice are phenotypically normal (103) .

$G_{12/13}$-COUPLED CHEMOREPELLANT S1PR2 MEDIATES AN ANTI-ANGIOGENIC ACTION

S1PR2 is quite different from S1PR1 and S1PR3, and is the first G protein-coupled receptor to be identified that mediates inhibition of cell migration (99, 102,113,118-120). The repertoire of G protein coupling by S1PR2 overlaps with that of S1PR3, however, S1PR2 robustly couples to $G_{12/13}$ to induce potent activation of RhoA (16,19-23,99,101-104,113,118-120). At the site downstream of $G_{12/13}$-RhoA, S1PR2 potently inhibits its chemoattractant-induced Rac activation via stimulation of Rac GTPase-activating protein (Rac GAP) activity to inhibit cell migration toward these chemoattractants (19-23,99,102-104,118-120). Endogenous S1P2R expressed in B16 melanoma cells mediates inhibition of Rac, cell migration and invasion in Matrigel, which are abolished by S1PR2-selective antagonist, JTE013 (118).

S1PR2-mediated, $G_{12/13}$-coupled RhoA activation also leads to activation of Rho kinase or ROCK, and ROCK-dependent activation of PTEN, with consequent reductions in 3'-phosphorylated phosphoinositides, PIP_3 and PIP_2, which are required for Akt activation (121). Inhibition of Akt via S1PR2 leads to inhibition, rather than stimulation, of cell proliferation by S1P (121-124). S1PR2 mediates relatively weak activation of Ras/ERK and PI3K pathways via G_i (16,20-23,99,101-104,118), which may explain mitogenic actions of S1PR2 under certain conditions.

S1PR2-mediated, $G_{12/13}$-Rho-dependent inhibition of Rac and Akt underlies potent inhibitory action of S1PR2 on angiogenesis (123). Thus, S1PR2 expressed in murine lung microvascular endothelial cells (MLECs) mediates inhibition by S1P of their migration, proliferation, and tube formation in vitro, and angiogenesis in vivo in subcutaneous Matrigel plug assay and tumor angiogenesis. In S1PR2 KO mice tumor angiogenesis and tumor progression are significantly enhanced as compared to wild type (WT) littermates. Subcutaneous inoculation of tumor cells in WT mice together with MLECs obtained from S1PR2 KO mice markedly potentiated tumor angiogenesis and tumor growth as compared to tumor cell inoculation with WT MLECs, which implicates endothelial cell-autonomous function of S1PR2. In addition, the recruitment of BMDCs to tumor stroma, the expression of proangiogenic factors, and MMP9 activity were all enhanced in S1PR2KO mice as compared to WT littermates. Bone marrow transplantation experiments using $S1PR2^{LacZ/LacZ}$ mice demonstrated that S1PR2 expressed in tumor-infiltrating BMDCs, which include $CD11b^+$ cells implicated in tumor angiogenesis, play a crucial role in inhibiting tumor angiogenesis. Deletion of S1PR2 only in BMDCs significantly potentiated tumor angiogenesis and tumor growth in WT mice. These results indicate that S1PR2 expressed in endothelial cells and BMDCs in concert mediate inhibition of tumor angiogenesis and tumor growth (123).

Consistent with these observations, S1PR2 expressed in macrophages mediate S1P inhibition of their recruitment to inflammatory sites (123). However, S1PR2 in endothelial cells also mediates inflammatory effects of S1P, by upregulation of the proinflammatory enzyme cyclooxygenase 2 (COX2) and

downregulation of eNOS expression. In S1PR2 KO neonates that are subjected to ischemia-driven retinopathy, revascularization into avascular zones of the retina were augmented, whereas pathologic neovascularization in the vitreous chamber was rather suppressed, with reductions in endothelial gaps and inflammatory cell infiltration (117).

S1PR2 also mediates S1P stimulation of PLC and Ca^{2+} mobilization via Gq and $G_{12/13}$, (16,125), which in some vascular beds results in vascular contraction, contributing to normal hemodynamic regulation.

S1PR2 KO mice develop abnormalities in the inner ear, i.e., deafness and vestibular ataxia, in which hemodynamic derangement due to abnormally dilated capillaries in stria vascularis is involved (126). S1PR2KO mice also show occasional convulsion around weaning age with abnormal electroencephalogram (48), and spontaneous development of diffuse large B cell lymphoma in adulthood (127). Molecular mechanisms for these phenotypes are yet to be fully understood.

S1PR1/2 double null and S1PR1/2/3 triple null embryos show more severe defects in embryonic vascular formation and earlier death in utero, compared to S1PR1 single null embryos. S1PR2/3 double null embryos also show partial embryonic lethality and vascular abnormalities. These results indicate that coordinated functions of S1PR1, S1PR2 and S1PR3 are required for development of a fully functional vascular system during embryonic stage (126).

S1PR1, S1PR2 and S1PR3 are widely expressed in various types of cells. The overall outcome of S1P signaling in a given cell type largely depends upon relative expression levels of the S1P receptor subtypes. In addition, cross-talks between S1P receptor signalings and growth factor or cytokine receptor signalings have been reported. For example, S1PR1 and VEGF receptor activation in concert stimulate angiogenesis. Under certain conditions S1PR3 activation leads to activation of TGF-β signaling pathway and fibrosis (128,129). Update information regarding detailed cross-talk mechanisms is available in recently published excellent reviews (22,95,130,131).

STIMULATING S1P SIGNALING PATHWAY FOR TREATMENT OF POST-ISCHEMIC ANGIOGENESIS

Patients with occlusive peripheral arterial diseases suffer from limb ischemia, seeking for effective angiogenic therapy. For these patients, the clinical trials of either administration of proangiogenic growth factors such as VEGF, FGF-2 and HGF, or their expression vectors, or bone marrow mononuclear cell implantation which supplies myeloid lineage cells that produce proangiogenic factors and endothelial progenitor cells, have been conducted (132-136). These trials demonstrated that the angiogenic therapy conferred some beneficial effects such as reduced pain and decreased need for amputation, however, the effects are not satisfactory and obtained only in a portion of patients. Some drawback has been reported in the angiogenic therapy: administration of VEGF causes increases in vascular permeability and resultant edema as a serious side effect.

We demonstrated for the first time that S1P was effective in stimulating post-ischemic angiogenesis in a mouse model of hindlimb ischemia after unilateral femoral artery resection (51,52). Daily intramuscular administration of S1P dose-dependently stimulated blood flow recovery, resulting in up to twice as much blood flow at 10^{-7} M of S1P, which was accompanied by 1.7-fold increase in the capillary density in ischemic muscle compared with vehicle control. The optimal S1P effects were comparable with those obtained with FGF-2. Differently from VEGF, S1P injections did not increase vascular permeability, which was evaluated by Miles assay. We also analyzed post-ischemic angiogenesis in SphK1-overexpressing transgenic (TG) mice. SphK1 TG mice showed 40-fold higher sphingosine kinase activity and 1.8-fold higher S1P content in skeletal muscle compared with WT mice (51). In SphK1 TG mice the post-ischemic blood flow recovery and angiogenesis were both accelerated compared with WT mice, without an increase in the vascular permeability. These observations suggest that S1PR1/3-mediated angiogenic signals are dominant over anti-angiogenic signal mediated via S1PR2, and indicate potential therapeutic usefulness of S1P for tissue ischemia. We then explored the way to achieve sustained delivery of S1P, and found that poly(lactic-co-glycolic-acid) (PLGA)-based S1P-containing microparticles (PLGA-S1P) are biodegradable and continuously release S1P (52). We studied the effects of PLGA-S1P microparticles and found that intramuscular injections of PLGA-S1P dose-dependently stimulated blood flow and increased microvessel density in C57BL/6 mice with injections every 3 days conferring the optimal result. In Balb/c mice, which show retarded blood flow recovery compared with C57BL/6 mice and exhibit limb necrosis with apparent functional dysfunction, injections of PLGA-S1P stimulated blood flow with alleviation of limb necrosis and dysfunction. PLGA-S1P microparticles did not induce edema in ischemic limbs but rather suppressed VEGF-induced edema. Moreover, we observed that PLGA-S1P promoted the coverage of vessels by smooth muscle cells and pericytes, thus stabilizing vessels. PLGA-S1P microparticles stimulated Akt and ERK

with increased phosphorylation of eNOS in ischemic muscle. Experiments with a nitric oxide synthase (NOS) inhibitor showed that the stimulatory effect of PLGA-S1P on blood flow recovery was in part dependent on NO production. PLGA-S1P also stimulated the expression of pro-angiogenic growth factors in ischemic tissues, and enhanced the recruitment of $CD45^+$, $CD11b^+$ and $Gr\text{-}1^+$ myeloid cells, which are known to contribute to post-ischemic angiogenesis through production of pro-angiogenic growth factors (52).

These results indicate that PLGA-based, sustained local delivery of S1P is a promising therapeutic modality for stimulating post-ischemic angiogenesis. It is expected that S1P receptor subtype-selective agonists and antagonists could confer better effects in therapeutic angiogenesis.

TARGETING THE S1P SIGNALING PATHWAY FOR CONTROLLING TUMOR ANGIOGENESIS

Tumor angiogenesis has been the target of an anti-VEGF monoclonal antibody (mAb)(bevacizumab or Avastin^R) (132) and inhibitors of receptor tyrosine kinases or multi-kinase inhibitors. These modalities have limitations, because of side effects, including perforating peptic ulcer, hypertention and proteinuria associated with anti-VEGF therapy, and acquisition by tumor cells of resistance.

Accumulated evidence provides molecular basis for anti-angiogenic therapy through targeting the S1P signaling pathway. Anti-S1P mAb (Sphingomab) (53,54), has been developed and shown to act as a molecular sponge to efficiently bind S1P, preventing its binding to cell surface receptors. The anti-S1P mAb inhibits tumor progression in mouse xenograft and allograft models through inhibition of tumor angiogenesis. Thus, the anti-S1P mAb blocked endothelial cell migration and formation of tubular structures in vitro, and inhibited blood vessel formation induced by pro-angiogenic growth factors and arrested tumor-associated angiogenesis in vivo (53). The anti-S1P mAb also inhibited mitogenic and anti-apoptotic effects of S1P in tumor cells and their release of proangiogenic cytokine (53,54). Since the antibody is capable of triggering S1P release from erythrocytes (71), which constitute a large reservoir of plasma S1P, suitable antibody delivery system that bypass bloodstream to target primary and metastatic tumor tissues would improve cost performance status.

Since S1P stimulates and inhibits tumor angiogenesis through S1PR1/3 and S1PR2, respectively, either S1PR1/3-selective antagonists or S1PR2-selective agonists are expected to be effective in inhibiting tumor angiogenesis and tumor growth. At present the former strategy is under investigation in experimental models whereas the latter not yet available.

As described above, downregulation of S1PR1 by FTY720-phosphate in lymphocytes inhibits lymphocyte trafficking. FTY720-phosphate also induces downregulation of S1PR1 in endothelial cells and suppresses angiogenic activity of S1P (50). Pretreatment of HUVECs with nanomolar concentrations of FTY720 or FTY720-phosphate downregulated S1PR1 and inhibited endothelial cell migration in response to subsequent S1P. FTY720 potently inhibited angiogenesis in a variety of in vivo models, including corneal micropocket assay, subcutaneously implanted chamber assay, and tumor angiogenesis, tumor growth and metastasis (55-57). Moreover, FTY720 at micromolar concentrations directly induced apoptosis of a breast cancer cell line (55). It is also reported in ovarian cancer cells that FTY720 induced autophagy and necrosis (137). These observations may be related to recently reported novel actions of FTY720, which include inhibition of SphK1 (138) and autotaxin, the latter being phospholipase D in the plasma responsible for production of the lipid mediator lysophosphatidic acid (139). Because of the fact that FTY720 is a potent immunosuppressant, it is concerned that its clinical use for cancer patients is compromised by suppression in anti-tumor immunity and increased risk of opportunistic infection. In addition, endothelial dysfunction with reduced eNOS activity and increased permeability could occur. It is reported that the induction of vascular leak by FTY720 is associated with ubiquitin-dependent degradation of endothelial S1PR1 in mice (139).

FUTURE PERSPECTIVES

Recently novel pharmacological S1P receptor antagonists with anti-angiogenic potential have been reported. These include the S1PR1/3 antaonist, compound 5, {sodium 4-[(4-butoxyphenyl) thio]-2'-[{4-[(heptylthio)methyl]-2-hydroxyphenyl}(hydroxy) methyl]biphenyl-3-sulfonate} which inhibited endothelial cell migration, proliferation and tube formation and also inhibited hypotensive effect of S1P in rats (140), and an FTY720 derivative 1-(hydroxymethyl)-3-(3-octylphenyl)cyclobutane (VPC03090), which show inhibition of tumor growth in vivo (141). An S1P derivative [N-((2S,3R)-3-hydroxy-1-morpholino-4-(3-octylphenyl)butan-2-yl)tetradecanamide] (NHOBT) also shows inhibition of endothelial cell tube formation and endogenous neovascularization of the chick embryo chorioallantoic membrane (142). It is expected that S1PR1/3 antagonists with potent anti-angiogenic activity but relatively

weak inductive activities of vascular leak and eNOS inhibition would become promising therapeutics for controlling tumor angiogenesis. S1PR2 agonist, if developed, is also a promising candidate of anti-angiogenic medicine.

Investigation in-depth into molecular mechanisms for S1P-mediated regulation of angiogenesis, in combination with development of S1PR subtype-targeted, selective agonists and antagonists and their optimal drug delivery system, are expected to improve outcomes of both pro-angiogenic and anti-angiogenic therapies in the future. Their possible interference with biomolecules with angiogenic potentials, such as nerve growth factor (144-146) and leptin (147-149), might also be evaluated.

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REGULATION OF GLUCOSE METABOLISM BY CENTRAL INSULIN ACTION

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Insulin has been known to act on the hypothalamus, in particular the arcuate nucleus, in the central nervous system. Such central insulin action is not only involved in the regulation of energy metabolism via the regulation of food intake and heat production, but also plays an important role in glucose metabolism by regulating hepatic glucose production and glucose uptake by skeletal muscles. Studies on the intracerebroventricular administration of PI-3K inhibitors or sulfonylureas have demonstrated that hyperpolarization of agouti-related protein neurons induced by the activation of PI-3K signaling/K_{ATP} channels in the hypothalamic arcuate nucleus plays an important role in the suppression of hepatic glucose production mediated by central insulin action. Cutting of the vagus nerve overrides the suppression of hepatic glucose production by intracerebroventricular insulin administration, which suggests the involvement of autonomic nerves in central insulin action in the liver. The central insulin action-mediated suppression of hepatic glucose production is associated with decreased gene expression of enzymes involved in hepatic gluconeogenesis, and both increased interleukin-6 expression in hepatic non-parenchymal cells induced by central insulin action and associated activation of hepatic STAT3 play an important role in the suppression of gene expression of hepatic gluconeogenesis-related enzymes. In animal models of obesity and insulin resistance, the central insulin action-mediated hepatic glucose production control mechanism is impaired in both the hypothalamus and liver. Increased hepatic gluconeogenesis in obesity and type-2 diabetes has been attributed to impaired hepatic insulin signaling and increased expression of enzymes involved in hepatic gluconeogenesis due to hyperglycemia, but may also be partially attributed to the impairment of the central insulin action-mediated suppression of hepatic gluconeogenesis. ***Biomed Rev*** **2011; 22: 31-39.**

Key words: central nervous system, gluconeogenesis, glucose, insulin, STAT3 signaling

INTRODUCTION

Homeostasis of energy metabolism is maintained by the close interaction between the central nervous system (CNS) and peripheral tissues. In response to changes such as food intake, a stimulus is sent from peripheral tissues to the CNS to regulate energy intake, and this is followed by the transmission of a command from the CNS to the peripheral tissues to regulate energy metabolism, such as increasing heat production (1, 2). In such CNS-peripheral tissue interactions, a variety of humoral

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factors play an important role especially in the transmission of information from peripheral tissues to the CNS. Previous studies have demonstrated that factors such as leptin, adiponectin, both secreted by adipose tissue, and glucagon like-peptide 1 secreted by intestinal tissue, play an important role in energy metabolism through their action on the CNS (3-5). Insulin, a potent regulator of glucose metabolism acting on the liver, skeletal muscle, and adipose tissue, has also been shown to regulate energy metabolism through its action on the CNS. Recent studies have also demonstrated that insulin action on the CNS not only regulates energy metabolism via the regulation of food intake and heat production, but is also involved in the regulation of glucose metabolism (6,7). These observations indicate that insulin regulates glucose metabolism via both the direct action on peripheral organs and the indirect regulation through its action on the CNS. The purpose of this article is to discuss the role of central insulin action in the regulation of glucose metabolism, with a focus on its recently-discovered action on the regulation of hepatic glucose production.

CENTRAL INSULIN ACTION AND ENERGY METABOLISM

Studies using ^{125}I-labeled insulin have demonstrated that circulating insulin acts on limited areas of the brain such as the hypothalamus, in particular the arcuate nucleus, and the choroid plexus (8-10). In particular, insulin action in the hypothalamic arcuate nucleus, which has both insulin and leptin receptors (11), plays an important role in the regulation of energy metabolism (Fig. 1). Brain endothelial cells are connected to each other by tight junctions to form the blood-brain barrier (BBB), which inhibits the entry of molecules larger than 400 Da into the CNS (12). Several mechanisms of how insulin, a molecule of some 5800 Da, is transported into the brain have been reported and include diffusion from periventricular regions lacking the BBB, such as the median eminence near the arcuate nucleus (13), and the involvement of insulin receptor-mediated transport mechanisms in the BBB (12). Studies using hyperinsulinemic euglycemic clamps have shown that an increase in insulin level in cerebrospinal fluid is limited, even in the presence of a significantly high level of

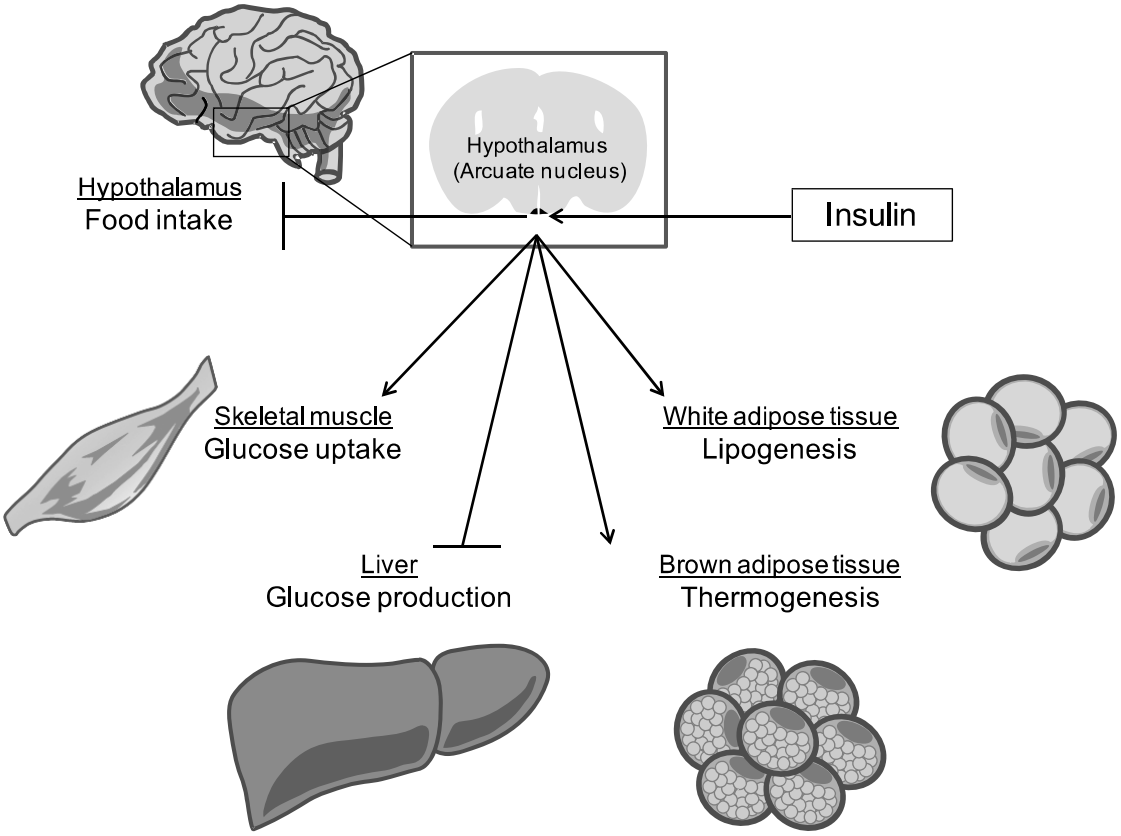

Figure 1. Central insulin action and energy metabolism. *Insulin acts on the hypothalamus, especially the arcuate nucleus. Hypothalamic insulin action suppresses food intake, induces glucose uptake in the muscle, decreases hepatic glucose production, and increases thermogenesis in brown adipose tissue and lipogenesis in white adipose tissue.*

circulating insulin, indicating the saturation of insulin transport into the CNS. These observations suggest the involvement of not only simple diffusion, but also carrier-mediated transport mechanisms in the saturable transport of insulin into the CNS (14-16).

Central insulin action plays an important role in food intake regulation. In a study using monkeys, intracerebroventricular administration of insulin led to decreases in food intake and body weight (17). Rat studies have also demonstrated similar effects following the intracerebroventricular administration of insulin or low-molecular weight compounds that can activate the insulin receptor (1,18). The neuron-specific insulin receptor knockout (NIRKO) mouse is an animal model of impaired central insulin action (19). The female NIRKO mouse exhibits a significant increase in food intake, and both males and females increase adipose tissue and body weight and develop insulin resistance (19). Such central insulin action-mediated regulation of food intake has been attributed to an action on neuropeptide tyrosine (NPY)/agouti-related protein (AgRP) neurons, which mediate orexigenic effects, and pro-opiomelanocortin (POMC)/cocaine-amphetamine-regulated transcript (CART) neurons, which mediate the anorexic effects in the hypothalamus. Intracerebroventricular administration of insulin leads to decreased expression of NPY and increased expression of POMC, resulting in suppressed food intake (1,6,20). When insulin binds to its receptor, it transmits information to cells by activating the phosphoinositide-3-kinase (PI3-K) and mitogen-activated protein kinase/extracellular-regulated kinase (MAPK) signaling pathways (21). The PI3-K signaling pathway has been shown to play an important role in the suppression of food intake mediated by insulin signaling in the CNS. Actually, studies have demonstrated that the anorexic effect of intracerebroventricular insulin administration can be inhibited by intracerebroventricular pretreatment with a PI3-K inhibitor (22). Leptin, which acts on the hypothalamus, suppresses food intake, and regulates energy metabolism, is reported to activate the hypothalamic PI3-K signaling pathway (23). Leptin regulates the expression of AgRP and POMC through activation of the PI3-K signaling pathway and it also regulates the expression of signal transducer and activator of transcription-3 (STAT3) signaling (24). STAT3-dependent regulation of AgRP and POMC expression is suppressed by competitive binding of each transcriptional promoter by Forkhead box O1 (FoxO1) (25), which is inhibited by the activation of PI3-K signaling (26, 27). Given that insulin does not activate hypothalamic STAT3, insulin regulates AgRP and POMC expression via inactivation of hypothalamic FoxO1 by PI-3K signaling (25,28). Central insulin action also plays an important role in increasing heat production following food intake via the regulation of sympathetic activity. The dietary control of sympathetic activity is critically mediated by insulin action in the ventromedial hypothalamus (VMH) (29). This finding is supported by the observation that the destruction of the VMH by the administration of gold thioglucose leads to suppression of heat production following food intake and impaired regulation of sympathetic activity (29, 30). Studies have suggested the involvement of hypothalamic PI-3K and MAPK in the regulation of sympathetic activity mediated by central insulin action. In particular, regulation of sympathetic stimulation of brown adipocytes, a major source of heat production in mice, has been suggested to be critically mediated by the activation of hypothalamic MAPK (31).

The involvement of central insulin action has also been suggested in the regulation of lipogenesis in white adipose tissue (32). A 7-day continuous intracerebroventricular insulin administration with an osmotic pump resulted in increased adipose tissue weight and increased expression of lipoprotein lipase in adipose tissue (32). However, the detailed role of central insulin action in white adipose tissue has not been elucidated. These observations indicate that insulin plays an important role in the homeostasis of energy metabolism by decreasing food intake and increasing heat production through its action on the hypothalamus.

CENTRAL INSULIN ACTION REGULATES GLUCOSE METABOLISM

There is an increasing amount of evidence to suggest that central insulin action plays an important role in the regulation of glucose metabolism as well as energy metabolism. Obici and colleagues developed rats deficient in hypothalamic insulin receptors by intracerebroventricular administration of antisense oligonucleotides and found that these rats exhibited insulin resistance (33). A study using hyperinsulinemic euglycemic clamps has shown that insulin resistance produced by knocking down hypothalamic insulin receptors is not associated with impaired glucose uptake in muscles and fat tissues but is attributed to impaired suppression of glucose production; in other words, increased glucose production in the liver (33). The NIRKO mouse, which also exhibits insulin resistance, has been shown to exhibit increased hepatic glucose production in a hyperinsulinemic euglycemic clamp study (34). Another study has demonstrated that the intracerebroventricu-

lar administration of insulin in rat or mouse models leads to suppressed glucose production in the liver (35,36). Central insulin action, acting mainly on the hypothalamic arcuate nucleus, has been shown to regulate hepatic glucose production *via* the vagus nerve (33). Cutting of the vagal hepatic branch has been demonstrated to result in reduced hepatic glucose production following intracerebroventricular administration of insulin (36). These studies suggest that the central insulin action regulates glucose metabolism through the suppression of hepatic glucose production via the vagal nerve. It has been reported that central insulin action activates hepatic glycogen synthesis (37). Considering that hepatic glycogen synthase is known to be activated by hepatic vagal nerve activation (38), central insulin action also regulates hepatic glycogen synthesis via the vagal nerve.

Hyperinsulinemic euglycemic clamp studies with insulin administration at high concentrations are used to examine in detail the effect of insulin on glucose uptake by peripheral organs, including skeletal muscles (39). A recent study using the euglycemic clamp with high-dose insulin administration has shown that the inhibition of central insulin action does not alter glucose uptake by adipose tissue, but reduces glucose uptake by skeletal muscles (40). Increased synthesis of glycogen in skeletal muscles has also been observed following intracerebroventricular administration of insulin in mice (41). Thus, the central insulin action may also play a certain role in glucose metabolism in skeletal muscles.

The PI-3K signaling pathway in the hypothalamic arcuate nucleus plays an important role in the regulation of glucose metabolism mediated by central insulin action. In rats, the intracerebroventricular administration of a PI-3K inhibitor to inhibit central insulin action leads to a decrease in insulin-dependent hypoglycemic response and an increase in glucose production in the liver (35,42). The use of an adenovirus vector to induce overexpression of a constitutively active mutant of Akt, which is activated by PI-3K, in the arcuate nucleus results in an enhanced hypoglycemic response to insulin with no change in food intake (42). These findings suggest that activation of the PI-3K signaling pathway in the CNS, especially in the hypothalamic arcuate nucleus, leads to suppressed glucose production in the liver and thus plays a certain role in the hypoglycemic response to insulin.

The K_{ATP} channels in the hypothalamus have been shown to play an important role in the suppression of hepatic glucose production mediated by the central insulin action. The intracerebroventricular administration of K_{ATP} channel blocker sulfonylurea can reverse the suppression of hepatic glucose production by central insulin action (35). Insulin has been shown to induce hyperpolarization of hypothalamic neurons by opening K_{ATP} channels via PI-3K (43). This is further supported by the fact that insulin opens K_{ATP} channels in both AgRP and POMC neurons and induces hyperpolarization of these neurons (44,45). It has also been demonstrated that MAPK inhibitors do not affect insulin-induced, hypothalamic K_{ATP} channel-dependent hyperpolarization (43). AgRP neuron-specific insulin receptor-deficient mice exhibited increased hepatic glucose production, whereas in POMC neuron-specific insulin receptor-deficient mice, insulin successfully suppressed glucose production in the liver (45). These results suggest that hyperpolarization and inactivation of AgRP neurons, resulting from the activation of PI-3K/K_{ATP} channels, play an important role in the suppression of hepatic glucose production mediated by the central insulin action.

The involvement of central insulin action has also been suggested in the regulation of glucose uptake by skeletal muscles, but its mechanism has been unclear (40). Leptin, which also acts on K_{ATP} channels in the hypothalamus (43), has been shown to increase glucose uptake by skeletal muscles by activating 5'-AMP-activated protein kinase (AMPK) via the sympathetic nervous system (46), and the hypothalamic administration of leptin has been shown to increase glucose uptake by adipose tissue (47). Intracerebroventricular administration of sulfonylurea in a euglycemic clamp study with a high-concentration insulin infusion resulted in increased hepatic glucose production and reduced glucose uptake by skeletal muscles due to blockage of central insulin action by K_{ATP} channel blockade (40). At the same time, no change in glucose uptake was observed in adipose tissue or the heart, and no significant change in AMPK activity was observed in skeletal muscles (40). These results suggest the regulation of glucose metabolism in skeletal muscles mediated by central insulin action has different mechanisms than the regulation of glucose metabolism in skeletal muscles mediated by central leptin action.

As it becomes increasingly clear how the central insulin action regulates hepatic glucose metabolism, studies have suggested that the contribution of this mechanism varies depending on the type of animal model. A dog model has been developed where insulin is infused from the carotid or vertebral artery to selectively increase the level of insulin circulating in the head compared with the peripheral insulin level (48). This model, where the insulin level in the head was selectively increased to a level 4 times higher than that achieved by the

standard hyperinsulinemic euglycemic clamp, demonstrated the same level of suppression of hepatic glucose production as the standard hyperinsulinemic euglycemic clamp. This finding indicates that the central insulin action may exert a milder suppressive effect on hepatic glucose production in dogs than in mice and rats.

MECHANISM OF CENTRAL INSULIN ACTION-MEDIATED REGULATION OF HEPATIC GLUCOSE PRODUCTION

Hepatic glucose production is composed of glycogenolysis and gluconeogenesis. Central insulin action-mediated suppression of hepatic glucose production has been attributed primarily to a decrease in gluconeogenesis (36). Hepatic gluconeogenesis is strongly regulated by the expression of the genes of related metabolic enzymes, and the central insulin action suppresses gene expression of enzymes involved in hepatic gluconeogenesis, such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. Gene expression of hepatic gluconeogenic enzymes is regulated by a number of transcription factors, including cyclic-AMP response element binding protein (CREB) and FoxO1 (49). Insulin decreases the gene expression of hepatic gluconeogenic enzymes by the inhibition of transcriptional activity of CREB and FoxO1 (50-53). Central insulin action also suppresses hepatic gluconeogenic gene expression as described above, and hepatic STAT3 plays an important role as a transcription factor involved in this suppression by central insulin action (34). Hepatic STAT3 suppresses gene expression of enzymes involved in hepatic gluconeogenesis when activated by interleukin-6 (IL-6) (54,55). This is further supported by liver-specific STAT3-deficient mice that develop insulin resistance as the gene expression of hepatic gluconeogenic enzymes increases, leading to glucose intolerance under the condition of obesity induced by high-fat diet feeding (54). Activation of hepatic STAT3 induced by the central insulin action has been demonstrated to be due to increased secretion of IL-6 from hepatic non-parenchymal cells (34). However, the role of the vagus nerve in hepatic STAT3 activation by central insulin action has not been fully elucidated. These observations indicate that insulin inhibits the expression of hepatic gluconeogenic genes by the direct action on the hepatocyte inhibiting CREB and FoxO1 activity and by central insulin action activating hepatic STAT3 (Fig. 2).

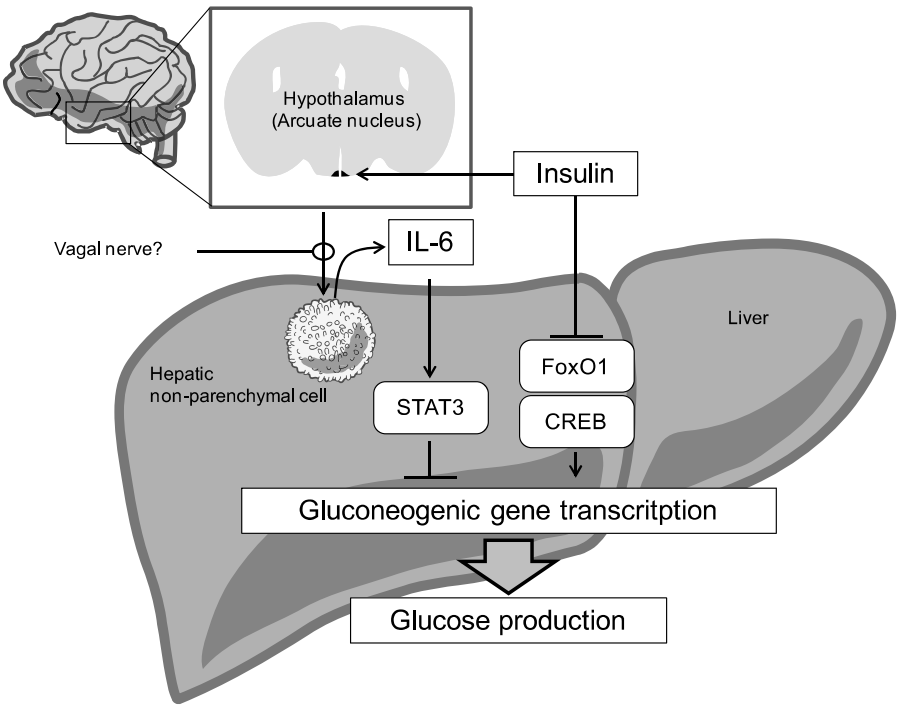

Figure 2. Direct and indirect regulation of hepatic gluconeogenic gene expression by insulin. *Insulin inhibits the transcriptional activity of FoxO1 and CREB and suppresses hepatic gluconeogenesis in a PI3-K signaling-dependent manner in hepatocytes. In addition to the direct effect on hepatocytes, central insulin action increases IL-6 expression in non-parenchymal hepatic cells, which activates hepatic STAT3 in a paracrine manner and decreases expression of hepatic gluconeogenic enzyme genes.*

Species difference has been suggested regarding the central insulin action-mediated suppression of hepatic glucose production, as evidenced by the lack of such suppressive mechanisms in dogs (37, 48). However, the central insulin action-mediated activation of hepatic STAT3 has been shown to be preserved in dogs as well as in mice (37, 56). This suggests that the contribution of hepatic STAT3 to the regulation of gene/protein expression of hepatic gluconeogenic enzymes varies among species.

CENTRAL INSULIN ACTION-MEDIATED REGULATION OF HEPATIC GLUCOSE PRODUCTION AND TYPE 2 DIABETES

Increased hepatic gluconeogenesis has been observed in obese individuals with type-2 diabetes (57). The question then arises as to the role of the central insulin action-mediated suppression of hepatic gluconeogenesis in the presence of obesity and insulin resistance. In a rat study, maintaining a high level of peripheral blood insulin did not lead to a substantial increase in central insulin action, probably due to saturation of the transport mechanisms (1). This suggests that an increased blood insulin level associated with insulin resistance does not lead to a substantial increase in insulin action in the CNS. With regard to insulin action in the hypothalamus, a study using obese Zucker rats with leptin receptor abnormalities has demonstrated that insulin fails to induce hyperpolarization of hypothalamic neurons in these rats (43). It has also been demonstrated that the intake of obesity-inducing high-fat diets, even short-term intake, induces activation of S6 kinase in the hypothalamus and thereby inhibits insulin signaling (58). A study using db/db leptin receptor-deficient obese mice has shown that the activation of hepatic STAT3, an effector of the central insulin action, is inhibited by endoplasmic reticulum stress associated with insulin resistance in obesity (59). This is further supported by the reversal of both the central insulin action-mediated suppression of glucose production in the liver and the promotion of glucose uptake by skeletal muscles in obesity model mice bred with a high-fat diet (40). Increased hepatic gluconeogenesis in type-2 diabetes has been attributed to impaired suppression of hepatic glucose production due to impaired hepatic insulin signaling and increased expression of enzymes involved in hepatic gluconeogenesis due to increased activity of CREB associated with hyperglycemia (60), but may also be partially attributed to the impairment of the central insulin action-mediated suppression of hepatic gluconeogenesis.

CONCLUSION

Studies have demonstrated that the central insulin action regulates not only food intake and energy metabolism, but also glucose metabolism via the regulation of hepatic glucose production. Hepatic glucose production plays a key role in glucose metabolism, as evidenced by the observation that increased hepatic glucose production leads to impaired glucose tolerance (61). Thus, hepatic glucose production is controlled by insulin's direct action on the liver and indirectly via its action on the CNS. The fact that various animal models of impaired hepatic insulin signaling exhibit profound glucose intolerance indicates that the direct action of insulin on the liver plays a central role in the homeostasis of glucose metabolism. At the same time, insulin resistance exhibited by CNS insulin receptor-deficient mice (19) and liver-specific STAT3-deficient mice (50) suggests a role of the central insulin action-mediated regulation of hepatic glucose production in glucose metabolism.

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MORPHOLOGY OF THE RAT CAROTID BODY

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The carotid body (CB) is the main peripheral arterial chemoreceptor that registers the levels of pO_2, pCO_2 and pH in the blood and responds to their changes by regulating breathing. It is strategically located in the bifurcation region of each common carotid artery. The organ consists of "glomera" composed of two cell types, glomus and sustentacular cells, interspersed by blood vessels and nerve bundles, and separated by connective tissue. The neuron-like glomus or type I cells contain numerous cytoplasmic organelles and dense-cored vesicles that store and release neurotransmitters. They form both conventional chemical and electrical synapses between each other and are contacted by peripheral nerve endings of petrosal ganglion afferent neurons. The glial-like sustentacular or type II cells sustain physiologic neurogenesis in the adult CB and are thus supposed to be progenitor cells. This new source of adult stem cells may be potentially useful for tissue repair after injury or for cell therapy against neurodegenerative diseases. The CB is a highly vascularized organ and its intraorgan hemodynamics possibly plays a role in the process of chemoreception. There is also evidence that chronic hypoxia induces marked morphological and neurochemical changes within the CB but the detailed molecular mechanisms by which these affect the hypoxic chemosensitivity still remain to be elucidated. Dysregulation of the CB function is implicated in various physiological and pathophysiological conditions, including ventilatory altitude acclimatization and sleep-disordered breathing. Knowledge of the morphological and functional aspects of the CB will contribute to our better understanding of respiratory homeostasis in health and disease. ***Biomed Rev*** **2011; 22: 41-55.**

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INTRODUCTION

The carotid body (CB; also known as the *glomus caroticum*, carotid corpuscle, carotid ganglion, and carotid gland) is a neural crest-derived paired ovoid mass of tissue, around 2 mm in diameter in humans and less than 1 mm in rats. Its small size explains why it was referred to as *ganglion minimum* in the first anatomical report on its existence in the human body, Hartwing Taube's Doctoral Thesis in 1743, although its discovery is attributed to his mentor, the great German physiologist Albrecht von Haller (1). However, for centuries its function had been completely unknown to scientists. The pioneering studies performed by the Spanish histologist Fernando de Castro (2, reviewed in 3,4) and the Flemish physiologist Corneille Heymans, 1938 Nobel Prize Winner in Physiology or Medicine (5,6) constituted the basis for its acceptance as a sensory receptor for chemical changes occurring in the blood. The glomus organ is situated bilaterally at the bifurcation of the common carotid artery (Fig. 1). This location is strategic for monitoring blood chemicals just before they reach the brain, a organ that is critically sensitive to oxygen and glucose deprivation.

The CB is the main peripheral chemoreceptor that registers the arterial blood levels of pO_2, pCO_2 and pH, and responds to their changes by regulating breathing (7,8). It plays an essential role in initiating an appropriate respiratory and cardiovascular response to hypoxia, hypercapnia and acidosis. It has also recently been shown that the CB is a glucose sensor activated by hypoglycemia (reviewed in 9).

The CB works in concert with the opposing afferent nerve endings of the petrosal ganglion (PG) cells and they together form a functional unit, the CB chemosensory system. In response to hypoxia CB sensor cells release a variety of neurotransmitters which activate chemoafferent nerve endings of PG neurons. The latter provide the afferent link between the CB chemoreceptors and respiratory nuclei in the brainstem, thus ensuring the transmission of the chemosensory information from the chemotransductive cells to the central nervous system. The efferent limb of the chemoreceptor reflex arc is formed by solitary axons projecting to the respiratory centers, distributed in a ponto-medullary respiratory network. They control the coordinated contractions of the abdominal, thoracic and laryngeal respiratory muscles and upon hypoxia stimulate breathing (10).

Much of the available evidence suggests that the CB dysfunction and altered oxygen homeostasis are involved in the pathophysiology of several human diseases, some of which are of a high incidence. Thus, a better knowledge of the basic morphology and physiology of the CB in a rat model will contribute to our understanding of respiratory homeostasis in health and disease.

GENERAL STRUCTURE OF THE CAROTID BODY

Normal histology

For decades rats have widely been used to study the morphology and physiology of the CB. The general organization of the CB parenchyma in islets of cells was originally described by Kohn (11). The organ is structurally complex and composed of four principal components: cell clusters, blood vessels, connective tissue and nerve fibers (12,13) (Fig. 2A). The small clusters, also known as glomeruli or glomoids, are the basic morphofunctional units of the CB. As originally described (14), they are formed by two juxtaposed cell types: type I or glomus cells (2-12 in each glomerulus in rats, an average of four cells), incompletely invested by 1-3 type II or sustentacular cells (Fig. 2B; see also 5A). Both parenchymal cell types can be clearly distinguished from each other, even at the light microscope level. The principal cell type, the neuron-like glomus cell, is considered the chemosensory cell of the organ and contains secretory granules packed with putative neurotransmitters. Glomus cells, like sympathetic neurons and chromaffin cells of the adrenal medulla, originate from the neural crest (15); therefore the CB was initially regarded a paraganglion (16). By stereological methods, Laidler and Kay (17) determined that the CB of the adult rat contains 11,500 ± 2500 glomus cells (mean ± SE). Chemoreceptor cells are round to oval in shape and their size usually varies between 8 and 16 μm. They have a clear, round nucleus and a copious and distinctly granular cytoplasm. Type II cells (~15-20% of all cells) are typically located at the periphery of the cell cluster. They are glial-like cells possessing long-shaped bodies with elongated hyperchromic nuclei, a thin cytoplasmic layer and extended processes that envelop groups of glomus cells. Classically, type II cells were considered to be supporting cells within the organ which play a role in the metabolic support but recently they have been assumed to be the CB stem cells (18–20). The CB also contains some autonomic microganglion cells, embedded within or located at the periphery of the CB (2,21,22). In rats, the number of these neurons varies from 10 to 20 (21). They mainly provide innervation for the blood vessels but may also have an efferent regulatory action on glomus cells (21,23).

The cell clusters are separated from each other by septa of connective tissue, which converge on the surface to form a capsule for the whole organ (Fig. 3A). Generally, there is rela-

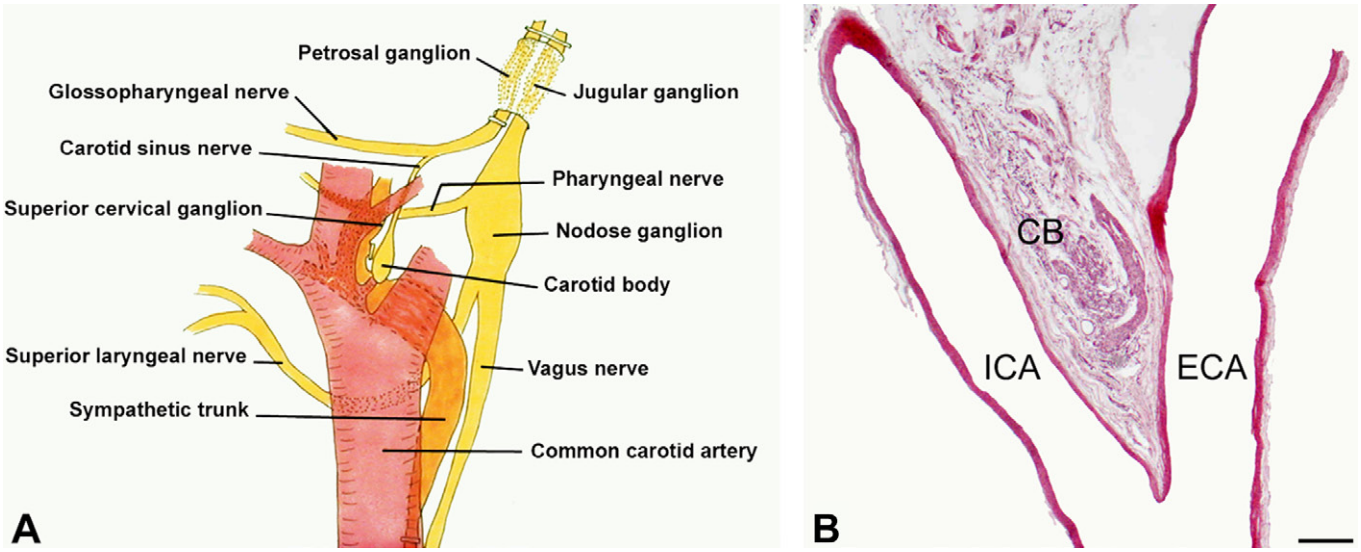

Figure 1. *Location of the carotid body (CB) of a rat. (**A**) Schematic diagram of the bifurcation area of the left common carotid artery. The glomus organ is positioned between the external carotid artery (ECA) and the internal carotid artery (ICA). (**B**) A low-magnification H&E stained section showing the strategic location of the CB between the ECA and ICA. Scale bar = 500 µm (**B**).*

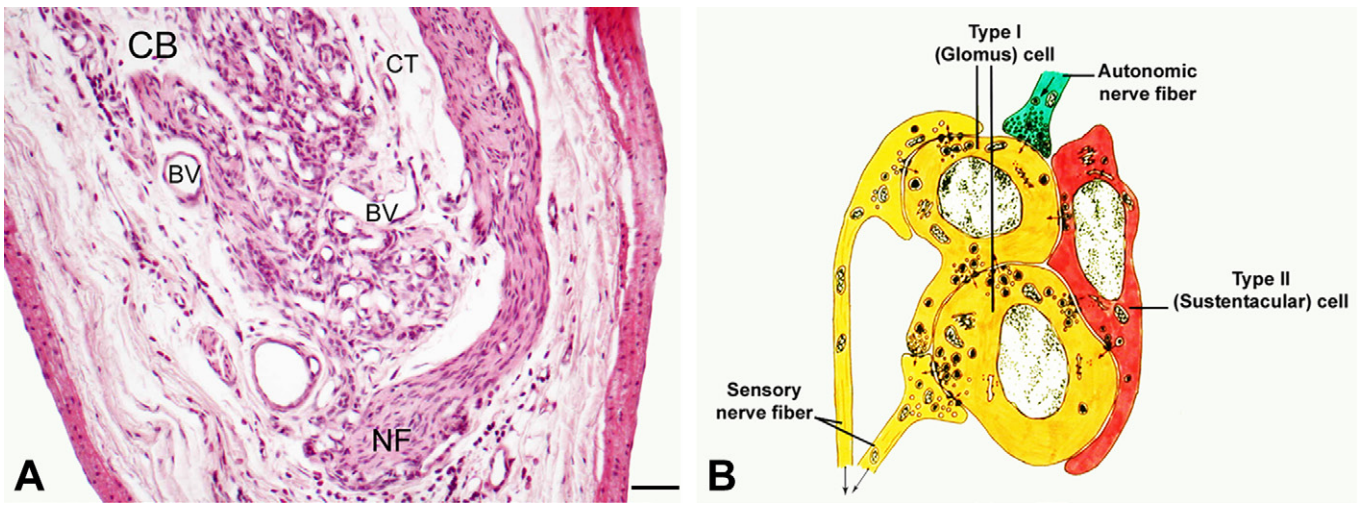

Figure 2. *The general structure of the carotid body (CB). (**A**) H&E staining illustrates the structural organization of the rat CB. The glomic tissue is arranged in cell clusters, glumeruli. Note that a large number of blood vessels (BV) are seen in the CB parenchyma, and some nerve fibers (NF) can also be observed in the surrounding connective tissue (CT). (**B**) Schematic representation of a CB glomerulus showing the type I (glomus) and type II (sustentacular) cells. Note that neuron-like type I cells are partially enveloped by glial-like type II cells. The glomus cells are dually innervated by both sensory and autonomic nerve fibers. Scale bar = 100 µm (**A**).*

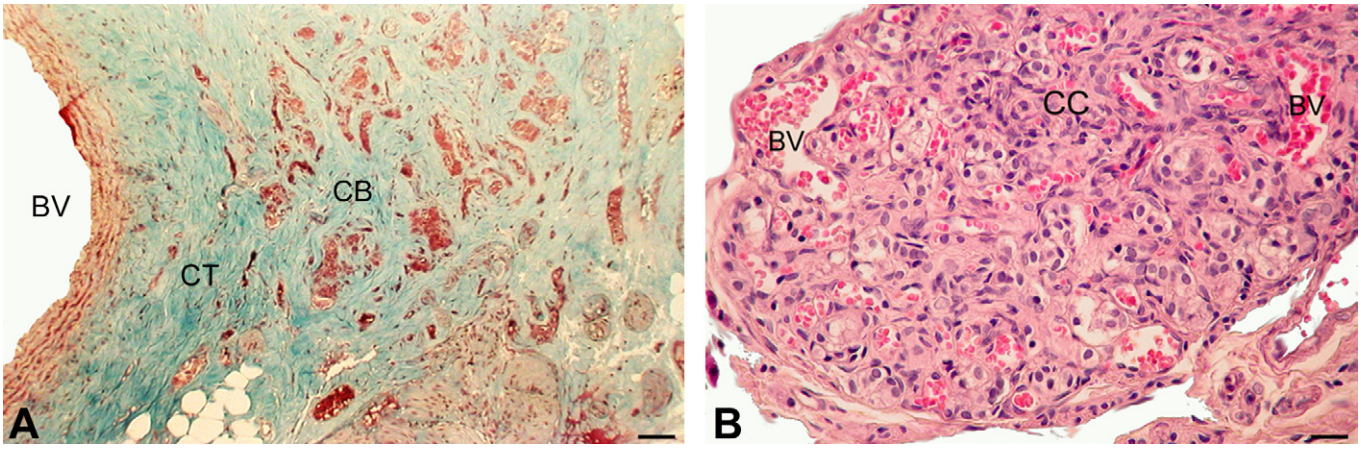

Figure 3. *(**A**) Low-power photomicrograph of a representative Azan-stained rat CB section showing the septa of collagen fibers with vascular connective tissue (CT) which surround tightly packed glomeruli and build up the glomic capsule. (**B**) A dense network of blood vessels (BV) is also dispersed in the CT. Note the intimate contact of capillaries with the cell clusters (CC). Scale bars = 200 µm (**A**) and 50 µm (**B**).*

tively little connective tissue in the CB of most young animals and its amount increases with age constituting 50-60% of the total volume of the adult CB. The stroma around the lobules contains relatively large blood vessels and nerve bundles.

In fact, the most striking anatomical characteristics of the CB are its rich vascularization and dense innervation. The CB is one of the most irrigated organs in the body and receives blood supply through a short branch, called the glomic artery, arising from the external carotid artery (13). A profuse capillary network travels in the walls of the connective tissue surrounding the CB glomeruli (Fig. 3B) and giving a pink-colored appearance to the CB. The capillaries emerge from the CB, anastomose with venules of variable diameters that form a dense venous plexus on the surface of the organ. The venous drainage of the CB is via one or two small veins, emerging from this superficial plexus, which empty into the internal jugular vein or one of its branches (7).

Since the pioneering work of Fernando de Castro (2) it has been known that the glomus cells are dually innervated by both sensory nerve fibers and autonomic fibers *via* the ganglioglomerular nerves (Fig. 4). The sensory nerve fibers which convey chemosensory impulses from the carotid body into the brainstem are mainly supplied by the carotid sinus nerve (also known as Hering's nerve) and their cell bodies are located in the PG of the glossopharyngeal nerve (24). In rats, there are about 450–750 axons in the carotid sinus nerve and the majority of them are unmyelinated fibers (22). Entering the cell cluster, each of these branches so as to innervate more than 20 glomus cells (Fig. 4A). In addition, the carotid body in the rat receives sensory innervation from the superior (jugular) ganglion and inferior (nodose) ganglion of the vagus nerve (24,25). As can be obtained from Fig. 4, the sympathetic nerve supply is provided by postganglionic neurons from the closely located superior cervical ganglion (SCG) (26,27). Most sympathetic nerve fibers are thought to supply blood vessels and a few of them may also innervate glomus cells (13). Parasympathetic neurons scattered around the carotid body have been described as the (internal) carotid ganglion (28).

ELECTRON MICROSCOPY OF THE NORMAL RAT CAROTID BODY

Ultrastructure of the parenchymal cells

Ultrastructural studies have shown that glomus cells have the morphological characteristics of actively synthesizing cells (12). Indeed, as seen with the transmission electron microscope, their cell bodies contain a large round, euchromatic nucleus and an abundant pale cytoplasm with numerous organelles (Fig. 5B). Amongst them, most notable are the multiple free ribosomes and polysomes, the flattened cisternae of rough endoplasmic reticulum, the well-developed Golgi apparatus and a large number of compact mitochondria. Another remarkable ultrastructural feature of these cells is the presence of osmiophilic dense-cored vesicles in their cytoplasm, where they are not randomly distributed. Indeed, they are rare in the Golgi region and occur in large groups tending to accumulate in the periphery of the cells (Fig. 5C). The size of the dense-

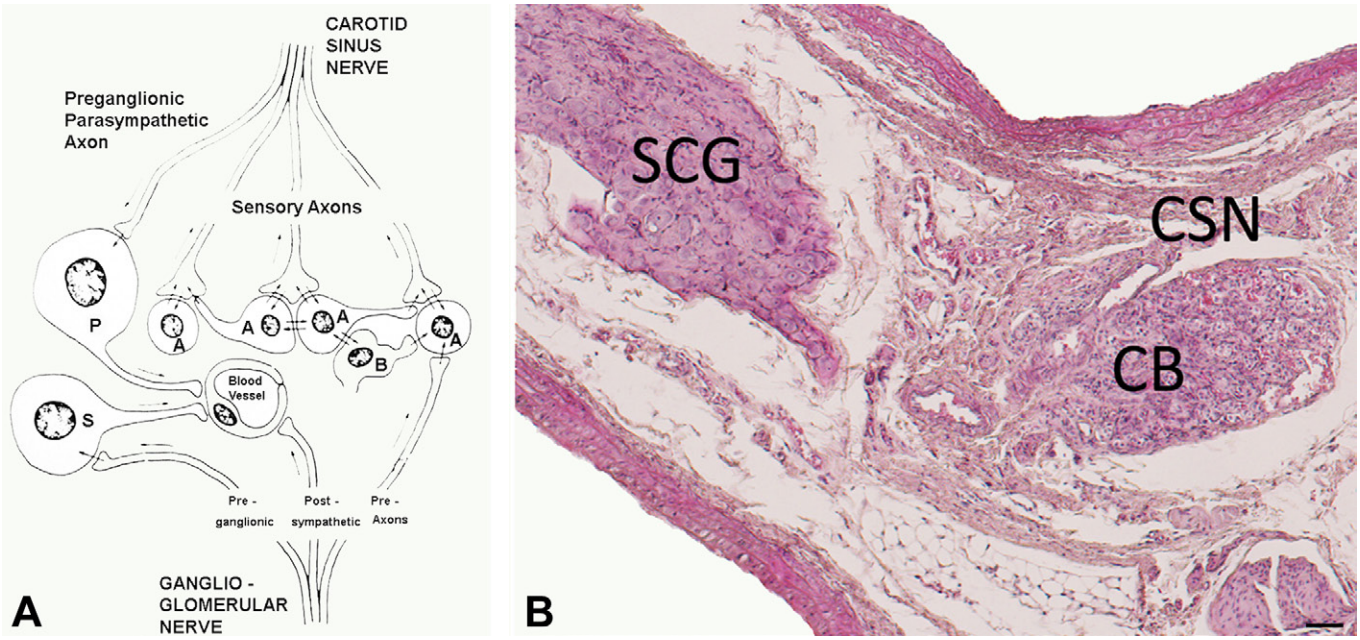

Figure 4. *(**A**) Schematic drawing of the rat carotid body (CB) innervation. The sensory nerve supply of the chemosensory glomus cells is provided by PG neurons through the carotid sinus nerve (CSN). The sympathetic innervation is performed by postganglionic neurons from the closely located superior cervical ganglion (SCG) via the ganglioglomerular nerve. They mostly supply the blood vessels although some of them may also innervate the CB parenchyma. (**B**) Histological picture of the rat CB indicating its innervation from the adjacent SCG. CSN, carotid sinus nerve. Scale bar = 150 µm (**B**).*

cored vesicles in the rat ranges from 50-200 nm (mean diameter about 100 nm) (21,29). Although smaller in size, they closely resemble the granules of paraneurons belonging to the diffuse neuroendocrine system cell family. In particular, the cytological features of the glomus cells are quite similar to the adrenal chromaffin cells and ganglionic small intensely fluorescent (SIF) cells. Similar to them, the glomus cells contain various biogenic amines and neuropeptides in the dense-cored granules (7,8,25,30). This similarity, together with findings from developmental studies as already noted, has lead historically to the concept of classical "Paraganglion" (18) and recently to that of "Paraneuron" (31). Therefore, all this is in favor of the proposal that the carotid body can be regarded as a secretory organ.

On the basis of the size and staining properties of their dense-cored vesicles, McDonald and Mitchell (21), and independently Hellström (32) categorized two subtypes of glomus cells: type A or large vesicle cells (mean vesicle diameter 116 nm) and type B or small vesicle cells (mean vesicle diameter 90 nm). The authors estimated that in rats type A cells comprise 51 ± 10% (mean ± S.D.) of the glomus cells. Besides, the population density of these granules varies between glomus cells, and this may be indicative of differing states of secretory activity within the CB. Smaller in number and size (about 40 nm in diameter) clear vesicles also occur in rat glomus cells (29). They tend to accumulate in the cell processes and occasionally in the regions facing the nerve endings.

The sustentacular cells contain a paucity of organelles in their cell bodies. The most distinguishing feature of these cells is the absence of secretory granules in their cytoplasm suggesting that they do not synthesize and store neurotransmitters. Therefore, despite their location in close proximity to the blood in the capillaries, they do not play a role in chemosensory function. Nonetheless, Golgi apparatus, ribosomes, scattered endoplasmic reticulum and occasional mitochondria are present, though developed to a lesser extent than in glomus cells (Fig. 5D). They also contain abundant, intermediate vimentin filaments and possess glial-like traits necessary to support and influence the behavior of glomus cells (33). Indeed, type II cells express glial markers such as the S-100 protein and glial fibrillary acidic protein (18,34). Another distinguishing feature of these cells is their possession of long cytoplasmic processes that extend away, partially envelop chemoreceptor cells and collectively form a protective network around them.

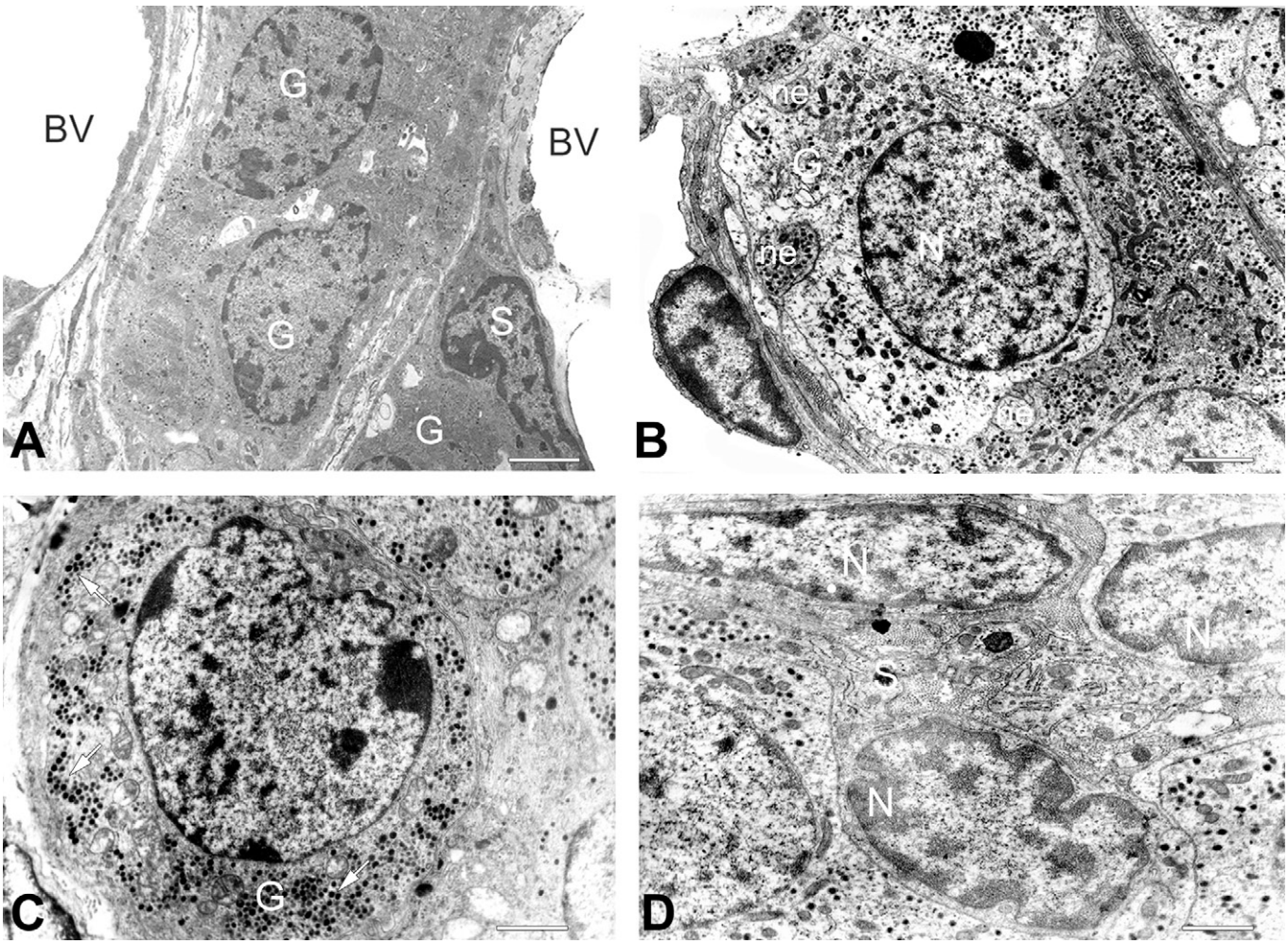

Figure 5. *Ultrastructure of the CB parenchyma. (**A**) An ultrathin section of a rat CB glomerulus indicating at a lower magnification a typical tightly packed cell cluster of glomus cells (G) adjacent to blood vessels (BV). Two glomus cells are partially invested by a sustentacular cell (S). (**B**) Electron micrograph of a glomus cell (G) with a round, euchromatic nucleus (N) and an abundant cytoplasm with numerous dense-cored vesicles. Note that some nerve endings (ne) are visible in the vicinity of the glomus cell. (**C**) shows the accumulation of dense-cored vesicles (arrows) in the periphery of a glomus cell (G). (**D**) Electron micrograph of a section through the peripheral region of a glomerulus. The sustentacular cells (S) possess elongated hyperchromatic nuclei (N) with a vesicle-free cytoplasm and long processes. Scale bars = 0.5 µm (**A**) and 1 µm (**B-D**).*

Like Schwann cells, they may completely ensheath single or small groups of unmyelinated nerve fibres in the CB, thus guiding the axons to the glomus cells in the space between the Schwann cells and cell clusters.

Synaptic organization

The synaptic connections of the organ have been characterized in the greatest details in the rat CB. The advent of the conventional electron microscopy has indicated that many adjacent glomus cells make "synaptic"-like somato-somatic contacts (Fig. 6A), thus explaining the characteristic morphological picture of cell clustering in the CB (7,12,13). The intercellular space between the contacting cells is about 20 nm. Notably, both large dense-cored vesicles and small clear vesicles accumulate at this synaptic junction. Recent studies by freeze-fracture electron microscopy (35) have additionally revealed the existence of gap junctions between some glomus cells in the CB which have been designated as electrical syn-

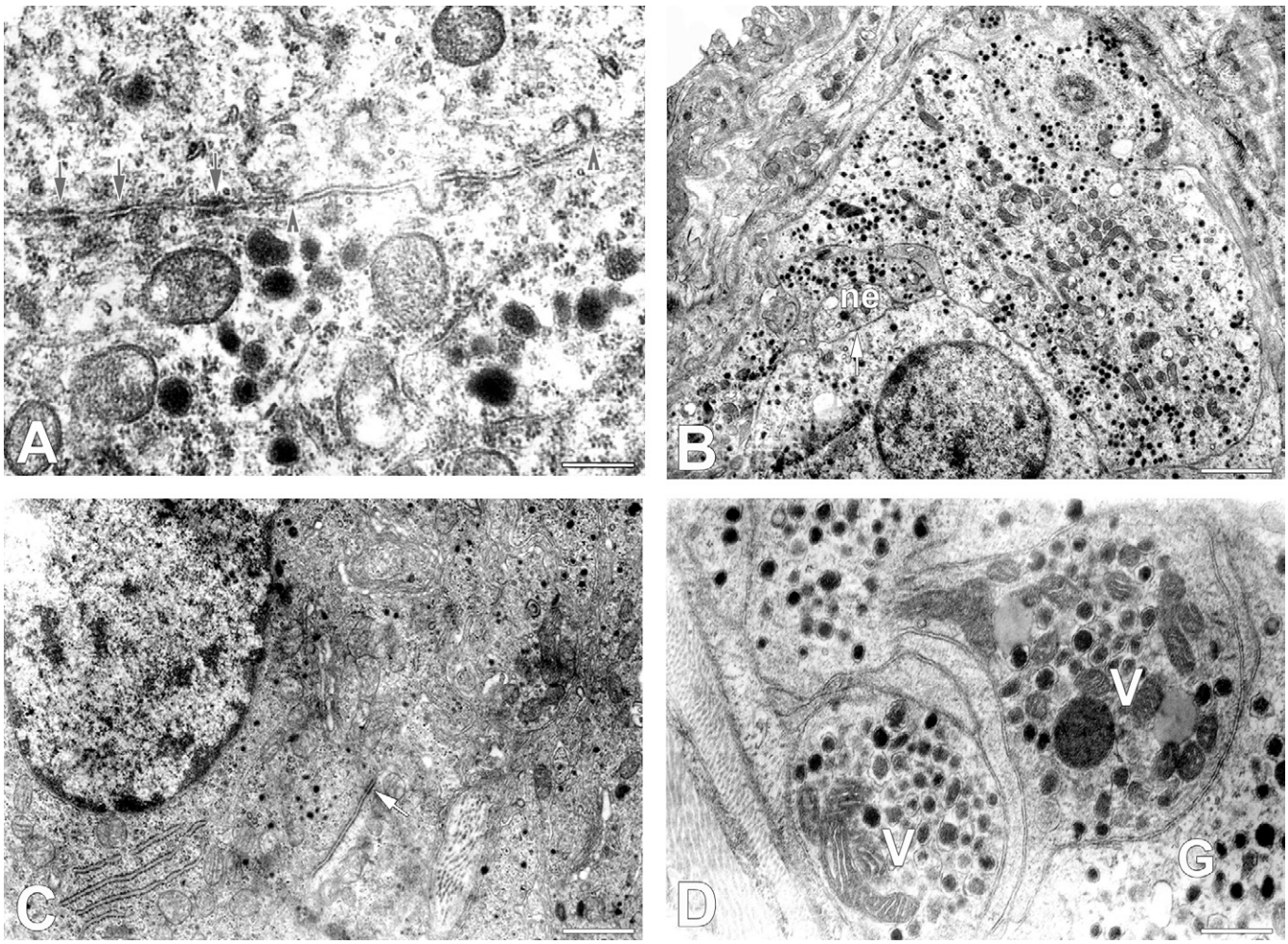

Figure 6. *Synaptic organization of the rat carotid body (CB). (**A**) Detail of the cell junction between two glomus cells illustrating zones of close membrane appositions representing gap junctions (arrowheads) and the synaptic-like synaptic contacts (arrows). Note the numerous small clear vesicles and a few large dense-cored vesicles at the periphery of the contacting cells. (**B**) Electron micrograph showing a sensory nerve ending (ne) making a synaptic contact (arrow) with a glomus cell. (**C**) A spherical clear vesicle-containing axon terminal forming a symmetrical synaptic contact (arrow) with a glomus cell body. (**D**) An autonomic nerve fiber with characteristic varicosities (V) containing numerous dense-cored vesicles and mitochondria in the vicinity of a glomus cell (G). Scale bars = 0.15 µm (**A**) and 1 µm (**B-D**).*

apses (Fig. 6A). Interestingly, gap junctions may also occur between glomus and sustentacular cells and such junctional specializations are observed between glomus cells and afferent nerve endings as well (36). The electrotonic coupling allows intercellular exchange of ions and small molecules and the passage of currents (37). Moreover, cell uncoupling increases the transmitter release, whereas tighter coupling reduces it (38). Besides, it has been found that the glomus cells are contacted by peripheral nerve endings of PG afferent neurons (Fig. 6B) (see 12,13). Sensory nerve endings on glomus cells may also appear as boutons "en passant", making multiple synaptic contacts (39). The presynaptic terminal contains a large number of mitochondria, numerous small (about 60 nm in diameter in the rat), clear vesicles (Fig. 6C) and a few large (usually 70-150 nm in diameter) dense-cored vesicles. Some larger boutons apposed to chemoreceptor cells and typically seen as axonal varicosities are filled with abundant densely packed small clear vesicles, large dense-cored vesicles and

mitochondria (Fig. 6D). They are considered preganglionic sympathetic efferent nerve endings, thus favoring the concept of the dual sensory and motor innervation of the CB (21,40,41). Such synaptic connections have also been shown on some ganglionic SIF cells (42). Sometimes (i.e. in about 10% of the cases), the "afferent" and "efferent" synapses are adjacent to each other forming reciprocal synapses in the CB (7,12). The synaptic contacts on glomus cells are with both symmetric and asymmetric membrane morphology and have functionally been described as bidirectional (12). It is likely that in response to natural stimuli peripheral processes of PG neurons release chemical substances at synapses triggering the exocytosis of one (or more) neurotransmitter(s) from the glomus cells (43). The released transmitter, acting on specific postsynaptic receptors, increases the rate of chemosensory discharge in nerve fibers of PG neurons projecting to the CB (7,44).

In addition to nerve-glomus cell contacts, nerve endings are occasionally observed to make synaptic contact with another nerve or nerve ending (Fig. 7A, B). The presynaptic profile possesses a few mitochondria, and always contains groups of

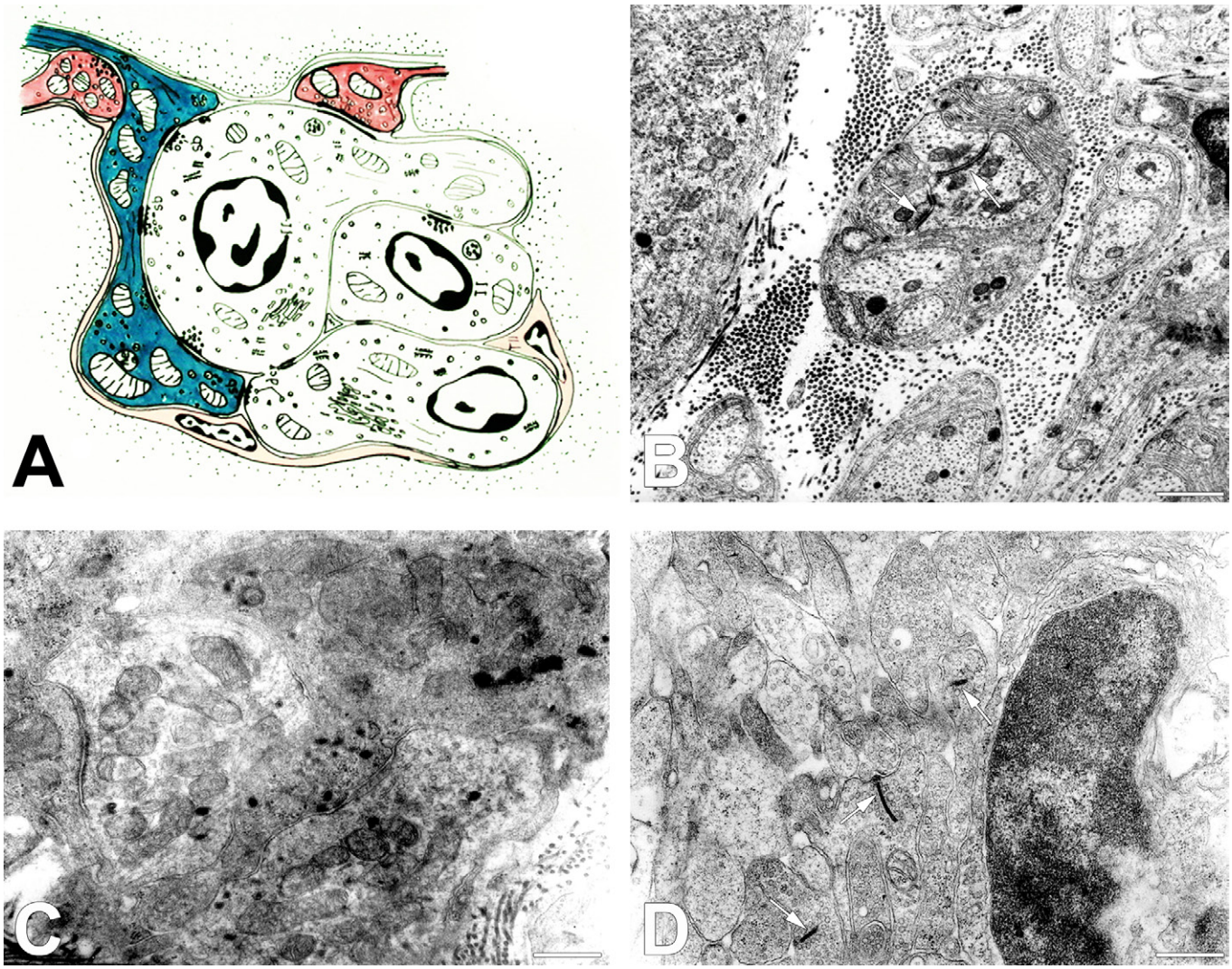

Figure 7. *Nerve-nerve ending relationships in the rat CB. (**A**) Schematic drawing of axo-axonic synaptic contacts with a symmetrical appearance. (**B**) Electron micrograph showing typical axo-axonic synapses in the rat CB. (**C**) The presynaptic profile contains a few mitochondria and groups of both small clear and occasional large dense-cored vesicles. (**D**) Ribbon synapses with the characteristic arrangement of electron dense structures (arrows) called synaptic body or ribbon in the presynaptic bouton apposing the postsynaptic terminal. Scale bars = 0.5 μm (**B-D**).*

clear vesicles as well as occasional dense-cored vesicles (Fig. 7C). The same structures, i.e. mitochondria, clear vesicles and a few dense-cored vesicles are present also in the postsynaptic nerve ending. Usually these axo-axonic synaptic contacts have a symmetrical appearance. Interestingly, a presynaptic dense body is sometimes seen denoting this synaptic contact as a ribbon synapse (Fig. 7D). This type of synapse typically links some particular sensory receptor cells. The ribbon has been proposed to shuttle synaptic vesicles to exocytotic sites, promote their release at the synapse and thus enable a rapid information processing.

Microvasculature ultrastructure

Most blood vessels in the CB are capillaries, which are abundant and closely packed. According to their morphological features and size, two types of capillaries have been identified in the CB (45–47). Type I capillaries are the prevailing type (60% of the total). They are convoluted, larger in size (8-20 μm in diameter) and have a thin wall, formed by a fenestrated endothelium with short microvilli, a basal lamina (50-100 nm in thickness) beneath and an incomplete covering of pericytes (Fig. 8A). Endothelial cytoplasm contains scant mitochondria, numerous micropinocytotic vesicles and occasional

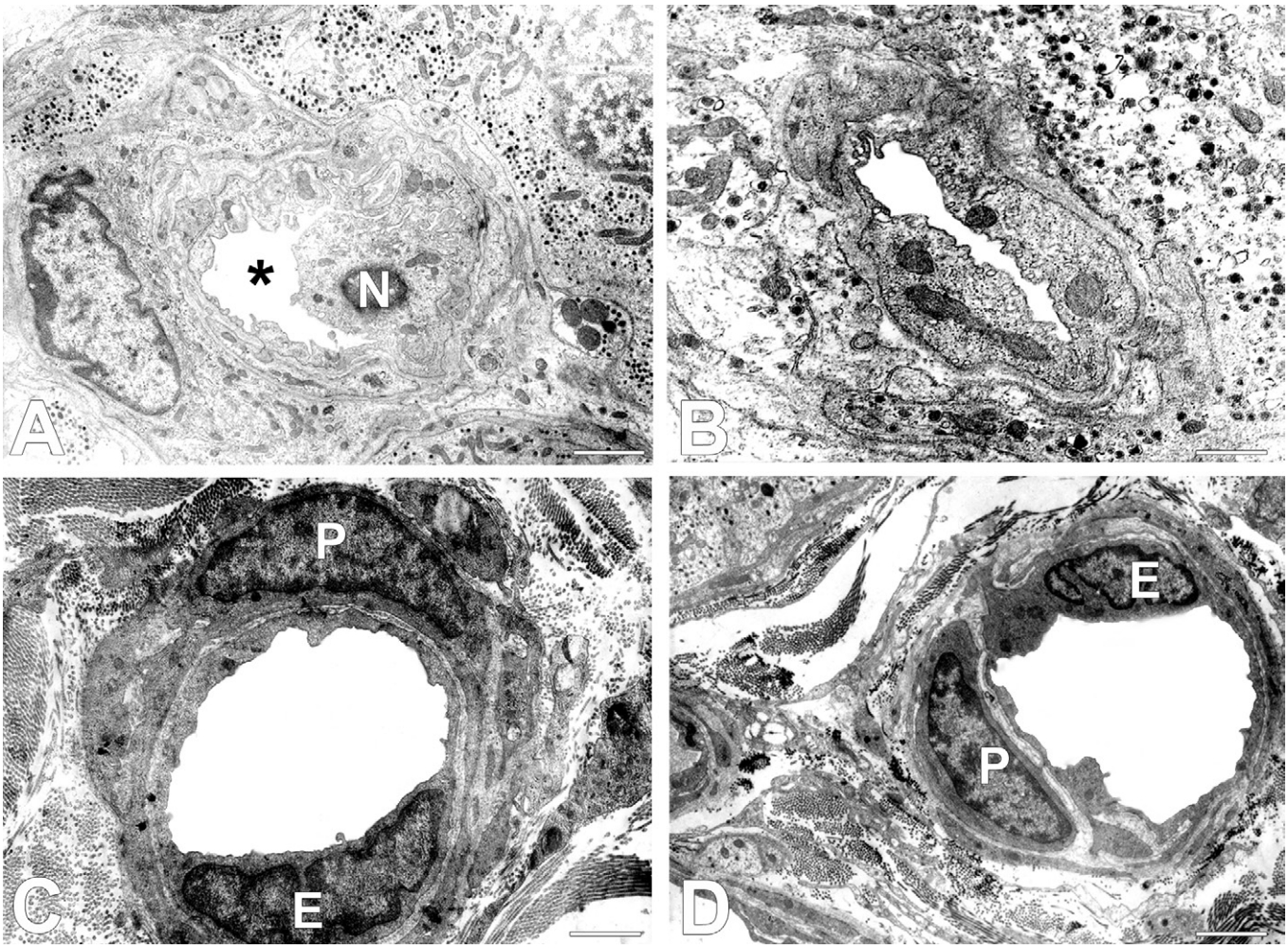

Figure 8. *Ultrastructure of capillaries in the rat CB. **(A)** A type I capillary (asterisk) closely associated with the cell clusters. Its thin wall is formed by a fenestrated endothelium containing the nucleus (N) and an attenuated part with short microvilli, lies on a basal lamina and is partially covered by a pericyte. **(B)** The endothelial cytoplasm of a fenestrated capillary with scant mitochondria and numerous micropinocytotic vesicles. **(C, D)** Cross section profiles of continuous type II capillaries showing the thick portion of the endothelial cytoplasm (E) and pericytes (P) investing them. Note the 'perisinusoidal' space filled with collagen fibers. Scale bars = 1 μm.*

Weibel-Palade bodies (Fig. 8B). These capillaries are closely associated with the cell clusters. Despite their morphological features, the fenestrated capillaries of the CB are not true sinusoids. They rather resemble the fenestrated capillaries of the adrenal medulla and other endocrine glands, mediating the characteristic hyperpermeability state in the CB. Type II capillaries are mostly straight, typically thinner (6-12 μm in diameter) and continuous. They are covered by pericytes and do not make contacts with cell clusters (Fig. 8C, D). The glomus cells are separated from the capillary endothelium by a 'perisinusoidal' space containing collagen and are lined on both sides by a basement membrane applied to the contiguous walls of glomus and endothelial cells (39). Collagen fibres are present not only in the "perisinusoidal" space but are also found to a variable extent between the glomus cells. Bundles of myelinated and unmyelinated axons are frequently seen in "perisinusoidal" and intercellular spaces.

MORPHOLOGICAL CHANGES IN THE HYPOXIC CAROTID BODY

Chronic hypoxia induces gene expression, leading to profound morphological changes in the CB. Hollinshead (48) was the first to describe cytological modifications of CB cells after a severe and sustained hypoxia, and similar investigations were also done with the electron microscope as early as 1958 by Hoffman and Birrel (49). Generally, the long term hypoxic exposure enlarges several-fold the size of the rat CB (50,51) causing glomus cell hypertrophy and hyperplasia (52–54). In addition, there is a decrease in the covering of glomus cells by sustentacular cells (55) and this alteration increases the potential area available for gap junction connections between the glomus cells, which have been shown to enhance glomus cell sensitivity (56). Although no structural changes in the sustentacular cells have been observed under such conditions, there is evidence that hypoxic adaptation of the rat CB involves proliferation of these cells as well (53,54). Also, systemic hypoxia changes the CB vascular structure, inducing marked (10-fold in rats) vasodilation (51) and the growth of new blood vessels (54,57). In fact, the number of blood vessels remains unchanged but a vascular remodeling and proliferation of endothelial cells of existing blood vessels occur during chronic hypoxia (58).

In humans such a physiological adaptive response to prolonged hypoxia occurs during acclimatization to high altitudes (54,59) or pathologically in patients suffering from systemic hypertension and/or cardiopulmonary diseases with concomitant hypoxemia (reviewed in 60).

NEUROCHEMICAL PLASTICITY OF THE CAROTID BODY IN CHRONIC HYPOXIA

In addition to the remarkable structural plasticity, chronic hypoxia induces changes in the neurotransmitter profile of chemosensory cells in the CB. It is well established that hypoxia causes glomus cells to depolarize and release (both excitatory and inhibitory) transmitters, which bind to autoreceptors expressed by type I cells or postsynaptic receptors on apposed chemoafferent nerve terminals (7). Multiple putative neurotransmitters are thought to mediate signals generated by hypoxia. The predominant excitatory transmitter synthesized and released by type I cells in response to hypoxia is still a matter of debate (7). Current evidence suggests that acetylcholine (ACh) and adenosine triphosphate (ATP) are two major excitatory neurotransmitter candidates in the rat hypoxic CB (for recent reviews, see 8,43). Based on observations accumulated during the first half of the 20th century, the so-called cholinergic and purinergic hypotheses for hypoxic chemosensitivity were introduced (see 61). Moreover, the co-release of ACh and ATP has been proposed to be the main mechanism mediating hypoxic chemotransmission in the rat CB (62,63), which constitutes the so-called cholinergic-purinergic hypothesis (61). Conversely, it has been reported that despite biochemical evidence for its excitatory action in the CB (7,43), pharmacological and physiological studies indicate that dopamine (DA), which is secreted by the glomus cells, has a primarily inhibitory role in rat CB chemoexcitation (8,54), the dopaminergic hypothesis (61). Other neurotransmitters present in the CB and postulated to be important in chemoreception, namely norepinephrine and serotonin, have not been shown to play an important role in hypoxic acclimatization of the CB to date (reviewed in 64). Our recent studies have proved the modulatory role of histamine as a transmitter in hypoxic chemosensitivity in rats (65,66). On the other hand, chronic hypoxia increases the inhibitory effect of nitric oxide (NO) and reactive oxygen species on glomus cells of rat CB (67). Based on these studies, it has been proposed that hypoxia augments the CB activity by inhibiting the NO-synthesizing enzyme, nitric oxide synthase (68). Altered peptidergic innervation of the rat CB also occurs during the course of hypoxic adaptation (69).

CAROTID BODY AND MECHANISMS OF DISEASE

Peripheral chemoreceptors have been implicated in various dis-

eases, including sleep-disordered breathing, congestive heart failure, and certain forms of hypertension (reviewed in 70). In the healthy fetus, the CB does not significantly contribute to fetal breathing, or in its activity necessary for establishing rhythmic breathing at birth (71). However during the early postnatal life, human infants seem to be particularly vulnerable to hypoxic and hypercapnic episodes during sleep and to changes in peripheral chemoreceptors resulting in altered chemosensitivity which may be one of the factors contributing to a higher incidence of sudden infant death syndrome (SIDS), a disease responsible for unexpected deaths in newborns (72). Indeed, smaller than usual in size CBs or abnormalities in their transmitter content have been reported in victims of the SIDS (73–75). Similarly, a tiny CB with a marked decrease in the number of glomus cells and their dense-cored vesicles has been seen in subjects with congenital central hypoventilation syndrome (76). On the other hand, abnormal enlargement of the CB and hypersensitivity to hypoxia has been shown in spontaneously hypertensive rats and in humans with essential hypertension but not with renal hypertension (77). Biochemical studies have additionally showed elevated catecholamines in the CB in essential hypertension (77). Available data suggest enhanced chemoreceptor reflexes in early stages of recurrent apneas, congestive heart failure, and certain forms of hypertension (see 70). Finally, CB denervation plays a critical role in the increased sympathetic activity found in patients with obstructive sleep apnea syndrome, an obesity-related disorder that can cause serious cardiovascular and neurocognitive problems (78,79). It is likely that the CB tends to maintain oxygen homeostasis by marked morphological and neurochemical changes and, thus, acts as a defense mechanism to prevent the progression of morbidity associated with these diseases.

APPLICATION OF CAROTID BODY STEM CELLS TO CELL THERAPY

Intriguingly, recent experimental data suggest that the mammalian CB is a neurogenic center and its stem cells could be potentially useful for cell therapy in Parkinson's disease (18). In fact, research in Dr López-Barneo's laboratory revealed that the adult type II cells are dormant stem cells that in response to physiologic hypoxia can proliferate and differentiate into new glomus cells (18-20). Detailed knowledge about the CB stem cells responsible for the neurogenic activity in the organ is needed since cells derived in vitro from progenitors exhibit the characteristic complex functional properties of mature glomus cells (18). Because of their dopaminergic nature, glomus cells have been used for intrastriatal CB transplantation studies in Parkinson's disease (80–83). Additional potential advantages of the CB tissue for cell therapy rely on its survival in hypoxic environments, similar to those existing in the brain parenchyma after a tissue graft (20). Intracerebral administration of CB cell aggregates or dispersed cells has also been tested for the treatment of an experimental model of stroke as the CB autotransplantation significantly reduces stroke-induced behavioral deficits and cerebral infarction (84). Therefore, expansion and differentiation of CB progenitors in vitro which can differentiate into functionally normal glomus cells may be a useful procedure for the production of a cell mass, thus permitting the successful development of neurological cell replacement therapy. Understanding the cellular interactions in the CB stem cell microenvironment (cell niche) and the molecular events responsible for the maintenance of multipotency of CB stem cells might settle the issue of small number of CB cells (see Fig. 9).

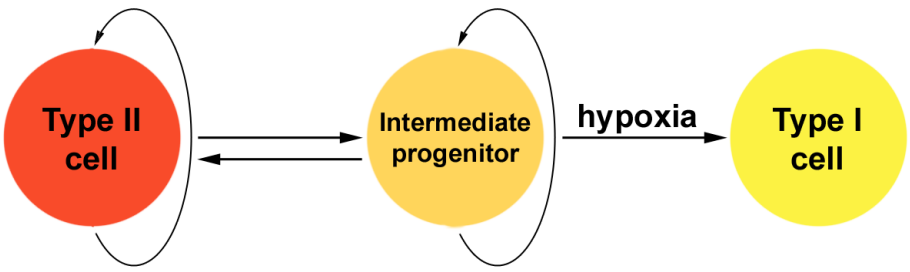

Figure 9. *Schematic drawing of cellular events taking place in the carotid body (CB) stem cell niche during a hypoxia/renormoxia cycle. The type II cells are quiescent (or slowly dividing) CB stem cells that can be reversibly converted into intermediate progenitors, which in turn, upon exposure to hypoxia, give rise to mature type I cells. Modified from Pardal et al, 2007 (based on ref. 18).*

CONCLUSION

Looking back on a large number of previous studies, the morphological and functional organization of the rat CB has been consistently demonstrated. Based on them, it has been widely believed that hypoxia is transduced by glomus cells organized in cell clusters where they make both reciprocal chemical and electrical synapses with each other. Glomus cells receive sensory innervation from PG chemoafferents and are intimately associated with sustentacular cells and the blood supply. Recent advances in CB research and in understanding morphological and physiological mechanisms that operate in it have revealed that chemoreception involves the interaction between glomus cells, between glomus and sustentacular cells, and, most importantly, between glomus cells and chemosensory nerve terminals. Such arrangement is ideally suited for both the autocrine and paracrine regulation of the glomus cell function (8). It can be inferred that the CB has an intricate internal structure and a remarkable ability to release in response to different chemostimuli a broad variety of transmitter agents that provide clues on its important role in the homeostatic maintenance of the whole organism.

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Dance Round

WE DANCE ROUND IN A RING AND SUPPOSE,
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

METALLOSIS: METAL ION RELEASE FROM METAL-ON-METAL JOINT SURFACE REPLACEMENT – CURRENT CONCERNS AND FUTURE PROBLEMS

Stephen R. Manning
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Since its innovation, joint replacement surgery has offered relief from the pain and functional limitation of destructive or degenerate joint disease. The search for the ideal material continues over 120 years later. Recently, using metal-on-metal bearings for younger patients has become the trend to avoid excess wear in high demand patients in the hope of reducing the need to revision surgery. Initial evidence suggested these prostheses offered a durable, functional safe joint that was less likely to be revised than the standard metal and polyethylene joint. A body of evidence is growing rapidly to suggest that metal-on-metal joints are associated with local tissue reactions – metallosis – cellular toxicity, increased serum metal ion concentrations, organ deposition of metal ions, higher rather than lower rates of revision surgery and no functional advantage over any other type of joint replacement. We will consider the reasons for metal ion release; the cellular, local tissue and systemic effects of metal ions and the patient risk and presentation. From the evidence reviewed, serious consideration should be given to the future use of metal-on-metal joint bearings and a suggested follow up plan for patients with such joints is identified and reproduced. ***Biomed Rev*** **2011; 22: 57-64.**

Key words: chromium toxicity, cobalt toxicity, metallosis, metal-on-metal joints, pseudo-tumor, prosthetic joint

INTRODUCTION

Joint replacement surgery is commonly performed by orthopaedic surgeons for patients with painful, destructive joint disease limiting their function and quality of life. It is little appreciated that joint replacement surgery was first described some 120 years ago. The Romanian born German surgical pioneer, Thermistocles Gluck (1853-1942) antedated the famous 20th Century pioneers such as Harboush (1), Wiltse (2) and Charnley (3) by more than 50 years. He was ahead of Küntsher (4) by some 50 years with the concept of intramedullary fracture fixation. His interest in bone defects was encouraged by his work in the Balkans as a wartime surgeon between 1877 and 1885. In his 1891 Treatise, Gluck, describes

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his observation of a rudimentary external fixator. This was made by a mechanic, and used on a femoral fracture, due to a gunshot in Bulgaria in 1885-1886 (5). Gluck sought to determine suitable material to replace diseased joints with. He describes implants made of aluminium, wood, glass, celluloid and nickel plated steel as being cheap and allowing healing without reaction. However, his favourite material was ivory. He described his experiments with cement "in particular the almost instantaneously congealing stone filler" which can be used as a filler or to provide security to his ivory implants. Even in 1891, with his excitement of pioneering a surgical advance Gluck urged caution. He foretold that wear and tear on the artificial joint could not be anticipated and loss of joint movements and ivory strength could result (5).

Wear of prosthetic joints remains a problem today. As we have passed through the 20th and into the 21st Century, joint replacements have evolved and become more common. Younger patients who expect higher levels of function and, naturally, have long life expectancies are being considered for hip surgery. A good functional result has been achieved based on the innovations of Sir John Charnley using polyethylene lined cups and metal femoral heads. However, high demand produces high levels of wear on the polyethylene cup liners and the potential need for revision surgery.

To withstand wear and provide a durable functional prosthetic joint metal-on-metal joints have evolved and procedures such as hip resurfacing offer a durable, functional, pain free joint whilst avoiding total hip replacement in the younger patient. Alternatively, total hip arthroplasty using metal components with a large femoral head to cup size ratio can be offered to the younger patient. Some studies show good functional results are achieved (6). Rates of wear with metal-on-metal prostheses have been shown to be 20-100 times lower than metal on polyethylene (7) and revision rates specifically due to loosening or femoral fracture are low over a 5 year period (2-3.6%) (8,9). These studies provide an argument in favour of the use of metal-on-metal bearings but evidence to the contrary may outweigh this.

The choice of metal implanted into the human body is governed by several factors. It must resist corrosion in the hostile environment of human tissue. It must be durable for its purpose. It must be inert or bio-compatible. It must be compatible with other components implanted with it. Metal alloys have been found to be most suitable. Stainless steel is the least corrosion resistant of the currently used metals and is used more for temporary purposes. Titanium and cobalt-chromium alloys do not corrode in the body however metal ions can diffuse out of the metals and into the body (10).

In this review we will consider the problem of metal ion release from implanted material; consider what, if any, risk it may pose for patients, and consider whether there is sufficient advantage of metal-on-metal bearings to justify any risks.

WEAR OF PROSTHETIC JOINTS

The articular surfaces of prosthetic joints are subjected to repetitive motion as part of their normal function. During their manufacture, joint surfaces are highly polished to ensure a smooth gliding surface. Any micro-imperfections may be eroded after implantation by localised friction, especially if the joint surfaces are not well lubricated by synovial fluid.

Correct placement of the joint components is vital, the geometry of the prosthetic joint place will influence its function and stability. It has been shown that a steeply inclined acetabular component or a small femoral head size can lead to an abnormal pattern of "edge loading." Edge loading is associated with increased rates of localised wear and in the case of metal-on-metal joints, higher serum metal ion concentrations (11-14). Small femoral head size is also associated with impingement of the femoral neck on the edge of the acetabular cup, resulting in restricted movement of the joint and, again, edge loading and excessive wear of the components (14).

Titanium alloy (Ti-6Al-4V) has been found to be susceptible to abrasive wear and it has been seen that one year after implantation the articular surfaces become covered in a "scratch and gouge" pattern. This is especially seen if any loosening of the components occurs, allowing loose acrylic debris from cement or wear debris from a polyethylene acetabulum liner to interpose between the articular surfaces. This pattern of damage is associated with localised release of metal (15,16).

Coupling of the components used in joint replacement is usually relatively straight forward – manufacturers provide both components as part of a set and the components are compatible with each other. Should any mis-pairing result then the outcome depends upon the metals involved. A reported case exists of an incompatible paring of cobalt-chromium and stainless steel leading to severe wear, local metal deposition and serum metal ion concentrations increased by a factor of 20 (17). Cases of excessive wear are in the minority, a 10 year

follow up study of 100 metal-on-metal hip arthoplasties has found survivorship rates for the femoral component of 98% and the acetabular component 96% at 10 years, that is not requiring revision surgery (18).

CELLULAR EFFECTS OF METALS

Chromium is a trace element, present in the human body. In its hexavelent form (Chromuim VI) it is readily absorbed by the lungs, skin and mucous membranes and is toxic. Cobalt is also a trace element and forms an integral part of vitamin B12. The effects of metal particles upon cell culture have been studied *in vitro*. Cell cultures have been exposed to cobalt and chromium separately and as an alloy. Particles have been generated to be representative of those seen with wear upon a prosthetic joint. It has been seen that nano-particles of cobalt-chrome alloy are more toxic to fibroblast culture that micron sized particles. Nano-particles were more readily absorbed into fibroblasts. They break down readily and form an electron dense cloud within the cell, inducing aneuploidy and cytotoxicity. The effects were greater than those seen with larger micron sized particles (19). Cytotoxic effects upon fibroblasts have also been seen to be greater with metals containing a high cobalt concentration (20) and fibroblast viability can be reduced by up to 95% with concentrations of clinically relevant sized particles of 50 micromoles per cell (21). Following exposure to cobalt, fibroblasts show morphological changes when examined under the microscope. Nucleoli become darker staining, cytoplasmic processes are withdrawn and chromatin condenses irreversibly (pyknosis). Within the cell culture, following exposure to cobalt a significant rise in lactate dehydrogenase is seen, suggestive of cellular injury (22).

When osteoblasts are exposed to cobalt or titanium particles, the secretion of interleukin 8 (IL-8/CXCL8) and monocyte chemotactic protein-1 [MCP-1/ chemokine (C-C motif) ligand 2 (CCL2)] is induced rapidly due to up-regulation of the corresponding chemokine genes. The effect of chemokine secretion is to induce macrophages and neutrophils to migrate to the area. Osteoblast synthetic function is reduced in the presence of cobalt ions, as seen by reduced alkaline phosphatise activity and calcium deposition. The production of type I collagen, the predominant form in bone, is inhibited and the production of osteocalcin, a unique and abundant calcium binding bone protein is inhibited by colbalt. Chromium also inhibits osteoblast alkaline phosphatase (23,24). Macrophages attracted to the area by the osteoblast secretions absorb metal particles and secrete IL-1β, IL-8 and tumor necrosis factor-alpha (TNF-α), in a particle dependant manner. These cytokines have been shown to have a bone resorbing effect (25). In particular, high levels of IL-1 producing cells have been found at the bone-implant interface during revision surgery for implant loosening. The role of TNF-α in bone resorption and implant loosening has lead to its consideration as a therapeutic target in those who present with aseptic implant loosening (26). Along with IL-1β producing macrophages, CD4+ T cells, helper T cells, are seen to be present in similar number but in excess of CD8+ T cells (27). The effect of metal debris T cell viability and function show some variability in the literature. Unlike fibroblasts viability has not been seen to be affected by metal debris in some *in vitro* studies, but proliferation has been seen to be inhibited by cobalt chromium molybedenum alloys, an effect not seen with titanium alloys (28). An opposite conclusion was reached by Hallab *et al* (29) who found *in vitro* that both Co-Cr-Mo and Ti alloys incubated with serum solutions led to a lymphocyte proliferative response, greatest when the metals formed metal-protein complexes. To add to this controversy, Akbar Brewer and Grant (30) found that at "clinically relevant" concentrations Cr^{6+} ions significantly decreased cell viability, proliferation and activation while increasing apoptosis. Co^{2+} also resulted in a decrease proliferation and cytokine response but no apoptosis.

LOCAL EFFECT OF METALS

At the local tissue level the effects of failing metal-on-metal bearings are well described. A frequent finding is blackening or grey staining of the tissues adjacent to the implant. These findings are described around the hip (31,32) the knee (33-35), the shoulder (36) and the spine (37).

Soft tissue masses in the vicinity of metal-on-metal bearings are not infrequently described. These masses histologically, have been found to consist of macrophages, metal particles, lymphocytes, fibrin and necrotic tissue (31,38). Microscopic examination of macrophages found in these pseudo-tumours has shown them to be laden with black metallic particles (39). Lymphocytes within pseudo-tumours and surrounding tissues may be found diffusely spread throughout or concentrated in the peri-vascular areas. It has been seen that with higher grades of diffuse inflammation around a failing metal-on-metal bearing is associated with an increasing extent of metal particles

in the tissue (40). As with inflammation from other causes, metallosis can lead to involvement of regional lymph nodes. Chromium containing histiocytes have been demonstrated in the enlarged pelvic lymph nodes of a patient 8 years after hip arthroplasty (41).

The size of pseudo-tumours is variable, in some the tumour mass itself is the reason for presentation (42) or in others the effect of the mass on nearby structures giving rise to symptoms may trigger presentation (43,44).

As we have already seen, the effect of metals upon macrophages and lymphocytes includes the secretion of bone resorbing cytokines at the implant-bone interface. This can lead to loosening of the implant and loss of optimum implant position (45-48), erosion of both trabecular and cortical bone (38,47,49,50) and trabecular micro-fractures of varying ages (51).

SYSTEMIC EFFECTS OF METALS

Metals released into the local tissues are absorbed into the blood stream. These metal ions are measurable in the serum and it has been seen that patients with metal-on-metal joint replacements have raised levels of cobalt and chromium in the blood and the urine (52). Blood levels can be several times the normal level but well within the limits of levels identified as toxic in metal industry workers. Metal ions are removed by the kidney and eliminated from the body in the urine (53). Chromium III ions have been seen, *in vitro* when mixed with human serum, to complex with albumin, transferin and immunoglobulins. The complexes formed were then more readily absorbed by macrophages than uncomplexed metal ions (54).

Titanium ions are released from implants slowly and despite being associated with local tissue metallosis, systemic diffusion levels are low. In sheep, following spinal fixation, in has been seen that at 24 months post surgery little systemic diffusion has taken place, at 36 months, titanium ions were present in all tissues (55). Human post mortem studies also demonstrate widespread distribution of metal ions throughout the tissues. Within lymph nodes evidence of fibrosis and necrosis associated with metal laden macrophages has been seen. The presence of metal containing macrophages within the liver and spleen has been shown and although the amounts of metal are higher than is seen in the lymph nodes, dilution in these larger organs makes the overall metal concentration less. Up to ten years after arthroplasty, no evidence of necrosis or fibrosis was seen in the liver or spleen (56). The alteration of T cell function and viability discussed previously when combined with lymph node fibrosis and necrosis could lead to local immune dysfunction. Small increases, compared to control subjects, have been seen in levels of metal ions found in the frontal cortex of patients with worn metal on metal prostheses (56). The effects of cobalt and chromium ions on nervous tissue described include 1 case of reversible polyneuropathy with histological evidence of axonopathy (57). Two cases of femoral nerve neuropathy due to a pseudo-tumour mass with histological evidence of complete nerve destruction are also reported (58). Consideration has been given to metal ions released following arthroplasty as potential carcinogens. In has been postulated that chronic stimulation and alteration of lymphocyte function could lead to an increased risk of lymphoma or leukaemia (56,59).

It has been seen that concentrations of metal ions is higher in patients whose prosthesis is worn or is loose. This leads to the question, could serum levels of metal ions be used to identify worn, failing or loose prostheses? It has been suggested that measurement of serum metal ions can be a useful adjunct to assessing metal-on-metal joints and a study has shown a good correlation between high levels of serum metal and wear on the joint surfaces (60). This may prove to be a sensitive indicator of wear if regular measurements are taken. The Medicines and Healthcare Products Regulatory Agency (MHRA) have recommended that patients with serum cobalt or chromium ion concentrations greater than 7μg/L should be further investigated. It was seen that 7 μg/L concentration of either ion, had a 90% specificity but only a 50% sensitivity for hip prosthesis failure (61).

HOW COMMON ARE PROBLEMS DUE TO METALOSIS?

The true incidence of metallosis is unknown as early pathology can be asymptomatic, diagnosis may be difficult, and reporting inaccurate (62). In 2011, the National Joint Registry for England and Wales reported that all cause revision rates at seven years for primary hip replacements regardless of implant type were 4.7% (of a total of 285,600 primary operations) however, metal-on-metal bearings had all causes revision rates of 11.8% for resurfacing and 13.6% for replacement joints (63). It can be seen therefore, that revision rates, within seven years of surgery, for metal-on-metal bearings are higher than revision rates in general, despite the previously quoted evidence of a

lower revision rate when looking specifically at revisions for loosening or fracture (8,9). The vast majority of joints have not needed revision during this time, but this is not to say that asymptomatic pathology has not developed.

HOW DO PATIENTS PRESENT?

Initial symptoms may be vague and patients may present with groin, buttock or lateral hip discomfort. They may present with the sensation of a lump around the hip which may or may not be visible. Sensations of "clicking," "clunking," instability or dislocation are less common and may follow a period of discomfort. The mean time of presentation has been seen to be 17 months post primary surgery in one study (64). Rarely, patients may present with serious local symptoms such as nerve compression or vessel damage (43,44,65).

The natural course of metallosis appears to be progressive once symptoms are present and revision surgery for deteriorating symptoms becomes necessary (66).

CONCLUSIONS AND FUTURE CONSIDERATIONS

At present we do not know how many patients will go on to develop symptoms following their metal on metal joint replacements or resurfacings, we may find that after several more years patients who had been initially asymptomatic run into difficulties. Long term effects of cobalt or chromium deposits in the liver are not well known. Although at 10 years, no necrosis or fibrosis has been seen in livers containing macrophages laden with cobalt or chromium (56) it is not known if this will be the case at 20 or more years. It has been suggested that there may be a link between metal-on-metal bearings and leukaemias or lymphomas (56,59) however, no definitive evidence has, to date, been described.

Revision surgery performed for metallosis is more difficult due to local tissue destruction and is associated with worse outcomes and more complications than revision surgery for other reasons including peri-prosthetic fractures (65,66).

Do the benefits of metal on metal bearings justify the potential complications and risks? Recent meta-analysis shows that functional outcomes following metal-on-metal joint replacements compared to metal on polyethylene or ceramic-on-ceramic joints offer no advantage but potentially metal-on-metal bearings have a higher revision rate (67).

What about those patients who, with the best intentions, have been given a metal-on-metal bearing? What should be done for them? The MHRA (68) and British Orthopaedic Association have issued guidance summarised by Fary *et al* (66) and shown in box 1.

When looking at general revision rates for metal-on-metal bearings rather than rates specifically for loosening or fractures we see that revision rates are higher than for traditional metal on polyethylene joints and, as discussed the surgery is more difficult with a higher incidence of complications and poorer functional results. Potential disadvantages are described at the cellular, tissue and systemic level. Taken with the unanswered questions on long term metal toxicity for a joint which is offering no functional advantage over any other currently used prosthesis we must seriously ask ourselves: should we be continuing to use metal on metal joint prostheses in our patients?

Box 1: ***Follow up of post metal on metal joint implants***

- Annual follow up for at least 5 years and more frequently if symptomatic
- Investigate any painful metal on metal joint. Measure serum cobalt and chromium, image the joint with MRI or ultrasound
- If there are concerns about component position or patients in cohorts with higher than expected failure then measure serum metals and image with MRI or ultrasound
- If serum metal ions are greater than 7µg/L then repeat at 3 months and image.
- If imaging shows soft tissue reaction, fluid collections or tissue masses then consider revision surgery.

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Dance Round

WE DANCE ROUND IN A RING AND SUPPOSE,
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

A LINKAGE OF MIND AND BRAIN: TOWARDS TRANSLATIONAL VALIDITY BETWEEN NEUROBIOLOGY AND PSYCHIATRY

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Aim: There are prominent discrepancies in the general approaches of psychology and psychiatry, many of them due to diverse and incompatible tacit positions on the mind-brain debate (MBD). For this reason we need to enhance the dialogue with neurosciences and other human sciences relevant to the problems of psychopathology. To achieve such goal we can reduce the level of diversity of mind-brain problem project-solutions as implied in different theoretical models and practices.
Arguments: I shall trace the MBD to the one of the most relevant for the modern psychopathology areas: the group of neurosciences. We seek the interference of the philosophical assumptions, the evidence of neuroscience and the development of psychopathology. We prove by a post rem analysis that the reduced group of predominant project-solutions of MBD excludes genuine forms of dualism and extreme forms of physicalism (like epiphenomenalism or eliminative materialism).
Conclusion: A predominant group of project-solutions is adopted including complementary combination of contemporary forms of physicalism: identity theory of mind applied to mental events and brain processes; supervenience principle applied to other mental phenomena. ***Biomed Rev* 2011; 22: 65-76.**

Key words: translation, neurobiology, psychiatry, validity

INTRODUCTION

What are we concerned about?

I shall attempt to raise two conceptual issues in this *Dance round*. The first of them is how a tacit position in the mind-brain debate reflects the actual knowledge in disciplines concerned in mental health (psychology, psychiatry, and neuroscience). The second issue is that of connectivity or translation between levels/domains of determination in respect the explanation of mental disorder. Both issues are antecedent or *ante rem* to a greater extent because an „anticipative" position in the mind-brain debate is implied in any kind of research or practice in mental health as inextricable though sometimes tacit predisposition. However it is also *post rem* or consequent, because the

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translation between levels of determination of psyche is a result of further interpretation of data, acquired in certain disciplinary domains and under different paradigmatic frameworks. The data of psychology, neuroscience etc. related fields is liable or not to transdisciplinary translation depending not on the evidence as it is but on the cognitive attitude to it. This is to say that similar as a cognitive content scientific data depend on the interpretation „angle" which, in turn is determined by the previously mentioned „preliminary" position in the mind-brain debate (MBD). Given data along with its theoretical rationale is incorporated in a ‚patchy way' into the disciplinary matrices of psychology, psychiatry and neurosciences. Then in a kind of circularity data post rem presupposes a philosophical position and also might justify / criticize it.

Such divergent and incoherent 'piecemeal' mental health knowledge fails to meet the criteria of scientific discipline, prescribed by the normative functions of psychiatry and psychology (1). That is why there is desperate need for transdisciplinary convergence of the views in the arena of mental health in order to facilitate the empirical convergence of the data emerging in different disciplinary domains. For instance, modern psychoanalysis makes an effort to develop non-classical dualist position; the gestalt psychology holds some peculiar variation of quasi-materialism, called "psycho physical isomorphism"; biological psychiatry is governed by eliminative materialism and so forth, as different traditions in respect to MBD imply contradictory approaches the diagnostic assessment and treatment in mental health, such as biological medication or psychoanalytic psychotherapy. So when they define a term like 'paranoia' or 'depression' or 'anxiety' they practically imply diverse and incompatible theoretical backgrounds. This was actually the reason for Carl Gustav Hempel and Robert Spitzer's escape into instrumental and operational taxonomy of DSM proclaimed to be 'atheoretical'.

I intend to present another resolution to the translational issue driven from more conformable and pragmatic approach to MBD. Eventually my claim will be that once we establish translational bridge laws between the domains of mentality and neural processes without specific polarization towards reduction or emergence, it may facilitate the cross-validation of the terms, notions and methods across different levels of explanation. In this context my intention is to state that this kind of inter-level vertical relationships is actually undermining the possibility of the conformable dialogue and will propose a kind of 'horizontal' complementary model which in my perspective is predisposed by a revised form of the identity theory of mind.

Historical background of MBD: a non-conventional epistemological perspective

In this section I shall apply the divergent-convergent method of Polikarov to the subject of MBD (2,3). There are several reasons for me to believe it is relevant to the topics posed herein: (*i*) as it will be demonstrated herewith MBD has evolved according to the divergent-convergent model, similarly to many other major problems in history of science, (*ii*) if considered in the context of convergence of the field of possible project solutions, the data of neuroscience (though sometimes controversial) delineate one major trend: towards formulation of a predominant group of materialist monism views (solutions of the MBP), and (*iii*) according to the empirical as well as meta-empirical evidence, the metamorphoses of dualism are false as well as some radical forms of the reductive physicalism (e.g. eliminative materialism or epiphenomenalism), and one plausible position might be outlined in a revised form of the identity theory of mind.

Azarya Polikarov (1921-2000) was an eminent philosopher of science in the XX century. One of the most significant and enduring of Polikarov's contributions is the introduction of the divergent-convergent method (DCM). It was published in 1973 in the Boston Studies in the Philosophy of Science, formulated as the "heuristic approach to problem- solving". According to DCM scientific problems are penetrated on two stages. At the first an extensive (divergent) "field of possible solutions" is formulated, some of them only hypothetical, others better supported by available data. At the second stage the field is "reduced" (or converged) to a more restricted area of "predominant group of project-solutions". The mechanism of convergence is usually logical and meta-empirical, i.e. based on scientific evidence. Polikarov defines two variations of predominant project solution: ultimate and alternative. The alternative type is subdivided into combined solutions with higher level of diversity (including radically alternative), and respectively lower level of diversity. DCM is still respected as a methodology of scientific pluralism (though the genuine focus of Polikarov was on heuristics) and its features of critical appraisal make it relevant to meta-empirical, theoretical and meta-theoretical studies.

Divergent-convergent method was recently adopted in the study of the mind-brain problem in psychology and psychiatry. I decided to combine the method of historical reconstruction and DCM in the analysis of the evolution of the mind brain debate. It is well known that a large number of diverse and sometimes radically alternative solutions of the mind - brain problem have been proposed in many scientific traditions:

psychoanalysis, behaviorism, neurophysiology, cognitive neuroscience, philosophy of language and mind. Some of these formulations are pure theoretical constructs; others are more empirical, i.e. supported by observable experimental or clinical data. Nevertheless the incoherence of the whole field, or the dominance of certain "monopolistic" solutions of the mind-brain problem, turned out to be a major source of shortcomings and controversies arising in the real practice and expertise in many professional areas concerned with neuroscience and mental health.

I aimed at reconstructing the debate at two historical and epistemological stages. In the stage of diversification (XVII-XIX century) the preliminary field of possible solutions was spanning from materialist monism to the different prototypes of dualism: Cartesian interactionism, psycho-physical parallelism and other minor bifurcations. My further analysis indicated that during the historical period of the most extensive mind-brain debate (end of XIX - beginning of XX century) a number of revolutionary changes of paradigms emerged, both in mental sciences and neurosciences. Worth mentioning are the neuronal doctrine of Ramon-y-Cajal, chemical neuro-mediation, functional and experimental neurosurgery synaptic ultrastructure and functional plasticity, behaviorist discoveries neuro-psychoanalysis, etc. These trends delivered evidence which made possible the "program integration" agenda of neuroscience and the medical branches of psychology, respectively psychopathology. This integration was promoted in Francis Crick's doctrine (4). I regarded this process as *ipso facto* convergence of the initial field of possible solutions of the mind brain problem to a reduced field of actually physicalist predominant group of project solutions. I have delineated two combined predominant groups according to the method of Polikarov. The one with higher level of diversity includes all contemporary variations of the materialist monism. The other, with lower level of diversity focuses on two influential types of physicalism: reductive and non-reductive. The main representatives of the reductive trend are the Australian Identity Group, eliminative materialis and epiphenomenalism. The non-reductive physicalism is represented in the supervenience theory, anomalous monism, and partly in the dual-aspect monism (see 20).

ARGUMENT

My core argument is divided into two counterparts. In the first I shall delineate the state-of-the-art in the area of the predominant group of project-solutions of MBP as configured in the DCM analysis from the first section of this essay. The second counterpart will engage with the story of the bridging laws as moderators in the MBD in respect to the utmost necessity in translational dialogue across disciplines in mental health (5).

Rendering dualism as significant: metaphysical vs. empirical significance

I shall adopt here the argumentation against modern dualism as elaborated by Kenneth Kendler in his fundamental paper for *American Journal of Psychiatry* (6). Kendler asserts that:

> "We need to reject definitively the belief that mind and brain reflect two fundamentally different and ultimately incommensurable kinds of "stuff." Rather, in accord with an overwhelming degree of clinical and scientific evidence, we should conclude that the human firstperson world of subjective experience emerges from and is entirely dependent upon brain functioning. The mental world does not exist independently of its physical instantiation in the brain. To reject Cartesian dualism … means to no longer consider the mental (or functional) to be fundamentally different thing from the biological (or organic). Rather, the mental and the biological become different ways of viewing and/or different levels of analysis of the mind-brain system"

Most of the other forms of the dualistic attempts have been falsified by natural evidence and are no more than intellectual speculations in respect to the scientific effort to explain and manage mental disorders. In this context my claim is that psychophysical dualism plays still an important metaphysical role *only* as a subject for meditations in both analytic and continental philosophy. On the other hand dualism is undeniably suspended with the data of neuroscience. It seems obvious that besides Sir John Eccles more than forty years ago, there is no other scientist from the field of empirical science to any more hold the position of dualism. At the same time dualism does not help to resolve and manage problems of the real people. And philosophy of psychiatry as it was conceived by Bill Fulford aims at the *philosophy-into-practice perspective*, i.e. a program to improve the mental health of the real people.

The type identity theory of mind: challenges and prospects

I shall ground my further arguments on the views of the British psychologist and philosopher of mind Ullin Thomas

Place (1920-2000). He states that empirical evidence is of critical importance for sustaining of a thesis in the MBD (7). Contrastingly to Davidson, Searle, Putnam, Kim or Churchlands, he used to be a practicing clinical psychologist for NHC throughout a period of 40 years. Hence Place had clear penetration into the obvious miss-understanding between the neuroscientists and the mental health care operators. They use theoretically diverse terms to indicate actually identical events in mental life corresponding to processes in the brain. I shall also refer to another critical construct for my analysis: the "perfect correlation" goal as stated elsewhere (8). So I believe that if patterns of "perfect correlation" are established at least for some of the mental-neural phenomena, this entails a type of identity, which may serve to improve the cooperation of the experts in the field of mental health.

I do not intend to construe a prior discussion as constituting an attempt to establish these forms of physicalism (type or token identity) as necessarily right. Rather I construe my prior analysis as determining these forms as plausible combined predominant group of project solutions of the mind brain problem. Briefly, I intend to set them as: (*i*) Relevant to the evidence of the modern neuroscience, and (*ii*) as feasible cognitive explanatory vehicle that can reinforce „*materialism as a scientific hypothesis*" (7). In summary my goal is to demonstrate a cognitive 'route' for heuristic and pluralistic way of solution of the mind-brain problem in efficient dialogue with neuroscience.

Originally, the type identity theory of mind was grounded on a group of evidence from experimental and clinical neuroscience, the excitation of the c-fibers in the nervous system correlated with the psychic experience of pain, contrastingly to the excitation of the A delta fibers as a correlate of the mere reception of the pain. Moreover a variety of other considerations were raised from empirical material, such as the evidence of the 'blind-sight'; prefrontal lobotomy; administration of Lysergic acid diethylamide and other biological agents inducing changes in experience and behavior.

As far as many authors define and understand the issue of psychophysical identity in their own manner, let me try to summarize my assumption for this subject of analysis. Mental states (processes and events) do correlate in time and space with brain processes (or events - dependent on the experimental paradigm). This assumption seems not to be challenged so far. One may find it appropriate to specify that these correlations are revealed in humans (and mammals) in one possible reality (world) to be assessed by certain methods. These limitations as introduced by Putnam and other anti-reductionists actually serve to delineate some features of feasibility the identity or any other robust physicalist thesis. Still the implication of the anti-reductionists goes far beyond such denomination of 'feasibility'. They use the idea that mental states might be composed of different elements in another possible reality as argument to undermine and deny the very foundations of the strict physicalism. There is made an attempt in this *Dance Round* to demonstrate how meta-empirical analysis may question this approach. This entails the conclusion that whilst materialism in some of its modern expressions still underpins the constructions of psychiatry and psychology as robust scientific disciplines, dualism and the radical forms of materialism remain of metaphysical significance without correspondence to the practical and scientific reality.

Another contested facet in the identity theory of mind is the role of causality. It is the causal implication made from brain processes to mental phenomena and backwards which delineates the distinctions between the different kinds of physicalist theories of mind. I consolidate these two points to argue why dualism and epiphenomenalism (6) and eliminative materialism (9) are false as well.

Further I shall define the range of the plausible pluralistic account of mind brain problem has been limited to identity theory of mind and supervenience. Finally I shall employ Thornton's critical account on supervenience as incapable to capture the 'interface problem' to further converge the field of possible project solutions of the mind-body problem.

In his 1960 paper "Materialism as a scientific hypothesis" U.T. Place writes:

> "What is important is that there must be some logical criteria which we use in deciding whether two sets of correlated observations refer to the same event or to two separate but causally related events. The problem of deciding what these criteria are is a logical problem which cannot be decided by experiment in any ordinary sense of the term; and since we cannot be certain that the criteria are satisfied in the case of sensations and brain-processes unless we know what the criteria are, the issue is to that extent a philosophical issue […] …but let us assume that the identity of things is established empirically, while the identity of concepts is established either deductively … or empirically, as in the case of temperature and molecular motion, by the empirical verification of a scientific theory within which it is possible to define one concept in terms of the other. I prefer to regard the temperature, lightning,

> and sensation-brain-process cases as examples of a special variety of the identity of things in which an identity is asserted between a state, process, or event and the microprocesses of which it is composed."

That is the precise original formulation of the type-type identity of mind-and-brain. In his earlier paper entitled "*Is consciousness a brain process?*" Place examines logical and metalinguistic aspects of identity. He believes that compositional identity refers to the logical distinction introduced by Gilbert Ryle between the 'is' of definition and 'is' of composition. Place specifies that in his reflection of identity he actually refers to the *compositional identity*. Further Place introduces another cognitive limitation. He argues in his analysis, again in the sense of the Oxford School that there is substantial difference between events, states and processes and the type identity is valid only in the case of statements about mental events and brain processes. That means that identity thesis is applied to e.g. events of sensations and distributed processes in the brain. It is an important point to be emphasized. Historical predecessors if identity such as Pierre Cabanis in the late XIX century and some more radical reductionists of the XX century like Smart, Armstrong and Feyarabend do not share any cognitive limitations to their convictions of reduction. Very often they are simply looking at anatomical loci of certain aspect of consciousness. This is the exact proposition that Place is arguing contra (7):

> "…But the empirical problem is not, as Smart seems to think, simply a matter of determining the precise anatomical location of this physiological process. It is still an open question whether there is, even in this relatively circumscribed area, a process which satisfies the logical criteria required to establish its identity with the sensation process. Even assuming that we know what these criteria are and are satisfied that they are applicable in this case, we cannot regard the question as finally settled until a process satisfying the necessary criteria has been discovered or until we are sure that we know enough about the brain to be certain that no such process exists. Until such time as this issue is settled by further psycho-physiological research, materialism remains an empirical hypothesis – the hypothesis that there exists, presumably in the brain, a physiological process which satisfies the logical criteria required to establish its identity…" [1960 ibid]

Hence, Place assumes explanation of mental phenomena in terms of biology in the same way as lightning is seen as electric discharge or heat as a molecular motion. There is a crucial question raised besides the explanatory relation of the lower level models (brain processes) to construction of the psyche, which seems "self-evident" according to Place himself. Most philosophers of mind will not rule out some kind of emergence. The actual question addresses the ontological or translational reduction. If one may eventually agree that higher level (whole person) experiences are undoubtedly composed of lower level (neuronal) processes, most of the identity theory opponents disagree whether the person level in composed only and exclusively of neural processes. The position of the ontological reduction is that not only specific process in the brain causes certain experience or behavior on the personal level but more importantly the backwards implication, namely that every certain type of mental phenomenon to one and the same brain process. Therefore the claim of reduction is inextricably bound to the claim of the causal power. In the intuition of the theorists of mind-brain identity as well as of the other types of reductive materialism *mentality has no causal potential in itself at all.* This implies over-determination of the mental from physical. The configuration essentially excludes any potential causal capacity of the mental hence any autonomy of human behavior and consciousness in general. In my perspective this should be one genuine subject of the debate. As it has been outlined by Kenneth S. Kendler:

> "That is, changes in the brain can directly affect mental Functioning…we commit ourselves to the concept ofmind-to-brain causality. In ways we can observe but not yet fully understand, subjective, first-person mental phenomena have causal efficacy in the world. They affect our brains and our bodies and through them the outside world." (Am J Psychiatry 2005; 162:433– 440)

It is also worth mentioning here that the 'classical' identity theory of mind as presented by Place does not take part in the debate of the causation at all. The assignment of causal power to the neural correlates of consciousness is an exclusive merit of the later identity theorists like David Armstrong. My own understanding is that we have not sufficient evidence to take a sound position on the causation of mentality and thus we should embrace the pragmatic bi-directional causation as proposed by Kendler and later supported with robust data from neuroscience.

The other genuine subject to debate should be the issue of translational vs. ontological reduction. One of the outstand-

ing proponents of the identity theory Jack Smart is inclined to believe that the ontological reduction is an ultimate goal:

> "…I should say that this is an ontological, not a translational physicalism. It would be absurd to try to translate sentences containing the word 'brain' or the word 'sensation' into sentences about electrons, protons and so on." (10)

This is not however only way in which we understand or interpret the notion of translation. In some perspective 'translational' properties of given phenomena are seen not just as a *linguistic* issue, but mainly as *epistemological* one. When I say epistemological I imply the justification of the use of inter-disciplinary shared terms and notions. Now imagine that a term like "emotions" is frequently employed in many disciplinary systems, such as psychology, psychopathology, and neurosciences. Therefore "emotions" is a shared construct with all derivative terms which are used to describe human experiences in health and disease, e.g. "depression" or "anger", "grief" and so forth. Disciplines which seem to employ this wide range of terminology are ascribed to explain different levels in mind-brain relationships, both vertical and horizontal. Vertical relations of biological properties and mental phenomena are subject to comprehensive interest and examination. My intuition is that there exists another kind of important *horizontal* structures in mental health knowledge which require consistent translation. We can define and understand the realm of mentality of its own right but any cognitive structure in it (regularity or notion representing certain aspects of consciousness) has to be underpinned with correspondent (identical in Boring's sense of correlation) cognitive structure in the realm of neural processes

Let me try to illustrate this configuration. It is used one provisional example on the following picture with two different classes of mental and respective neural phenomena, where A stands for realm of mentality and B stands for realm of neural processes (Fig. 1).

My claim is that no connection at the level of mentality (*not* equivalent to intentional realm in McDowelian's terminology) may exist if it is not underpinned by a corresponding connection at neural level or in the neural domain. In other words, in the rectangular structure as illustrated above not only its basic elements (the items in the separate angles) are connected with bridge laws, but the *very laws* (both vertical and horizontal) are 'stabilized' by homological bridges, say isomorphic lines of connection. This does not necessarily imply that anything in mind-brain relationships is governed under such rules. I

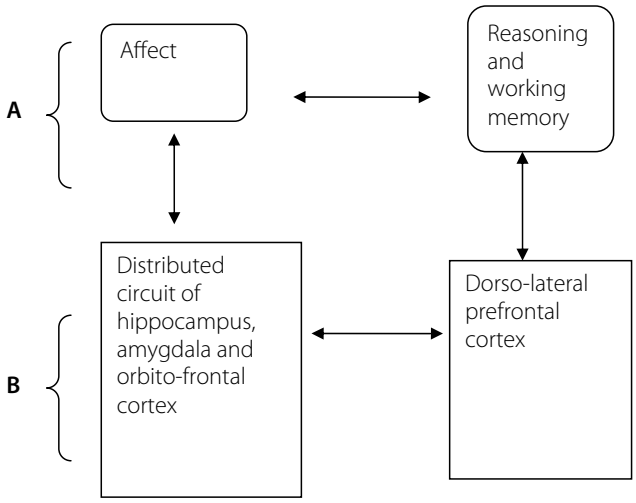

Figure 1: *Interrelations of the neurobiological and mental domains*

suggest this configuration just for the 'scientific' descriptions of observable phenomena which claim to be 'evidence' both in neuroscience and psychology and hence need to face the respective demands for scientific stability (reliability) and inter-disciplinary validity. In this sense I have no claim at such robust structures when it concerns the values and trans-personal relationships at the level of the whole person and intentionality. This is consistent with other similar views on the construction of mentality. This is to some extent the case with Psycho-biological theory of personality of Claude Robert Cloninger (11). He defines two integrative domains of personality. One of them is explicitly underpinned with neuro-biological and genetic mechanisms and is named *temperament*; it is connected closely to other biologically derived concepts such as Eysenck's theory of personality (including e.g. neuroticism and extroversion as personality traits). The other domain is related to the character and specifically to '*humanistic and trans-personal style' of communication and relating to others*. The latter domain explores such aspects of personal profile Self-directednes, Cooperativeness and Self-transcendence which obviously belong to the intentional realm. Self-transcendence becomes later the basis of Cloninger's '*science of well being'*. My intuition is that there is a variety of other mental phenomena besides the 'temperament' in the terms of Cloninger which correspond to causal substrate in the brain and thus are liable to translational reduction. Still there are a number of other irreducible intentional entities which transcend beyond my configuration and which are therefore outside the frontiers of this analysis. These entities belong to

the realm of socio-cultural values; whilst my deep concern as it has been outlined in the introduction is within the realm of facts (or evidence).

In the following section I shall further defend identity theory from two extreme forms of reductive physicalism as well as from some anti-reductionist responses.

Eliminative materialism and epiphenomenalism are false

Two more radical theories in the convergent field of project solutions of the mind-brain problem are regarded as influential in the past two decades. These are epiphenomenalism and eliminative materialism.

Objections at eliminative materialism

One of leading proponents of eliminative materialism Paul M. Churchland (9) asserts that:

> "The identity theory was called into doubt not because the prospects for a materialist account of our mental capacities were thought to be poor, but because it seemed unlikely that the arrival of an adequate materialist theory would bring with it the nice one-to-one match-ups, between the concepts of folk psychology and the concepts of theoretical neuroscience, that intertheoretic reduction requires. The reason for that doubt was the great variety of quite different physical systems that could instantiate the required functional organization. Eliminative materialism also doubts that the correct neuroscientific account of human capacities will produce a neat reduction of our common-sense framework, but here the doubts arise from a quite different source.
>
> As the eliminative materialists see it, the one-to-one match-ups will not be found, and our common-sense psychological framework will not enjoy an intertheoretic reduction, because our common-sense psycholological framework is a false and radically misleading conception of the causes of human behavior and the nature of cognitive activity. On this view, folk psychology is not just an incomplete representation of our inner natures; it is an outright activities. Consequently, we cannot expect a truly adequate neuroscientific account of our inner lives to provide theoretical categories that match up nicely with the categories of our commonsense framework. Accordingly, we must expect that the older framework will simply be eliminated, rather than be reduced, by a matured neuroscience".

My objections at Churchland's vision are twofold. The first one is the subscription to the construct of fuzzy and indefinite construct of "*folk" or "commonsense psychology*". This commitment is not unusual in philosophy of psychology and psychiatry even in contemporary pieces of research (12). Eliminative materialism regards "folk psychology" as fuzzy and indefinite and therefore hopes that 'matured neuroscience' complemented with computational technologies can eliminate it. Nonetheless I stress that the *very notion* of folk psychology is delineated in a fuzzy and indefinite way and we have not specific borderline between what is assumed as „folk psychology" and what serves as ‚scientific psychology" (cognitive, clinical etc) and that is not justified in Churchlands' claim.

My worry however is also that such stipulation does not help to improve the standards for '*scientific*' psychology and hence psychiatry. Although some authors assume the extrapolation of the issues from folk to cognitive psychology as possible and necessary (13) this is also a kind of 'piecemeal' approach to the constitution of evidence in areas of mental health knowledge claiming at scientific value of their data. Second, many branches of psychology and psychiatry (clinical, behavioral, cognitive etc.) insist on their commitment to positive science. There is collected considerable evidence in these frameworks. Although it is criticized for the limited trans-disciplinary capacity of the cognitive content (14, 15) it meets some 'internal' criteria for validity for different reasons like utility (16). This means that we are less concerned with the internal validity but the capacity for 'translation' of data between different disciplinary languages. Hence we are concerned more in patterns of identity therefore of correlations of mind-and-brain activities than in complete denial of all contemporary psychological knowledge as proposed by Churchland, regardless whether it is "commonsense" or "scientific". Second, eliminative materialism is much more precise that the ultimate replacement of the overall "mental' vocabulary is on its way. They tend to believe without any hint at reservation that every single construct of psychology is to be replaced with neuro-computational categories. Whilst Place seems to be much more cautious in his predictions (emphasized and underlined in the quotations in this essay) that materialism is nothing but a 'scientific hypothesis" and that just a certain class of mental constructs are liable to reduction. He is inclined to accept that there is also a class of irreducible entities, a view I completely share with him. I have developed a couple of case studies to endorse this point herewith.

Case study:

Thesis : identity of the pain experience with the c-fibres activation : a claim for possibility of universal explanatory reduction.
Anti-thesis:
(a) "false" pain: many survivors after limb amputation report pain experience from the area of the the same limb that has been eliminated;
(b) Kandinsky-de Clerambault syndrome, sensory type: patients inform about senstaions (very often pain sensations) without any activation of c-fibers. Two worries emerge: what determines the pain experience in De Clerambault s-me and where is the causal connection of the mind-and-brain complexity?

Case report 1: Sasho V., 50 years old
Hospitalization (July 2007) of a patient with depressive-paranoid syndrome, basically assessed a recurrent depression with elements oh hypochondria.
Extraction from the narrative from the last interview (Oct 30th, 2007):
"I felt aches...initially I thought I've got a cancer. When the pains begin (puts his arms around the epigastria and the lateral abdominal area)...it is just something glowing, sometimes very hard and embarrassing, than gradually faded...eventually I felt pain in the chest on the left side...like a heartache. It pressed and released, usually in the evening. Maybe it was caused by some kind of exterior power, influence from outside, like magic."
The numerous instrumental explorations, including EMG did not prove any organic cause for Sasho's complaints as well as there were revealed no data, associated with C-fibers excitation. On the other hand he responded to antipsychotic treatment.

Case report 2: Stefan S., 55 years old

Serial hospitalization in the clinic; this particular one - by the reason of
a legal expertise.
According to the morbid anamnesis it refers a case of schizophrenia in a stage of evolution of a paraphernia syndrome.
The latter is presented with pseudo-hallucinatory phenomena as well as with confabulatory megalomania signs and inventory delusions.
The further analysis of the pseudo-hallucinatory component shows that the patient shared experiences like aches in the internal organs.
Extract form the interview:
"…I am a great inventor, I created inventions for billions dollars. The foreign intelligence services prosecute me and want to destroy me… three men follow me with little machines implanted into my organs. They intend to hurt me – inducing undefined internal and of the left hand pain ", caused by "extra-sensorial equipment".
The investigation of the periphery segment of the pain sensor with EEG and EMG did not prove any excitation in the extra-lemniscal system.
Imaging techniques are not available in order to explore the CNS pain-related structures and function.

Synthesis:
(a) central, not peripheral activity of the neural system causes the pain sesnsation; pragmatic reductionism; bi-directional causation according to Kendler (2005) and Korf (2009)
(b) distributed functional systems involved, not local structure interactions,
(c) organo-dynamic dissolution of the higher mental functions, "liberation" of the archaic ones: e.g. automatisms in the sensory modality (pain perception).

In conclusion: (a) explanatory pluralism is necessary in the case, in combination with (b) token identity, applied to mental phenomena, already explored by the neuroscience and (c) supervenience to the ones, which nature is not clarified yet. (d) Psychopathological phenomena might be explained through organo-dynamic theory (H.Ey, 1962).

Last but not least in my revision of the identity theory, as indicated above. I attempt to say that identity has a 'horizontal' meaning besides the obvious 'vertical' implication. In this horizontal perspective there is no such demand at replacement of the vocabulary. Each of the languages in question is a nomothetic system of its own right, mental and physical, but we are aware of their correspondence (Bohring's) perfect correlation, and each expert operating with these languages is prepared for the inter-playability of the terminology employed in his discipline. Put in other words the upgrade of identity theory of mind is a prototype of a *,manual for translation'*.

Ruling out epiphenomenalism

The other radical theory of mind advocating a heavy reductionist program is epiphenomenalism. I am inclined to rule it out as well. As it has been articulated by Ken Kendler (6):

> "The core assertion of epiphenomenalism is that the mental world is without causal efficacy, our mental life being simply froth on the wave or steam from the engine. Thoughts, feelings, and impulses occur within our subjective experience, but they do nothing. All the causal action occurs at the level of brain function. For the present purposes, I wish to simply assert its falsity and argue that thoughts, feelings, and impulses matter not only because they are responsible for huge amounts of human suffering but because they do things."

In later studies this point has been confirmed empirically in the continuous contributions of Jacob Korf, who demonstrated experimentally that mental experience, though generated by electric activity of the brain, can also affect brain function by top-down causation. Korf has illuminated that the signal detected via neuro-imaging techniques (like PET or fMRI) is actually the restorative *iso-energetic* response of the neural networks after performance of certain mental activity, i.e. it *comes as an effect from, not as a cause for the psychic phenomena* (17, 18) .

Commentary on some anti-reductionism assumptions

I shall try to explain herewith why a large body of criticism of the identity theory of mind is inappropriate in the context of philosophy of psychiatry. Three critical objections targeted: presented by Davidsson, Putnam and Kripke (e.g. 19). The 'multiple reliability" or 'Twin Earth" arguments are among the most cited anti-reductionist points along with the anomalous monism of Davidsson and Kripke's argument. All three critical assertions are well known in the philosophy of psychiatry literature and have been exposed in an extensive analysis (and still there is discarded the critical role of the identity theory of mind). This is why I shall not go in-depth with their reproduction but present just a brief disagreement. It is precisely that any of these anti-reductionist accounts is inevitably leading into *metaphysical confusion*. If unfold the most common anti-reductionist statements lead either into some form of psychophysical dualism or directly into idealistic monism. These two specific positions in MBD, though perhaps significant in themselves for philosophy are not relevant to the modern evidence of neuroscience and can not contribute to the "philosophy-into-practice" perspective as mentioned earlier. In fact they are grounded on completely speculative assumptions such as the possibility of the existence of another parallel reality in the "Twin Earth" thinking experiment by Hillary Putnam. In my understanding philosophy of psychiatry (including philosophy of mind in the same context) have not to consider 'multiple' realities and mental organizations. It is implied by the very subject of the mental disorder as something concerning *human* mentality in *this* world. This does not mean that I am rejecting the significance of these arguments in general philosophy of mind and analytic philosophy.

Toward a revised identity theory of mind

In previous sections I have *danced round* my view that there is a "horizontal" aspect of the identity thesis which is not that committed to the reduction vs. emergence debate as is the "vertical" identity. My implication for the update of the identity theory of mind looks like that: "*we have two measures, e.g. clinical assessment depression rating scale and neurobiological measurement of some brain process, e.g. binding potential for some causally efficient for depression brain protein. If we explore them simultaneously we shall reveal unquestionable convergence between the two scores: depression scale will correspond to some equipotent value of the brain measure*." (20) Identity thesis as defined by Boring seems to be consistent:

> „... a perfect correlation is identity. Two events that always occur together at the same time in the same place, without any temporal or spatial differentiation at all, are not two events but the same event. The mind-body correlations as formulated at present do not admit of spatial correlation, so they reduce to matters of simple correlation in time. The need for identification is no less urgent in this case" (8).

My tentative argument is that the modern neuroscience disre-

gards the critical importance of the concordance of the time correlation (currently the brain scans are performed in different time and in different space from the clinical evaluation) and so to claim that improvement of the methodological framework and protocols will possibly help us to demonstrate at least *some patterns* (or '*patchy reduction*' in the terms of Kendler) of mind-brain identity. There is positive predicative evidence that we shall succeed at least in part of this program.

If we do then the issue is raised what will be the impact of the established models of identity. Modern mental and neuroscience hold respectively implicit transactionist dualism and eliminative materialism positions. Eventually we shall discover that such terribly diverse constructs in modern neuropsychiatry as paranoia in psychoanalysis (tacit dualism) and neuro-physiology (tacit eliminativism) are in fact identical. I.e. we have different implications of one term in diverse branches of mental health knowledge (diverse in the sense of their tacit position in the MBD which proves to be in fact identical in different disciplinary languages, if bridged with cross-validity law-like translational structures. In turn this may facilitate the reconciliation of the paradigms in the field.

Is there any room left for supervenience?

Further we need to address one more query. Namely whether there is any room left for the supervenience theory of mind. I need supervenience applied to other mental phenomena, because of two reasons. The one is that, as it is stated specifically by Place (7) type identity refers to mental events and neural processes in the terms of Gilbert Ryle. I still need some explanatory framework for the mental processes and states, respectively neural events and states. Because of the higher level of complexity, which characterizes these kinds of relationships, type identity is not appropriate as explanatory model. However supervenience delivers some, let me say broader „*periphrasis*" of the reductive physicalism, which is feasible to fill in these gaps, so that they may remain protected against the speculations of the dualism or even of the epiphenomenalism. At the same time we need to be cautious in subscribing to supervenience. In his recent study of the interface problem as defined by Bermudez Thornton (21) for instance argues that:

> "… non-reductionist supervenience is not a stable middle point between a form of dualism that eschews supervenience or a reductionist physicalism that can also explain supervenience. That in turn suggests that supervenience cannot…provide a deflationary resolution of the interface problem".

In other words supervenience seems not to offer comprehensive ontological resolution of the mind-brain problem. This is why I prefer to operationally employ supervenience as well as the weak forms of reductive physicalism (e.g. token identity and 'patchy reductionism') in order to deliver pluralistic explanatory account of the more sophisticated and complex phenomena of the mental life as exemplified in the case studies in the previous section.

On the role of connectivity or the "bridge laws" in science as applied to the connections between neuroscience and psychiatry

Identity theory of mind as well as any form of reduction in science is inextricably bound to the construct of the so called bi-conditional Nagelian laws. Ernest Nagel (22) gives a specific definition for the role of reduction:

> "…Reduction, in the sense in which the word is here employed, is the explanation of a theory or a set of experimental laws established in one area of inquiry, by a theory usually though not invariably formulated for some or other domain."

Nagel distinguishes two types of reductions: (*i*) Homogeneous reductions: relations between two sets of statements that employ a homogeneous vocabulary, and (*ii*) Inhomogeneous reductions, where the subject matter of the primary science appears to be qualitatively discontinuous with the materials studied by the secondary science.

By all evidence wherever there exist psychophysical reductions bolstered by an updated identity theory of mind as exposed above, they should be a type of inhomogeneous connections between mental and physical terms and law-like structures (see 2.2. and 2.5.) According to the Nagelian concept, the nature of *bridge-laws* (the assumptions that assure connect ability) might be construed in three different ways: logical connections (ruled out be Nagel himself); conventions and factual or material connections (empirical connections). As we are aware since Hempel and Spitzer the logical connections along with conventions delineate the modern 'scientific psychiatry'.

Many studies have demonstrated that in practice this approach is to a great extent misleading and proto-scientific. My claim is that the bridge laws between psychiatry and neuroscience should be underpinned with factual connections based on real findings of clinical sciences and neuro-biology in health and disorder. Last but not least, a reservation in the adoption of

the Nageliean concept should be made. On one hand it seems to me inevitable to adopt bridge psychophysical laws where we have sufficient evidence to sustain them in order to establish patterns of identity and thus predispose reconciliation between paradigms in mental health. On the other I do not embrace Nagel's demand for ontological elimination of the reduced entities besides the most basic sciences (neuro-biochemistry in our case). I see no sensible need in this ultimate reduction from the angle of the cognitive pluralism. Also in the light of my previous arguments a program for ultimate reduction (like those proposed by the eliminative materialism and epiphenomenalism) is predestinated to failure due to a number of meta-empirical reasons.

CONCLUSION

Psychiatry definitely must focus on the personal experience and values. The person centered comprehensive assessment however needs to be the superstructure over a more robust scientific basis. The individual assessment is always unstable as it is unique for every person. That's why I regard it is a kind of superstructure, which should be grounded on a preliminary fundamental transdisciplinary structure of knowledge, stabilized with cross-validity "bridging" connections in the network of the basic explanatory sciences about mind-and-brain.

As an interdiscipline, psychiatry should meet the criteria for internal validity but also for independent external validity. This is why it needs cross-validation of its shared terms and methods with neuroscience. At the same time neuroscience is not likely to deliver evidence about the validity of the contemporary narrow categories in mental health. It is more likely that neurobiology can further validate broad diagnostic prototypes. Once stable prototypes are validated via explanatory connections from psychology (the group of 'mind' or 'mental' sciences) and neurobiology (the group of 'brain' or 'neurosciences') we can superstructure them with the personal narratives.

In summary, I propose a frame shift from mind-brain opposition (controversy) to mind-and-brain unity *without the eliminativist claim* for ultimate reduction and with due respect to the personal experience and values.

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Dance Round

WE DANCE ROUND IN A RING AND SUPPOSE,
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

ABO GENETIC SYSTEM, SEXUALLY TRANSMITTED INFECTIONS AND ADNDROGEN-ASSOCIATED DERMATOSES

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In the middle of 20th century, it was proved that ABO genetic system was the result of play of selection, including the infectious mortality due to two deadly epidemic collisions in the antiquity and with the main scene being Asian societies. It was discovered that plague tended to kill blood group O while smallpox blood group A carriers. Onwards no link was sought between this evolutionary phenomenon and blood group-related sexually transmitted infections and recurrent androgen-associated dermatoses (such as pityriasis versicolor and acne vulgaris) as well as sexual and fertility activity. Here we Dance Round such possible links. We found that these are expressed more strongly by blood group B carriers, and an attempt at translation of some relationships into population (intercontinental) level. We emphasize the genesis of blood group-related population gene pool equilibrium level and its attributes such as complex defense responses and co-operated immune reactions. ***Biomed Rev*** **2011; 22: 77-80.**

Key words: ABO genetic system, cell-mediated immunity, population gene pool, sexually transmitted infections, recurrent androgen-related dermatoses

INTRODUCTION

Apart from significant advances in the study of human sexual potency, many aspects remain to be elucidated. The difficulty is in the fact that such an essential biological function and sociocultural phenomena along with related sexual practices cannot be reduced to a common denominator. In the middle of 20th century, it has been established that blood group B (BG-B) is a factual marker of Eastern belonging. Blood group B distribution in India preserves approximately equal values: for 1942 – 34.8% (1), for 1966-1970 - 32-42% (2), and for 1997 – 37.4% (3). Values concerning Japan are even more stable: for 1933-1944 21.9-23.1% (1) and for 1966-1970 – 22.2% (2). Here the reasons consist in the emigration processes which do

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not alter BO polymorphism. Conversely, for the British Isles (4) and Germany (2), BG-B incidence increases on the expense of BG-A because of the intensive post-war immigration

Blood groups and antibody responses towards some sexually transmitted infections and their tropism to some androgen-associated dermatoses

Research on the relationship between blood groups and sexual potency continuously grow in depth and quantity (5). We were inspired to do this investigation when during the one-act mass screening seroepidemiologic testing in 2001 among a total of 536 young, 18-year-old Caucasian navy sailors. They wee examined a year before joining the Navy and then every season, i.e. 12 times during their 3-year service as well as every two years after dismissal. Telephone interviews as well as air-mailed or e-mailed questionnaires were used.

The purpose of the study was to establish if some well-known recurrent androgen-associated dermatoses (RAAD) such as *pityriasis versicolor* (RPv) (when starting during navy or permanently, Pm) and *acne vulgaris* (RAv) were expressed more strongly by BG-B carriers. It was established that BG-B carriers were higher generators of elevated antibody titres (> 1/20) against sexually transmitted infections (STI) such as herpes simplex virus-2 (HSV-2) ($p<0.05$ - $p<0.001$) and cytomegalovirus (CMV) ($p<0.001$) (Table 1) than the other BG carriers. The diagnosis of CMV infection was made by a classic hemagglutinin test while that of HSV-2 by additional clinical monitoring concordant in more than 85% of the cases with antibody responses. The possible BG-B-related higher sexual potency was tested during a 10-year long (2000-2009) randomized longitudinal population follow-up study. In order to achieve a higher preciseness we examined under homogenous endogenous and exogenous conditions such as matched gender, age, secondary educational level, nutrition, military stress level, inhabitants in the coastal area as well as origin from classic population morphs (CPMs) i.e., hereditary villagers (HVs) and hereditary town dwellers (HTDs).

The cause for discriminating the town-village hybrids (TVHs) will be stated later on. The concept that ABO group polymorphism results from the play of the natural selection, i.e., the infectious mortality from plague and smallpox (2-4) goes with our data where BG-B dominates by 3.58 times among HTDs (n=107) (in 43%) than among VHs (n=429) (in 12%) ($p<0.001$) being a consequence of the naturally rare disposition of the latter to these fatal infections.

As shown on Table 1, these RAAD gravitated by 1.25-2.16 times and by 1.65-2.0 times ($p<0.01$ - $p<0.001$), respectively, more strongly towards BG-B carriers than towards the other BG ones. A fascinating example was the unique strong BG-B carriers' tropism towards double (RPv and RAv) RAAD ($p<0.05$ - $p<0.01$). On the other hand, there was no similar correlation concerning the general recurrence rate of dandruff (RDf). This is in accordance with the hypothesis that Df, an abridged version of seborrhoic dermatosis, is a particular kind of eczema aggravated by added commensal, *Mallassezia* yeast rather than an infection *sui generis* as considered by some authors (6,7).

Concerning the aggregations of RAAD among BG-B carriers such as RPv+RAv, RPv+RDf, and RAv+RDf it is evident that Df is closer to Pv than to Av, however, there is no correlation to the high antibody responses towards HSV2 and CMV. Apparently, epidemiodynamics of Pv directly correlates with BG-B population frequency rate. Our data from illustrate a statistically significant BG-B domination by two times ($p<0.001$) among Gypsy recruits of proven Indo-Asian origin (n=325) (in 32%) than that among Bulgarian ones (n=850) (in 16,2%). RPv incidence rate among Gypsy recruits is of 10.4% but among Bulgarian ones is of 6%. More interesting, generalized Pv (in one third of the cases on the face, too) is by 3.6 times more common among Gypsies (in 5.29%) than

Table 1. *Relationships between ABO genetic system and sexually transmitted infections (STI) and recurrent androgen-associated dermatoses (RAAD) during navy (DN) and after navy (AN)*

Blood group	n	Titres of STI		RAAD			RDf	Co.RAAD and RAAD+RDf		
		HSV2	CMV	DN and AN				DN and AN		
		≥ 1/20	≥ 1/20	RPv		RAv		RPv+RAv	RPv+RDf	RAv+RDf
				Start DN	Pm					
		n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%	n/%
O	157	21/13.4	26/16.6	30/19.1	0/0	27/17.2	65/41.4	7/4.46	25/15.9	10/6.36
A	217	72/33.2	104/47.9	25/11.5	6/2.76	45/20.7	58/26.7	6/2.76	7/3.22	8/3.68
B	108	52/48.1	63/58.3	26/24.0	6/5.55	37/34.2	13/13.5	19/17.6	14/13.0	0/0
AB	54	0/0	11/20.4	6/11.1	0/0	0/0	14/25.9	0/0	0/0	0/0

among Bulgarians (in 1.44%). A similar Pv dissemination patterns are typical of the Indian population (8.9) dominated by BG-B carriers (1-3). According to the first outpatient's attendance by students in East Africa, Pv is more common among men than among women as well as among Asian people than among individuals from other countries (10). Av sex-related tropism displays similar features (11,12).

Blood group-related population gene pool differentiation level and its attributes: complex immune and defense (health/disease) responses

All of these BG-associated diseases are multifactorial, i.e. a result from a complex interplay of polygenes and multiple environmental triggering factors. They are, however, not inherited in a simple Mendelian fashion and not associated with chromosomal abnormalities at all. Thus they are an area of supreme priority and challenge facing the contemporary medical genetics. Taking into consideration the basic laws of genetics formulated in 20th century (13-15) and seminal insights of Ajalla and Kriger (16), we recognize that there is another evolutionarily strong but missing key player that governs, subordinates and predetermines not only the well-known and suspected pathways of the pathogenesis and epidemiogenesis of the diseases but also the general outcome of health-disease responses' balance. This substantial common denominator represents an epigenetically assigned major phenotype-related population gene pool (PGP) differentiation level. The latter functions either as a harmonious co-adaptive genetic (allele and interlocus) equilibrium, or as a disharmonious interlocus interaction, the so-called epistatic genetic suppression. The first one represents the normal adaptation, i.e. health by itself, and is typical of CPMs and their BG-B carriers. The second one represents the disadaptation, i.e. the pathological states or diseases by themselves are typical of TVHs as an abridged version of Western hybrid societies (WHSs). That is why WHSs turn into the most powerful generators of chronic, refractory and recurrent diseases, highly pathogenic flora carriers, infections and allergies.

The concrete PGP balance level was assessed through its sounding via some complex defensive traits such as multiple (triple or double) infectious allergic resistance (MIAR) or susceptibility (MIAS) to grippe and grippe-like conditions (GGLC), RTp, and allergy. The individual and population genetic make-up as causative factor possesses a predetermining g role for human defence and immune homeostasis through the interactions between the genes. These interactions are epigenetically (evolutionarily) assigned via concrete type and intensity of the population mating as compatible inbreeding or abnormal urbanogenic interbreeding and intrinsically related to it genetic suppression. It is due to the fact that in ancient times, BG-B carriers suffered a much more severe pressure of the selection (infectious mortality) which enhanced PGP harmonization and resulted in better defense and immune capacity.

Measurements of stability and power of basic and specific cell-mediated immunity (CMI) were accomplished by intradermal testing with Candidin (C), phytohemagglutinin (PHA) and Trichophytin (T). Individuals with positive delayed skin allergy, the so-called CMI towards C and PHA at one and the same time were classified as such with co-operated basic CMI (Co.BCMI) while those with marked Co.CMI and T were considered as such with co-operative specific CMI (Co. SCMI) (Table 2). BG-B carriers who survived the plague and smallpox epidemics responded not only with a higher Co.CMI ($p<0.01$ - $p<0.001$) but also with an implied general biological compensatory reflex, i.e. with accelerated fertility. This higher fertility goes with our data showing that the parents of the examined BG-B carriers have more than two children in 75% of the cases while among those of the other blood groups this occurs only in 40-50% of the cases ($p<0.001$). Hence one

Table 2. *Relationships between ABO genetic system, defense polymorphism and co-operated basic cell-mediated immunity (Co.BCMI)*

Blood group	n	Co.CMI		Defense polymorphism				PGP differentiation level
				MIAR		MIAS		
		Co.BCMI C+PHA	Co.SCMI C+PHA+T	triple	double	triple	double	general outcome H/D responses' ratio
		n/%	n/%	n/%	n/%	n/%	n/%	%/%
O	157	82/52.2	26/16.7	13/8.28	82/52.2	31/19.7	31/19.7	60.5/39.5
A	217	108/49.8	10/4.6	97/44.7	42/19.3	18/8.29	60/27.6	64/36
B	108	72/66.6	18/16.6	36/33.3	36/33.3	18/16.6	18/16.6	66.7/33.3
AB	54	23/42.6	0/0	18/33.4	12/22.2	12/22.2	12/22.2	55.6/44.4

might suppose that hyperandrogeny which determined this phenomenon was coded by evolution in the genome of BG-B carriers. There is no doubt that the intense sexual potency and fertility in Eastern societies resulted in a huge population density and, logically, sufficient sex was given the status of a cult (see e.g. Camasutra and other treatises).

CONCLUSION

Altogether, through a10-year longitudinal monitoring and a multitheoretical scanning of the processes (17) we establish a significant link between BG-B carriers and some STI and implied evolutionary gene-encoded hyperandrogeny and fertility, respectively. This is confirmed by a striking relationship between BG-B and long-known RAAD such as Pv and Av. We hold the opinion that ABO group polymorphism is the result from a powerful pressure of the selection by a selective BG-related infectious mortality (18-20) in ancient times. We found a confirmation of the ideas and hypotheses not only in the dramatic domination of BG-B and high sexual activity among Eastern societies but also in publishing data about a strong domination of some RAAD among them. The BG-B carriers having passed through the deadly epidemics are, logically, owners of a relatively higher Co.BCMI and Co.SCMI homeostasis and PGP-related harmonization due to a systematic tinkering and coining of evolution. Long-term prospective research is needed to better elucidate these essential topics.

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Commentary

A LINKAGE OF MIND AND BRAIN: SIR JOHN ECCLES AND MODERN DUALISTIC INTERACTIONISM

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Our minds, constituted by conscious experiences, are both the most familiar and most mysterious aspect of our lives. Despite the large amount of clinical evidence suggesting an intimate relationship between the brain function and the mind, the nature of this relationship remains poorly understood. In this Commentary we discuss some of the problems faced by the classical mind-brain identity theory and explain how the quantum dualistic interactionism proposed by Sir John Eccles could resolve these problems. ***Biomed Rev* 2011; 22: 81-84.**

Key words: mind, conscious experience, brain, materialism, dualism, quantum mechanics

MIND-BRAIN PROBLEM IN CLASSICAL PHYSICS

Our *minds* are constituted by subjective *conscious experiences* through which we access ourselves and the surrounding world. Examples of conscious experiences are the pain of the toothache, the smell of the rose, or the perceived blueness of the blue sky. Large amount of clinical evidence suggests that there is an intimate relationship between the brain function and the mind, because discrete lesions in the brain could impair our cognitive abilities and change the way we experience the world (1). Thus it is clear that our minds should depend somehow on the brain states. Nevertheless, this is the best that classical materialism can say on the subject. Asking further questions within the framework of classical materialism leads to paradoxical conclusions, which contradict experimental observations and our common sense. Several important questions are:

(i) How the mind can affect the physical brain?
(ii) How can we have free will if we cannot choose between several alternatives?
(iii) How can we be responsible for our actions if our choices are predetermined?
(iv) Why the brain processes produce any experiences at all?

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(v) Why during anesthesia the brain processes do not produce experiences?

According to classical materialism all brain processes can be reduced to molecular chains of causes and effects, which although immensely complex operate as a deterministic clockwork mechanism (2). Because the material world is causally closed, if the brain states produce conscious experiences, then these experiences cannot be causally effective. According to the evolution theory however, something that is not causally effective cannot lead to evolutionary advantage and cannot be selected by natural selection. The *mind-brain identity theory* is an attempt to resolve this problem. It postulates that the mind states *are* brain states, which makes the mind states causally effective and capable of providing evolutionary advantage. The proposed solution however comes at a dear price. Because the mind-brain identity theory is based on classical materialism, the brain dynamics is completely deterministic. Therefore, the mind-brain choices are predetermined and there is no room for free will or choice making. If we cannot make choices we cannot be morally responsible for our actions, no more than a falling stone is morally responsible for breaking one's leg. Furthermore, by attributing mental properties to the material brain states, it is not easy to explain why there are brain processes that do not produce conscious experiences. For example, during general anesthesia, the conscious experience is safely erased, yet, an experimentalist can flash light into the eye of an anesthetized animal and still record evoked potentials from pyramidal neurons in the primary visual cortex (3). If mind states are identical to brain states, it should be impossible to turn them on or off using anesthetics, because the brain states always remain brain states.

In a Dance Round in this volume of *Biomedical Reviews*, it was suggested that the mind-brain identity thesis applies to "events of sensations" and "distributed processes in the brain" (4). Such a definition however is vacuous. There are various distributed processes in the brain that do not produce conscious experiences. Furthermore, the events of sensations are by definition mental and to say that conscious experiences are associated with brain states or processes that produce sensation is circular. One might be tempted to identify the events of sensations with the electrophysiological processes of inputting sensory information from the sense organs to the cortex, but certainly the latter processes are not always associated with sensory experiences as can be seen from the provided example with recording of visually evoked potentials from pyramidal neurons in the primary sensory cortex of anesthetized animals (3). Experiments with similar results were performed also in anesthetized human patients using auditory stimulation and EEG recording (5).

MIND-BRAIN PROBLEM IN QUANTUM PHYSICS

The ease with which one can construct arguments contradicting experimental observations and our common sense does not solely result from the postulated mind-brain identity. Instead, the roots of the problem lie within the causally closed deterministic structure of classical materialism. Fortunately, in 1920's with the birth of quantum mechanics, which describes the behavior of elementary physical particles, it became clear that classical materialism is not a correct description of the physical world. The behavior of elementary physical particles was found to be inherently indeterministic so that one cannot predict exactly the future state of an individual particle, only the probability with which given future state could occur. The dynamics of individual quantum particles is governed by a *wavefunction* ψ, which is obtained by solving the Schrödinger equation (6). The wavefunction ψ depends on the *boundary conditions* that include the initial position of the particle and its environment. If one knows the boundary conditions, one can solve the Schrödinger equation and see how the wavefunction ψ evolves in a deterministic way through space and time. What makes the theory extraordinary is the fact that the wavefunction ψ of a quantum system *cannot be observed*. The wavefunction ψ is sometimes described as *pre-probability* because it is the square of the wavefunction $|\psi|^2$ that gives the *probability* to find the quantum particle at a certain position at a certain time (7). Curiously, the wavefunction ψ is defined in a non-material multi-dimensional Hilbert space, in which each axis represents a possible state of the quantum particle. If the quantum particle can be in infinite number of possible states, the Hilbert space is infinite-dimensional (8,9). The indeterminism in quantum mechanics stems from the fact that when the particle interacts with other particles only one of the possible states is *actualized*. After the interaction the new position of the particle and its new environment will serve as new boundary conditions for solving the Schrödinger equation and obtaining new wavefunction ψ. The new wavefunction ψ will evolve again in a deterministic way through space and time until the next interaction, during which one of the multiple possibilities will be actualized with the square of the new wavefunction $|\psi|^2$ providing the probability to find the quantum particle at a certain position at a certain time. Undoubtedly such behavior of the elementary physical particles may look disturbing and incomprehensible at first encounter. Richard Feynman, who won the 1965 Nobel Prize in Physics for his work on quantum electrodynamics, repeatedly stressed that *nobody understands how the real world can be like this* (10). And when asked whether he likes the weird quantum laws, he answered that it is irrelevant what he likes or dislikes, the important thing is how the physical world is. The predictions of quantum theory are currently confirmed with such a high degree of precision

that there is little doubt that quantum theory is the correct physical description of the world. Sir John Eccles, who won the 1963 Nobel Prize in Physiology or Medicine for his work on the synapse, was one of the first who understood the importance of quantum mechanics for resolving the mind-brain problem, and proposed that mental events can cause brain events analogously to how the wavefunction ψ determines the probability $|\psi|^2$ for a given quantum particle to be found at a certain position at a certain time (11). Indeed if one considers the wavefunction ψ of a quantum particle as a non-observable mental state of pre-probabilities and the actualized position in space and time as an observable material state, then the dualistic interactionism proposed by Sir John Eccles is consistent with the modern vision of what the physical world is. Interestingly, in quantum mechanics a system of interacting quantum particles can have a wavefunction ψ satisfying the Schrödinger equation, whereas the individual particles in the composite system may not have individual wavefunctions ψ. Such state does not have a classical analogue, and is referred to as a *quantum entangled state* (9). If the human mind could be described by a global wavefunction ψ that results from quantum entanglement of different brain subcomponents, each of which does not have an individual wavefunction ψ, then one sees that it would be incorrect to say that the mind represented by the global wavefunction ψ is composed of simpler minds. Furthermore, in dualistic interactionism general anesthesia and loss of consciousness should not be understood simply as turning off the mental states. Instead following the analogy with split-brain patients, who have two independent minds in each cerebral hemisphere due to surgically severed corpus callosum (12), one can assume that anesthetic molecules disrupt the interactions and the quantum entanglements between individual brain molecules leading to zillion split-brain subcomponents, each of which possessing the simplest mental states available to individual quantum particles. Thus consciousness would not emerge out of nothing and would not disintegrate down to nothing. In its essence the dualism affirms that consciousness can have different level of complexity with simplest mental properties attributable to all elementary quantum particles.

The conceptual difference between the dualistic interactionism based on quantum mechanics, and alternative versions of dualism based on classical materialism, such as epiphenomenalism or psychophysic parallelism, is shown in Figure 1. Because quantum laws are indeterministic it is possible to construct a theory in which the mind and brain states interact: the brain state produces deterministically a mind state after which the mind state makes an indeterministic choice from multiple possible alternatives and selects a future brain state. This is in stark contrast with classical materialism where the brain states obey causally closed deterministic laws. In epiphenomenalism, the brain states produce the mental states but these mental states are unable to affect causally the brain states. The epiphenomenal mental states are just useless spectators. Even more ridiculous is the psychophysical parallelism where the brain states and the mental states do not interact at all, but which are set in a pre-established harmony created by a divine creator.

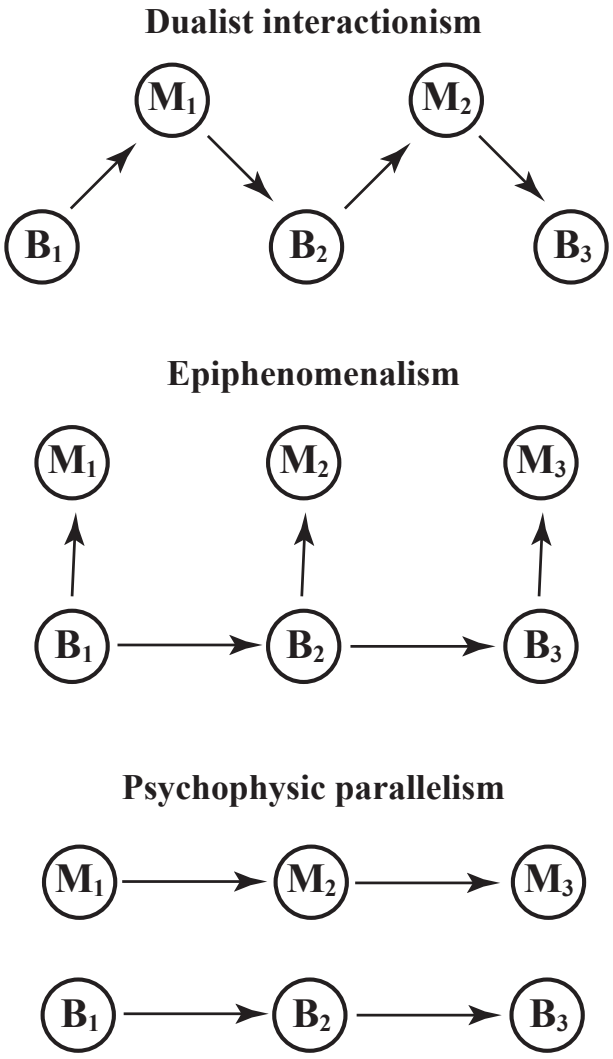

Figure 1. *Varieties of dualist causal interaction. Dualist interactionism affirms that brain states produce mental states, which may in turn select the future brain states. Epiphenomenalism affirms that brain states produce mental states, which do not affect causally the brain states. Psychophysic parallelism affirms that brain states and mental states do not interact with each other but are set in a pre-established harmony created by a divine creator. B_1, B_2, B_3, brain states; M_1, M_2, M_3, mental states; arrows indicate the direction of the interactions.*

CONCLUSIONS

In a Dance Round in this *BMR* volume, it was stated that dualism is "undeniably suspended with the data of neuroscience" and that "besides Sir John Eccles more than forty years ago, there is no other scientist from the field of empirical science to any more hold the position of dualism" (4). While any form of dualism based on classical materialism can be considered refuted, it is not true that dualism in general is refuted and that no present scientist holds such position. Currently, an increasing number of neuroscientists and physicists think that quantum theory could support dualist interactionism along the lines envisaged by Sir John Eccles. Attestation for that are the plethora of recent models based on the nanoscale organization of the neurons in which the mind states can affect the brain through quantum effects (13-19). Some support for the feasibility of such quantum models has been already provided by the experimental verification of quantum tunneling for hydrogen transfer in enzyme-catalyzed reactions (20-22), quantum quasi-particle assisted folding of proteins (23) and microsecond quantum coherence in the retina of some birds that are able to navigate by sensing Earth's magnetic field (24). The dualist interactionism envisaged by Sir John Eccles is not only viable, it could be our best bet for a physical theory of consciousness.

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Response

TOWARDS PRAGMATIC AND FUNCTIONAL UNIT OF MIND-AND-BRAIN

IN RESPONSE TO DANKO GEORGIEV'S "A LINKAGE OF MIND AND BRAIN: SIR JOHN ECCLES AND MODERN DUALISTIC INTERACTIONISM"

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Consciousness is an enigma, perhaps the greatest enigma of philosophy of science. It can be described as a multilevel phenomenon, where transition (from unconsciousness to consciousness) is not a compromise OFF / ON in neuronal activity, but involves a complex change in nerve function, which is mediated by the environment (1). For the analysis of consciousness, the Australian philosopher David J. Chalmers distinguishes the easy problem of the hard problem of consciousness. The easy problem to analyze issues such as discrimination between sensory stimuli, the integration of information to guide behavior, verbalization of internal states, the integration of sensory information with past experience, how to focus attention, and what distinguishes waking from sleep. On the other hand, the "hard problem" of consciousness is to explain how the physical brain gives rise to consciousness. This analysis deals with the latter. ***Biomed Rev* 2011; 22: 85-89.**

APPROACH TO THE THEORY OF MIND-BRAIN IDENTITY

Analysis the hard problem of consciousness begins by question how the mind (thoughts, feelings, etc) can be explained in terms of what we know *as matter*? The "Identity Theory" anticipated by the British philosopher Ullin Place to "Is Consciousness a Brain Process" in 1956, and John Smart with "Sensations and Brain Processes" in 1959, says that perceptions and consciousness are physical processes in the brain, just as the rainbow is a physical process in the atmosphere (appearance of a spectrum of different light frequencies on the sky) (2,3).

Smart and Place propose that mental processes are identical to brain processes, if and only if, mental states are something material - but not behavior, that should be assumed is identical to the internal physical states (4). But if the mind is ultimately matter, then how is it built? They identify conscious states with brain states, which raises the question: where interaction occurs between these states? Since conscious states and physical states are the same thing, there is no need to interact. They "become" together.

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Joy, for example, is a physical state (because the body produces dopamine, endorphins, oxytocin and serotonin) and conscious (produces shiny inner state) that causes some actions that are both states, physical and conscious (general welfare is generating high levels of energy and a strong willingness to constructive action, which the person who experiences it, reveal in their appearance, language, decisions and actions) (5). Related in this context, the concept of identity makes the conscious brain "primary and reflective" will be equal to mind.

Danko Georgiev in his Commentary in the present volume of *Biomedical Reviews* (6), states that it has been suggested in our *Dance Round* "… that the mind-brain identity thesis applies to "events of sensations" and "distributed processes in the brain". Such a definition however is vacuous. There are various distributed processes in the brain that do not produce conscious experiences. Furthermore, the events of sensations are by definition mental and to say that conscious experiences are associated with brain states or processes that produce sensation is circular" (6,7).

On the contrary, Capra (8) distinguishes two types of consciousness: (*i*) primary consciousness, which is the direct experience of perceptions, sensations, thoughts and memory contents and images, dreams and daydream, and (*ii*) reflective consciousness that is the conscious experience per se. The latter is necessary for self-consciousness, which involves realizing being a unique individual separate from others, with a personal and future history. The reflective consciousness includes the process of integration, namely, to observe one's own mind and its functions, in other words, know we know.

While it is true that the conscious experience in the normal adult human consciousness involves both primary and reflective consciousness, in this context we should argue that conscious experience is not always associated with brain states or processes that produce sensations. Therefore, the statement "to say the events of sensations are by definition mental and to say that conscious experiences are associated with brain states or processes that produce sensations is circular" is not always correct.

According to the American philosopher David Lewis in his *An argument for the identity theory*, and the Australian philosopher David Malet Armstrong the theory of identity might be extended to all that is mental, "not only awareness and perceptions: all states mental are physical states in the brain, all mental processes are brain processes" (9). Therefore, the mental processes are playing a "causal role": a mental state (e.g. happiness) can cause behavior, and it does because it is a brain state.

According to Hillary Putnam's argument not only humans but also for example, amphibians, or aliens if any, may have feelings. However it seems unlikely that all beings with the same feelings are in the same brain state: if this is not the case, then feeling can not be identical to a particular brain state. On this basis he argues that the identity theory lacks empirical foundation (10). "Even if it happens that a particular brain state is bound in each case with a single "mental state" in a person, the absolute correlation between mental state and brain state does not necessarily mean that both states are in fact one and the same" (10).

Further query is whether a state of mind (which is a brain state) is caused by and/or causes behavior. For example, the rainbow is not the only physical process of the atmosphere, but is caused for this physical process (the sun's rays suffer a breakdown when crossing water droplets contained in the atmosphere). Then, a mental state is defined by its causal role: what caused the mental state, which produced in turn for this mental state, and its relationship to other mental states. As we can see, consciousness is not a passive phenomenon in response to stimuli, but an active process of interpretation, construction and re-interpretation of data (11).

THE PROBLEM OF EVOLUTIONARY CAUSALITY.

According to the theory of evolution, things that are not causally effective not lead to evolutionary advantage and can not be perpetuated by natural selection (12). The theory of mind-brain identity is an attempt to resolve this issue, and argues that mental states are brain states, which make mental states are causally effective and capable of producing evolutionary advantage (12). This type of causality exemplifies very well the behavior of elementary physics particles that are governed by the wave function as it has been demonstrated in the *BMR* Commentary by Danko Georgiev (6 and references therein).

The individual particle can not have a single wave function because wave function can be found in an infinite number of possible states in the multidimensional-material space of Hilbert, where each axis represents a possible state. Ie, the particle may be in infinite states, and hence the Hilbert space is of infinite size. At this time, the particle is an unobservable mental state, described as "the time before being detected probabilistically."

When the individual particle interacts with other particles, each of these "possible states" is selected to be updated (determined), ie a system of interacting particles creates a wave

function (which depends on the boundary conditions, which include the initial position of the particle and its environment), so the new position of the particle and the new environment will serve as a condition for determining the position of the particle through the Schrodinger equation.

Sir John Eccles suggests that mental events are caused in the brain in a way analogous to how the wave function makes a particle being in a particular position at any given time, and asserts that consciousness can have different levels of complexity formed from simple mental properties attributed to quantum particles. As the quantum laws are indeterminate, it is possible to construct a theory in which the mind and brain states interact: brain states deterministically produce a mental state, mental state makes a choice of multiple indeterministic alternatives and selects a mental state in the future. This argument would explain why not all brain states cause mental states, why there are mental processes that do not accompany any behavior, and why there are mental states that seem to involve other mental states. Based on the foregoing, we affirm that interactionist dualism presented by Georgiev is not necessarily a dualistic view in the classic sense, but should be considered rather as a new type of non-reductive physicalism, which is to argue that it is not necessary to postulate for the soul or mind a second metaphysical entity. For this position, the soul or mind are physiologically expressed or embodied in our person. This proposal is intended to reconcile our views on body, soul, mind and brain. To that end we agree with the basic requirements of the presented model.

VALIDITY OF SCIENTIFIC RESEARCH OF CONSCIOUSNESS

In the research of consciousness, the validity has two main tasks: (*i*) the development and refinement of theoretical approaches of consciousness , which we have discussed in the preceding paragraphs, and (*ii*) the construction of instruments and procedures for measurement of the consciousness, which we discuss in the next part.

A reliable measure is measuring a construct consistent over time, people and situations, it is also one that measures what it purports to measure (13). A measure can be reliable without being valid. Therefore, the reliability is necessary but not sufficient to prove the validity (14). In research on consciousness, as in other studies, internal and external validity seem to contradict each other (14,15). To get an experimental design you have to control for all interfering variables. That's why you often conduct your experiment in a laboratory setting. While gaining internal validity (excluding interfering variables by keeping them constant) you lose ecological or external validity because you establish an artificial lab setting. On the other hand with observational research you can't control for interfering variables (low internal validity) but you can measure in the natural (ecological) environment, at the place where behavior normally occurs. However, in doing so, you sacrifice internal validity (14,15).

The apparent contradiction of internal validity and external validity is, however, only superficial. The question of whether results from a particular study generalize to other people, places or times arises only when one follows an inductivist research strategy. If the goal of a study is to deductively test a theory, one is only concerned with factors which might undermine the rigor of the study, i.e. threats to internal validity (14,15). One particular implication entailed from the interactionist perspective as exposed above is related to the poor validity of that model when applied for instance to the cases of shared psychiatric and neuroscience taxonomy (16).

OUR CONTRIBUTION

We propose a change in concept between the "mind-brain dissociation", for "pragmatic and functional unit mind-brain", without the pretense of the eliminativist reduction. Here we argue that interactionist dualism proposed by Karl Popper and John Eccles, where mind, though different from the material brain, interacts with the brain and depends upon it, is based on the principle that mind and matter are "substances" different from each other (material and immaterial substance), affirmation that for several centuries has been ruled out by face with the problem of the need for the existence of a place of interaction between "such substances" (17).

By mentioning that the interactionism was refuted for over 40 years, we refer that before reading the work of Eccles - *The Understanding of the Brain* (1973) - most neuroscientists intuitively had perceived a gap between mental and physical phenomena, which caused dualistic views that were replaced by purely materialistic positions, trying to better respond to questions such as where are performed the mental processes (17).

Moreover, we underline that one of the shortcomings of dualism was to propose the existence of mind-body like two supreme principles, uncreated, contours, independent, irreducible and antagonistic, and maintain the separation of an intelligible world of ideas, eternal, immutable and necessary, of the sensible world of matter, temporal, mutable and corruptible (soul encased in a body) delineating two orders

that claimed to be "essentially different". We believe this radical distinction between ideal and real being, normality and disease, between well thinking, and the thinking like the influences of the individual surroundings, restricts freedom of conscience and freedom for diagnosis, from the point of view that conscious activity assessment must be holistic and multi-influenced (17).

Although Karl Popper in his model of "demarcation of science" takes the psychoanalysis as an example to demonstrate the principle of falsifiability and qualifies as pseudoscience, he argues that it is rational and valuable as well. Remarking that a theory may well be meaningful without being scientific, and as such, "significance criteria" may not necessarily coincide with a "criterion of demarcation", proposing that falsifiability is a property of statements and theories, and in itself is neutral.

Returning to the dilemma between dualism and materialism, we speak in favor of materialism as a driver of a new philosophy that is proclaimed as a science by neurosciences and psychiatry, called to find out the truths that help with life skills and values guide, offering new ways to validate research and new methods to know the truth.

Similarly, we agree that the cure is independent of the method, but sometimes is not independent of conditions under which the method of healing is applied. We propose that stereotypes hinders diagnosis, we believe it is necessary to give answers to the reasons that hinder the lack of recognition, care and access to treatment for psychological problems in real people, so we argue that to improve the practice of psychiatry, the scientist needs to forget the point of view of all proposals that are reductive: mental states can not be reduced to behavior, brain states or functional states, but must be referred depending on the complexity of the individual (18).

This supports the eliminativist view that considers possible to reject the mind-body problem, because we think that it is wrong to ask how they fit the mental states and biological factors. We suggest that more should be accepted that human beings can be described in various ways: for example, mental or biological terms (19).

On the other side, from a purely scientific point of view, we consider that the "hard problem" of consciousness, namely, the physical processes that give rise to brain consciousness must be based on the special quality of what we call "mental", where there must be something that is, for that body, from the point of view that constitutes the first person perspective. This is critical because it allows us to see the deficiencies in other definitions of philosophy of mind; such as it is the dispositional mental or mental is functional, and so on. If it were simply a mental gear in a chain of more functional processes: neurophysiological, chemical, mechanical, etc., then any being that had implemented an algorithm and the necessary parts could perform these functions, such as a robot (20).

We also argue that materialism is far from being able to answer the "hard problem", as the subjective experience of consciousness means, ie the opposite of objectivity. In some writings of consciousness is considered synonymous with mind. However the mind includes unconscious mental processes (20).

We argue that consciousness is not a passive phenomenon in response to stimuli but an active process of interpretation and construction of external data memory, relating them in this context, we refer to "type of identity," which makes the conscious brain (primary and reflective) will be equal to mind. It also refers to Anglo-Saxon literature that uses two different words, in Spanish is often translated as consciousness. The first is "awareness", which translates into apperception; the second is "consciousness", which translates into consciousness. This distinction is important because there is the English expression "unconscious awareness" which translates to "unconscious apperception." Some authors define apperception as a state in which we access to information which can be used to control behavior (1, 20).

On the other hand, in that current alternative version called the "pragmatic and functional unit mind-brain," we propose that if the mind is the function performed by the brain, mental states would then be functional states, only if mental states are states functional regardless of specific brain states that occur. This supports the proposal by John Searle who insists that we must keep in mind that consciousness is caused by brain processes, but can not be reduced to these processes because it is a phenomenon of "first person", or subjective, while the brain processes are phenomena of "third person", i.e. objective ones (21,22).

We also address the evolutionary argument against epiphenomenalism, that if consciousness is a sequence of conscious mental states and each of these states experiences some specific content, then consciousness must have had an evolutionary purpose. We refer at this point to Nicholas Humphrey, who claims that *it is conscious to have feelings, as opposed to perceptions*. Although in evolution some states are full of affection (feelings) and other neutral with respect to the feelings (perceptions), both are enhanced by natural selection (mental Darwinism) (23).

Finally, we maintain our position on pragmatism of function, in order to help psychiatry to improve the validity of diagnosis of mental disorders, considered as the product of brain activity.

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Third International Symposium on Adipobiology and Adipopharmacology (ISAA)

25 – 27 October 2012, Burgas, Bulgaria

Organized by the Bulgarian Society for Cell Biology in collaboration with Municipality of Burgas, International Federation for Cell Biology, and Bulgarian Respiratory Society

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LETTER FROM THE CHAIRMAN

Dear Colleagues,

We have to meet and work together,
and together to believe - cry out, fall down,
Because it was we who suffered for the magic of the greeting.
The great significance of the plain shaking hands.

Hristo Photev (1934-2002),
From *Liturgy for the Dolphins*

It is our pleasure to invite you to Burgas for the Third International Symposium on Adipobiology and Adipopharmacology (ISAA), 25 - 27 October 2012. The Symposium offers a prestigious forum for the presentation of novel basic, translational and clinical research in the field. Many key scientists expressing very high BMI (Brain Mass Index) have already confirmed their participation as speakers, and several additional speakers will be selected from the submitted abstracts, to ensure a state-of-the-science symposium. Adipobiology investigates the role of adipose-secreted bioactive molecules (adipokines and others) in the pathogenesis of obesity and related cardiometabolic diseases (atherosclerosis, hypertension, type 2 diabetes mellitus, metabolic syndrome) and also diseases of the liver, ovary, bone and nervous system. Likewise, adipose-targeted pharmacology may create novel therapeutic approaches for these diseases.

We invite you to join us to create a stimulating scientific, educational and friendly event. I personally look forward to welcoming you to Burgas, my native city. Together we will capture the scope and depth of the exciting field of adipobiology and adipopharmacology. And friendship (friendomics).

On behalf of the Bulgarian Society for Cell Biology
George N. Chaldakov, MD, PhD

PRELIMINARY PROGRAM

The format of the Symposium will include 5 oral sessions, each session initiated by the state-of-the-science (SOS) speakers, followed by speakers selected from submitted abstracts. Venue: Cultural Center, Burgas.

Cultural Center, Burgas, Bulgaria

Thursday, 25 October 2012

Registration: 3 – 6 PM
Fee (payable *in situ,* in Burgas): 200.– Euros
For Bulgarians: 70.– BGN
For students: no registration fee.

Registration fee includes (i) participation in all scientific sessions, (ii) Symposium bag including a copy of volume 4, 2012 of *Adipobiology* (an International Journal of Adipose Tissue in Health and Disease) and of a book entitled *SOS for Homo Obesus. Science, Adipoeconomics, and Adipopolitics*, (iii) opening reception, (iii) lunch on Friday 26 October and Saturday 27 October, (iv) gala dinner, (v) trip to Nessebar, an ancient and unique city along the Black Sea, 40 km North from Burgas, and (vi) closing dinner.

Opening reception and candle light lecture: 7 PM

Friday, 26 October 2012

Opening: 8:30 AM
Sessions, including coffee break: 9 AM – 1 PM
Lunch: 1:15 – 2:15 PM
Sessions, including coffee break: 2:30 – 7 PM
Gala dinner: 8 PM

Saturday, 27 October 2012

Opening: 8:30 AM
Sessions, including coffee break: 9 AM – 1 PM
Lunch: 1:15 – 2:15 PM
Trip to Nessebar via Pomorie, another ancient city, where *Vitis vinifera* is traditionally trans-differentiated into high-quality red wines.
Closing dinner: 8 PM

ABSTRACT AND MANUSCRIPT SUBMISSION INFORMATION

Abstract submission deadline: 25 August 2012
Manuscript submission to *Adipobiology* volume 4, 2012: deadline 5 August 2012
Registration: open including *in situ* at the Registration desk in Burgas

Abstract/manuscript submission to:
chaldakov@yahoo.com
atontchev@yahoo.com

Please organize your abstract to include title (in capitals), authors/affiliations, and e-mail address of the corresponding author, followed by 2 double-spaced pages, font 12, Times New Roman, written in complete sentences, without subheadings, including 3-4 References if required, following the Instructions to Authors (see below). All abstracts will be peer-reviewed and ranked on the basis of scientific merit. The Organizers will use these rankings to develop the final program of the Symposium.
The Abstracts will be published in *Adipobiology* 4, 2012.

Please organize your manuscript for *Adipobiology* 4, 2012 following the Instructions to Authors (see Instructions for *Biomedical Reviews*, pages 95-96).

Advertisements (one page) of companies producing drugs, biomedical products and/or laboratory instruments will be published in *Adipobiology* 4, 2012. A first come-first served advertisement assignment begins on 1 June 2012.
For more details and/or for sponsorship opportunities kindly contact:
atontchev@yahoo.com
chaldakov@yahoo.com

DATES TO REMEMBER

Abstract submission deadline: 25 August 2012
Manuscript submission deadline: 5 August 2012
Opening reception – Candle light lecture: Thursday, 25 October 2012, 7 PM
Gala dinner: Friday, 26 October 2012, 8 PM
Closing dinner: Saturday, 27 October 2012, 8 PM

Biomedical Reviews
An International Journal of Cell Biology of Disease

INSTRUCTIONS TO AUTHORS

General Information

***Biomedical Reviews (BMR)* is an official Journal of the Bulgarian Society for Cell Biology (BGSCB).** *Biomedical Reviews* publishes articles focused on updated knowledge in disease-oriented molecular cell biology. The following types of contributions are published:

(i) *Review* articles summarize state-of-the-science on a given biomedical topic. Contributors to Reviews are, in general, invited by the Editors and the Editorial Board, but idea proposals are welcome. Potential authors are invited to submit a letter of interest to the Editor. Proposals should contain an outline of the contents, including an abstract, a list of 20 relevant articles including from the proposer's own research, and a brief statement on why now is a good time to review the topic in question. Reviews will not be accepted for editorial processing unless pre-approved for submission.

(ii) *Dance Round* articles are short, position papers that are intended to focus observations that seem to point the field in a new direction, to give the author's personal views on a controversial topic, or to direct soundly based criticism at some widely held dogma in biomedicine.

(iii) *Topic issues* aimed at clustering contributions to a biomedical cutting edge within one issue. Guest Editors of such issues are, in general, invited by the Editors and the Editorial Board, but idea proposals are welcome.

Multiple-part papers are discouraged. Manuscripts submitted under multiple authorship are reviewed with the understanding that all listed authors concur in the submission and that the final manuscript has been approved by all authors. If accepted, the article shall not be published elsewhere in the same form, in either the same or another language, without the written consent of the Editors and Publisher.

Organization of the Manuscript

Text of manuscripts must include an abstract, an introduction, followed by the body of manuscript, a conclusion, acknowledgments, a list of references, and, if available, figure legends, and tables. Pages should be double-spaced, Times New Roman should be used throughout, sized at 12 pt. The text file should be submitted in either Word or PDF format to the Editor at *chaldakov@yahoo.com*

Title Page

Please organize a title page as the first page of the text file to include the following:

- Title
- Abbreviated title
- Authors and their affiliations.
- Corresponding author with complete address, including telephone and fax number, and an e-mail address
- Number of figures, tables and pages
- Up to 6 key words that do not appear in the title

Abstract

The abstract (typically about, although not strictly restricted to, 250 words) should provide a concise summary of the data to be reviewed and major conclusions of the study. It should be written in complete sentences, without explicit subheadings. Citing references should be avoided.

Introduction

The introduction should briefly indicate the background of the topic, and explain the objectives of the paper.

Captions should be used within the body of the manuscript to outline important points.

Conclusion

This section should be as concise as possible and should summarize the data discussed in the paper, and possibly, should contain a statement of their significance and future biomedical implications.

References

Only published and "in press" references should appear in the reference list. The latest information on "in press" references should be provided. Any "in press" references that are relevant for reviewers to see in order to make a well-informed evaluation should be included as a separate document text file along with the submitted manuscript. "Submitted" references as well as personal communications should be cited only in text. Authors are responsible for all personal communications and must obtain written approval from persons cited before submitting the paper to the *Journal*. Proof of such approval may be requested by the *Journal*.

References should be each numbered, ordered sequentially as they appear in the text, and cited in parentheses: "text (1)". In case of with multiple references, these should be cited starting from the smallest number: "text (1-3)". In the list of references, papers should be listed numerically. The name (surname first) of the author(s) should be followed the by the full title of the paper as it appeared in the original, the source of the reference, together with the year, volume number, and the first and last pages. If the author list for a paper exceeds 6, *et al* (in oblique font) should be added after the sixth author. References to web-only journals should also provide URL in full or DOI if known. Book titles are in oblique font with all main words' first letter being capitalized. The following illustrates the format to be used:

- **Journal article**
  Iwamoto Y, Koide H, Ogita K, Nishizuka Y. The protein kinase C family for the regulation of cellular functions. *Biomed Rev* 1992; 1: 1-6.
- **Book**
  Author A. *Book Title*. Publisher name, 2000.
- **Chapter in a book**
  Author A. Chapter title. In: Author A, editor(s). *Book Title*. Publisher name, 2000; 1-10.

Abbreviations of journal titles should follow those listed in the *Index Medicus*. Responsibility for the correctness of the references lies with the author(s). After manuscript revisions, authors should double check that all in-text citations are in the reference list and that all references on the reference list have at least one corresponding in-text citation.

Illustrations
All figures must be cited in the text and numbered consecutively (Fig. 1, Fig. 2, etc.). Each figure should be submitted as a separate file. For vector graphics, EPS (Encapsulated PostScript) files are the preferred format. TIFF (Tagged Image File Format) is the recommended file format for bitmap, greyscale and colour images. When supplying TIFF files please ensure that files are supplied at the correct resolution:

- line artwork = minimum of 1000 ppi
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- combination artwork (line/tone) = minimum of 500 ppi

Authors should be aware that using color figures will incur an additional charge for color in their reprints.

Preparation of Tables
Each table should be double-spaced with an explanatory title and will appear at the end of the text of the manuscript. All tables must be cited in the text (e.g. "Table 1").

Figure Legends
Each figure should be accompanied by a title and an explanatory legend. The title should be part of the legend and not lettered onto the figure itself. Legends should be concise.

Abbreviations
Use abbreviations if a term appears three or more times. Spell out all abbreviations at first occurrence, and then introduce them by placing the abbreviation in parentheses. The metric system should be used for all volumes, lengths, weights, etc. Temperatures should be expressed in degrees Celsius (centigrade). Units should conform to the International System of Units (SI).

PROOFS, OFFPRINTS, MISCELLANEOUS

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Contents

Front cover: *Illustration of the bone marrow, blood, and brain tissues. CXCR4+ HSC are anchored to SDF-1+ stromal cells of the bone marrow. The administration of G-CSF combined with CXCR4-antagonist plerixafor leads to hypermobilization of endogenous HSC into the blood circulation along with stimulation of neutrophilic granulocytes. The blood-borne HSC are recruited to the injured brain by chemotaxis. Inside CNS, bone marrow cells improve neuroprotection, plasticity, and brain repair.* From Milena Penkowa's review, pages 1-6.