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Natural Secretory Products of Human Neural and Microvessel Endothelial Cells

Implications in Pathogenic "Spreading" and Alzheimer's Disease

Yuhai Zhao, Jian-Guo Cui, and Walter J. Lukiw*

Neuroscience Center of Excellence, Louisiana State University Health Sciences Center, New Orleans, LA

Abstract

Neurons, glia, and endothelial cells of the cerebral microvasculature co-exist in intimate proximity in nervous tissues, and their homeostatic interactions in health, as well as coordinated response to injury, have led to the concept that they form the basic elements of a functional neurovascular unit. During the course of normal cellular metabolism, growth, and development, each of these brain cell types secrete various species of potentially neurotoxic peptides and factors, events that increase in magnitude as brain cells age. This article reviews contemporary research on the secretory products of the three primary cell types that constitute the neurovascular unit in deep brain regions. We provide some novel in vitro data that illustrate potentially pathogenic paracrine effects within primary cells of the neurovascular unit. For example, the pro-inflammatory cytokine interleukin (IL)-1β was found to stimulate amyloid-β (Aβ) peptide release from human neural cells, and human brain microvessel endothelial cells exposed to transient hypoxia were found to secrete IL-1β at concentrations known to induce Aβ42 peptide release from human neural cells. Hypoxia and excessive IL-1β and Aβ42 abundance are typical pathogenic stress factors implicated in the initiation and development of common, chronic neurological disorders such as Alzheimer's disease. These data support the hypothesis that paracrine effects of stressed constituent cells of the neurovascular unit may contribute to "spreading effects" characteristic of progressive neurodegenerative disorders.

Index Entries: Alzheimer's disease; amyloid-β 42; human neural cells; IL-1β, microvessel endothelial cells; neurodegeneration; neurovascular unit; paracrine effects; primary culture; spreading effects.

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*Author to whom correspondence and reprint requests should be addressed. E-mail: wlukiw@lsuhc.edu

Introduction

Maintaining the homeostasis of the cerebral microenvironment is essential for the normal function of brain, and this balance is achieved through subtle interactions among perivascular neurons, astrocytes, and endothelial cells lining the cerebral microvasculature *(1–12)*. Because of the close proximity among these cells in deep brain regions, they constitute a functional unit, termed the *neurovascular unit* (Fig. 1; refs. *1–4*). During both normal development and pathological conditions, cell types of the neurovascular unit secrete various species of potentially neurotoxic peptides and bioactive factors into the extracellular and Virchow-Robin space, and these are thought to ultimately trigger changes in the homeostasis of the local cerebral environment. Abundant experimental and clinical evidence suggest that vascular factors contribute to the pathogenesis of neurodegenerative disorders such as Alzheimer's disease (AD) *(1–9)*. For example, cerebral vascular amyloid-β (Aβ) protein deposition is a feature common to AD and cerebral amyloid angiopathy *(5,7–10)*. However, little research attention has been paid to the progressive "spreading effects" of the AD process throughout the limbic system. The perivascular neurons, astrocytes, and endothelial cells of the neurovascular unit each possess several distinctive biosynthetic capabilities and neurosecretory properties that are internally regulated and contribute to brain cell microenvironment.

This article summarizes current knowledge concerning the secretory products of human brain microvessel endothelial and neural cells, their potential paracrine effects on adjacent brain cell types, and their possible contributions to pathological mechanisms characteristic of AD. Additionally, we provide some novel data concerning the secretory products in the culture medium of human brain microvessel endothelial cells and human neural cells (a mixture of primary human neurons and glia) in vitro under both normal growth and stressed conditions and explain how these may contribute synergistically to the development of AD-type change.

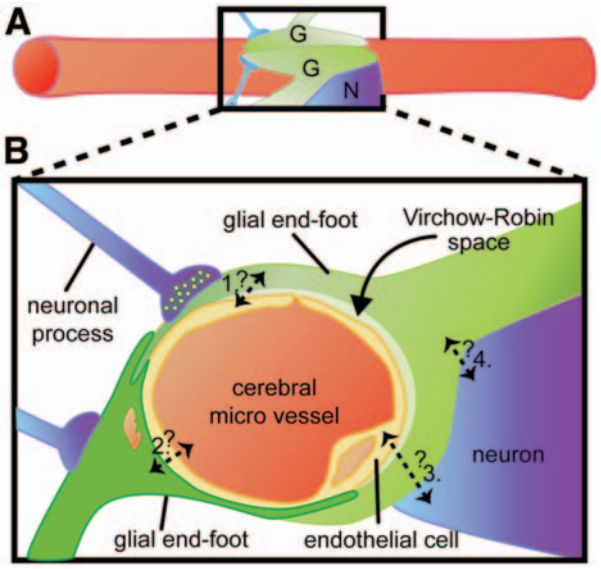

Fig. 1. Primary cell types and geometry of the neurovascular unit in deep brain regions. **(A)** Longitudinal schematic of a cerebral microvessel and **(B)** cross-section. The hippocampus and association neocortex of the human brain are nourished by an extremely dense network of microvessels approx 6 μm in diameter. This is the same diameter as a healthy erythrocyte, thereby allowing the passage of only single trains of red blood cells at a time through the cerebral microvasculature. No neuronal or glial cell lies further than 10 to 12 μm from this deep brain microcapillary system (1-9). The brain microvasculature is noticeably devoid of smooth muscle cells abundant in higher caliber cerebral blood vessels. Neurons (blue), glia (green), their processes and endothelial cells (yellow-red) constitute the primary cell types that define the neurovascular unit (1-4). White areas in **(B)** contain brain parenchyma, omitted here for clarity. Dysfunctional cerebral vasculature is intimately associated with the onset and development of AD (5-9). Intercellular interactions between (1) cerebral endothelial cells and glia, (2) glia and bioactive factors within the lumen of the cerebral microvessel, (3) perivascular neurons and cerebral endothelial cells, and (4) perivascular neurons and glia (dashed lines with arrows) are not well-understood. We propose that dysfunctional microvessel endothelial cells that line the brain's microvasculature, as well as closely juxtaposed neurons and glia, may synergistically contribute to pathogenic mechanisms characteristic of the AD process.

The Concept of Neurovascular Unit

One foremost anatomical feature unique to the cerebral microvasculature is its extensive and intimate network of interactions with neurons and glia *(1–4,8–12)*. In vertebrate brains, the major cerebral arteries branch into smaller pial arteries that run along the brain surface and penetrate into the brain as still smaller intracerebral arteries. Larger caliber arteries of the brain consist of an endothelial cell layer, a smooth muscle cell layer, and an outer layer called the adventia, separated by the cerebral spinal fluid-filled Virchow-Robin Spaces and the glia limitans membrane formed by astrocytes, thereby isolating brain interstitial fluid from the cerebral spinal fluid. Large-caliber arteries are innervated by perivascular nerves and/or local interneurons and their synapses, which can regulate blood flow through release of vasodilators and vasoconstrictors *(1,2,9)*.

As intracerebral arteries branch deeper into the brain parenchyma, they gradually anastomose into intracerebral microarterioles and microcapillaries averaging about 6 μm in diameter, allowing the passage of only single trains of erythrocytes. Here, brain microcapillaries, which form the anatomical basis for the blood–brain barrier (BBB) and provide the majority of blood-borne nutritive support to neural cells, are mainly comprised of just a single layer of endothelial cells surrounded by interconnected foot processes of astrocytes and perivascular neurons (Fig. 1; refs. *1,2,* and *9*). Although it is controversial whether or not perivascular neurons have direct contact with capillary endothelial cells, they are in extremely close (approx 2μm) proximity as their cell bodies and processes formulate a neuronal–astrocytic–endothelial syncytium. Endothelial cells of the BBB, connected by tight junctions, are in stringent control of molecular transport between brain interstitial fluid and the systemic circulation. Therefore, perivascular neurons, glia (astrocytes, microglia, and oligodendrocytes), and vascular cells (endothelial cells, smooth muscle cells, and pericytes) are structurally juxtaposed, and because each is capable of secreting multiple neuroactive and vasoactive substances, they are also functionally interconnected, forming the basis for cerebral homeostasis and the stringent regulation of the cerebral micro-environment *(1–4,9–11)*. Therefore, the term "neurovascular unit" highlights the integrated structural and functional relationship among these cell types; indeed, their molecular genetic patterns of neurophysiological reactions in both health and brain cell injury appear to be highly coordinated.

As described more fully in the following sections, normally functioning neurons, glia, and endothelial cells of the neurovascular unit each secrete a complex array of peptides and bioactive factors that when misregulated may contribute to altered homeostasis in adjacent cell types. By recognizing these cellular subtypes together as an integrated functional unit, we may better understand the initiation and "spreading" mechanisms common to many neurological disorders, such as AD, and other related neurodegenerative diseases with cerebral vascular involvement.

Natural Secretory Products of Neural Cells: Amyloid-β Peptides and AD

Neurons

Functioning as the fundamental synaptic signaling processors of the nervous system in multicellular eukaryotes, neurons also secrete an array of growth and neurotrophic factors, cytokines, chemokines, and neuroactive peptides *(8,12,13)*. Major secretory products that have implicated neurons in the etiology and pathogenesis of AD are a series of highly insoluble and self-aggregating Aβ peptides, 39 to 43 amino acids in length. Aβ peptide deposition and aggregation in the brain parenchyma form the senile plaque, one of the major pathological hallmarks of AD affected brain *(14–17)*. Senile plaques are also overproduced in an age-related fashion in transgenic animal models, such as Tg2576

mice that overexpress Aβ precursor protein (βAPP; refs. *14–18*). Therefore, Aβ peptides are the ragged cleavage products of βAPP, a type I transmembrane protein of several different isoforms ranging from 695 to 710 amino acids *(19,20)*.

The most abundant form in brain (APP695) is produced mainly by neurons *(19–22)*. There are two different pathways for APP cleavage, including the α-secretase pathway in which APP is cleaved by α-secretase, releasing secreted (s)APPα from the cell surface and leaving an 83-amino acid carboxyl-terminal APP fragment *(19–22)*. Generation of (s)APPα is generally considered to be a response to neural activity and may help modulate synaptic plasticity *(19,21)*. Alternately, via the β- and γ-secretase pathway, Aβ is produced after sequential cleavage of APP by β-amyloid cleavage enzyme (BACE) and γ-secretase. The byproducts of this process also include secreted (s)APPβ and an APP intracellular domain fragment, whose neural functions are not well-understood *(19,22)*. Two prominent species of Aβ peptides are generally produced via the β- and γ-secretase pathways; the majority are Aβ40 or Aβx-40 peptides, all bearing the same carboxyl terminus at residue 40, with only a minor fraction as the "long Aβ" that ends at residue 42 *(20–26)*. Aβ42 peptides aggregate much more readily than Aβ40 and are considered as the seed of senile plaque formation *(23,24)*. Although Aβ40 is mainly distributed in amyloid plaques in the cerebral microvessels, Aβ42 often dominates within parenchymal senile plaques *(25)*. According to the different stages of its assembly process, Aβ can be roughly classified into soluble Aβ (the newly secreted one or freshly dissolved synthetic Aβ), aggregates, Aβ oligomers (also known as Aβ-derived diffusible ligands or ADDLs), protofibrils, and fibrils (the main form of Aβ in senile plaques; ref. *19*). Recent evidence implicates soluble ADDLs and protofibrils rather than mature amyloid plaques as the most potent toxic forms of Aβ *(26–28)*, and this helps to explain some of the weaknesses in the amyloid cascade hypothesis—for example, the poor correlation between mature amyloid plaque densities and cognitive deficits in patients with AD and transgenic mouse models of AD *(26)*.

One prevailing theory for AD pathogenesis, the amyloid cascade hypothesis, suggests that Aβ deposition is the initiator of AD and that other pathological changes such as neurofibrillary tangles and neural inflammation are consequences of excessive Aβ production and release *(22,29)*. Although Aβ peptides may be directly toxic to neurons, they also increase neuronal vulnerability to oxidative and metabolic stress *(19)*. Neurotoxicity of Aβ peptides are manifested by induction of oxidative stress, impairment of calcium homeostasis, and energy metabolism in neurons *(19,30)*. Aβ may interact with redox-active metals such as Ca^{2+}, Fe^{2+}, Zn^{2+} and Al^{3+} that not only promote Aβ peptide aggregation but also release peroxides and other reactive oxygen intermediates (ROIs) via Fenton chemistries *(31–33)*. When interacting with cellular membranes, ROIs induce lipid peroxidation and oxidative modification of membrane transporters, receptors, and ion channels, leading to further disruption of normal physiological cellular metabolism *(19, 24–30)*. ROIs also stimulate β- and γ-secretase-mediated production of amyloidogenic Aβ peptides *(22,30)*.

Accumulation of Aβ in mitochondria induces mitochondrial dysfunction, disruption of cellular energy supply, and activation of pro-apoptotic caspases via the release of cytochrome *c* into cytoplasm through compromised mitochondrial membranes *(34–36)*. Oxidative damage to cellular membrane or endoplasmic reticulum can both cause calcium homeostasis dysregulation through enhanced Ca^{2+} influx into cytoplasm, leading to hyperactivation of Ca^{2+}-dependent proteases and kinases *(22–24)*. Another largely unexplored aspect of Aβ peptide neurotoxicity is the induction of pro-apoptotic, pro-inflammatory, and pro-angiogenic signaling and pathogenic gene expression *(13,33)*. All these reactions involving Aβ peptide may synergistically combine to induce cellular inflammatory and apop-

totic cascades, ultimately resulting in neuronal cell death.

Glial Cells

The significance of glial cells in the initiation and development of AD has been progressively expanding since their pivotal support roles in both brain cell homeostasis and pathophysiology have become increasingly understood *(10,11,37–41)*. Two main groups of glial cells in the brain appear to co-operate in pathophysiology; microglia, and macroglia. Microglia are macrophage-like cells that regulate the inflammatory response of the neural tissues to injury or infection, whereas macroglia include oligodendrocytes, which form myelin in the brain, and astrocytes, which stain positive for glial fibrillary acidic protein *(10,13)*. As integral components of the neurovascular unit, astrocytes send processes to both neuronal synapses and vascular endothelial cells while connecting with other astrocytes through gap junctions *(37)*. More recent studies suggest a pivotal role of astrocytes as intermediary processors in functional hyperaemia and neurovascular coupling *(41,42,44)*. The mechanism for this event is simplified as the following: glutamate released by neural activities may activate metabotropic glutamate receptors in cortical astrocytes, leading to an increase in intracellular Ca^{2+} and activation of members of the cyclooxygenase gene family *(41,43,65)*. Astrocytes subsequently release vasoactive substances such as K^+, nitric oxide, adenosine, prostaglandins, and epoxyeicosatrienoic acids, which eventually lead to vasodilation *(2–4,41)*. Astrocytes not only modulate synaptic transmission by uptake and release neurotransmitters such as glutamate but also provide essential nutritive support such as lactate to neurons *(11,44)*. During development, astrocytes modulate angiogenesis and endothelial tight junction formation by releasing growth and neurotrophic factors and in adult brain participate in maintaining BBB integrity by ensheathing the endothelium with astrocytic endfeet *(37)*. Chronically activated microglia and astrocytes can kill adjacent neurons by the secretion of highly toxic products such as ROIs, nitric oxide, pro-inflammatory cytokines such as IL-1β, proteolytic enzymes, complement factors, and excitory amino acids *(37–40)*. Both fibrillary and diffusible Aβ can activate astrocytes to produce cytokines (such as IL-1β), IL-6, and RANTES *(40,45)*. Further in vitro studies have shown that astrocytes can release pro-inflammatory chemokines such as tumor necrosis factor (TNF)-α, monocyte chemotactic protein-1, and IL-1β upon activation by Aβ42 *(38)*. Combinations of cytokines such as IL-1β or TNF-α and interferon-γ are further able to stimulate generation of Aβ40 and Aβ42 from astrocytes *(46,47)*. Microglia, functioning as cerebral macrophages, can recruit and stimulate astrocytes; however, unlike astrocytes, microglia are more likely to be activated by fibrillary Aβ peptides and the "mature" forms of Aβ peptides associated with senile plaques *(38,45)*. Activated microglia also produce toxins and pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α; the chemokine IL-8, macrophage inflammatory proteins; MCP-1; growth factor macrophage-colony stimulating factor; and pentraxins *(38,48–50)*. These toxins and inflammatory factors, secreted by both astrocytes and microglia, further trigger inflammatory cascades, thereby exacerbating conditions in AD brain that may be either pathogenic or beneficial *(50)*. Interestingly, both astrocytes and microglia function in an attempt to catabolize Aβ deposits through either phagocytosis or enzymatic degradation *(39,51,52)*.

Endothelial Cells and Their Secretory Products in Neurophysiology and in AD

Brain endothelial cells are the third major cellular component of the neurovascular unit in deep brain regions. As the structural basis of the BBB, many interesting similarities are shared by both cerebral blood vessel endothelial and neural cells, such as intercellular signaling, differentiation, and anatomical patterning,

leading to the realization of close interaction and interdependence among these three brain cell types *(8,12)*. Indeed, emerging evidence suggests that neural cells and cerebral vascular endothelial cells are intricately connected during cerebral development, following metabolic challenge, and during pathological development *(1–4,9–12)*. Interestingly, when cocultured with neural stem cells, endothelial cells release endothelial growth factors that not only stimulate self-renewal but also induce neurogenesis *(53)*. When stimulated by vascular endothelial growth factor, endothelial cells further trigger neurogenesis through secretion of neural growth factors such as brain-derived growth factor *(8,54)*. Endothelial cells also release a series of highly bioactive factors, including leukemia inhibitory factor, bone morphogenetic protein-2, brain-derived growth factor, and basic fibroblast growth factor, which induce the differentiation of both astrocyte precursors and neural stem cells *(8,10–12)*. Vascular-derived neurotrophic factor artemin, a member of the glial cell line-derived neurotrophic factor family, functions in guiding sympathetic neurons toward their target tissues *(55)*.

Endothelial Cells, Cerebral Microvasculature and AD

The many common risk factors shared by both cerebral vascular disease and AD and the comorbidity of vascular diseases in the majority of AD cases are prime reasons for the speculation that a pathogenic link exists between the two *(5–12,56)*. More recent studies support a direct and essential contribution of cerebral vascular dysregulation to the pathogenesis of AD *(1,5,6)*. Several vascular hypotheses for the initiation of AD propose an imbalance between Aβ production and elimination across endothelial cells of the BBB *(7,9,57)*. Endogenously generated Aβ peptides in brain can be cleared either through enzymatic degradation by certain proteases such as insulin degrading enzyme, neprilysin, or plasmin or by transporting Aβ directly across the BBB into the peripheral circulation *(5,22)*. Low-density lipoprotein receptor-related protein, expressed in endothelial cells of the BBB, may be one major transporter responsible for Aβ efflux *(9,57,58)*. Vascular disease risk factors such as hypertension, diabetes, hypercholesterolemia, concentration of cholesterol oxides (such as 24S-hydroxycholesterol within the brain and in the systemic circulation), neural inflammation, and the APOE-4 genotype, as well as aging, all may bring about chronic damage to the brain capillary endothelial cells, which may further result in chronic hypoperfusion and dysregulation within the neurovascular unit *(7,59–64)*. The normal ability of the BBB to clear Aβ deposits from brain is compromised for many reasons, causing accumulation of Aβ in both brain parenchyma and blood vessels, allowing the expression of Aβ peptide's neurotoxic effects *(7,22,56,61)*.

After exposure of cerebral vasculature to Aβ peptides, endothelial angiogenic processes appear to be targeted, and soluble Aβ peptides also induce significant production of ROI in endothelial cells, generating oxidative stress within the cerebral vascular lining *(1,2,7,62,63)*. Additionally, endothelial cells in AD release high levels of inflammatory proteins such as IL-1β, IL-6, TNF-α, cerebrovascular transforming growth factor-β, and neurotoxic thrombin *(56,62–64)*. All these secretory products may collectively support neuro-inflammatory cascades, killing neurons and further damaging the integrity of the neurovascular unit, which in turn induces progressive release of toxic products, thus creating vicious self-reinforcing cycles and potentially enabling "spreading effects" among susceptible cell types *(7,67)*.

Involvement of Pro-Inflammatory Cytokines in the Natural Release of Amyloid-β Peptides From Aging Human Neural Cells

Cultured primary human neural (HN) cells have been highly informative for the in vitro

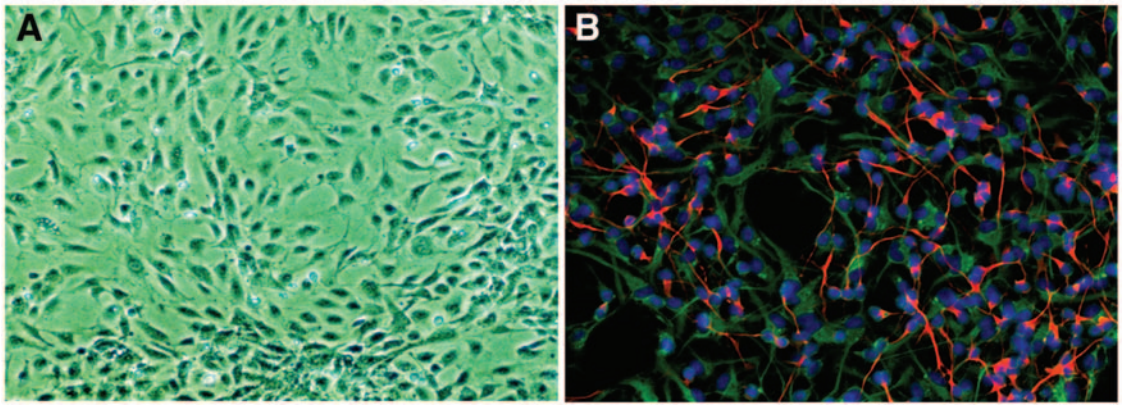

Fig. 2. Fluorescence microscopy of hBMEC and HN cells in primary culture. **(A)** hBMECs in primary culture stained briefly with 0.1% methylene blue (3 wk in culture, 70% confluent; ×20 magnification) and **(B)** HN cells, a primary coculture of neurons and glia, stained with anti-β-tubulin III, a neuron-specific marker (red) and with anti-glial specific fibrillary protein (GFAP), a glial-specific marker (green) (3 wk in culture, 40% confluent; ×20 magnification). Cells in **(B)** have also been stained with Hoescht 33258 (*bis*-benzimide) to highlight and quantify apoptotic features of either neuronal or glial cell nuclei (blue) (×20 magnification; ref. *13*)

study of the basic molecular genetic mechanisms that contribute to AD-type change (Fig. 2; refs. *13,43,60,* and *65*). During the 2-mo culture of HN cells, the surrounding incubation medium was harvested at weekly intervals, concentrated in the presence of a protease inhibitor cocktail and then analyzed by SDS-PAGE, Western blotting, and/or enzyme-linked immunosorbent assay using anti-Aβ40 or anti-Aβ42 antibodies *(13)*.

During normal cellular aging in vitro, researchers found that potentially neurotoxic Aβ peptides, both the Aβ40 and Aβ42 species, are released from HN cells in primary culture *(13,17,18)*. Interestingly, the release of Aβ40 and Aβ42 peptides by HN cells was found to steadily increase as these cells aged from 1 to 7 wk. The rate of Aβ40 peptide release was almost eightfold higher in 7 vs 1-wk-old HN cells, although both cultures contained approximately the same ratios of neurons and glia as determined by cell-specific fluorescent staining (ref. *13*; unpublished observations). Additionally, the ratio of the release of detected Aβ40 peptide concentration over Aβ42 peptide in the HN cell culture medium was found to be a constant 9- to 10-fold for all time-points tested (Fig. 3A,B). Interestingly, when the pro-inflammatory cytokine IL-1β was constantly present in the HN cell incubation medium at 10 ng/mL concentration, the release of both Aβ40 and Aβ42 peptides was found to be significantly increased over controls (Fig. 4). These findings suggest that the presence of physiological amounts of IL-1β throughout the natural growth of HN cells in primary culture in vitro promotes Aβ peptide release—and this may have consequences on Aβ peptide-mediated neurotoxicity. These findings support the contention that physiological stressors associated with normal brain cell function during aging stimulates progressive Aβ peptide release from neural cells and that under further cytokine IL-1β-induced stress the enhancement of amyloidogenic peptides released may further contribute to mechanisms associated with the initiation and/or propagation of amyloid-mediated neurotoxicity and brain cell degeneration.

Paracrine Effects in Hypoxia-Stressed Primary Human Brain Microvessel Endothelial Cells and HN Cells

As reviewed earlier, neurons, glia, and endothelial cells are each capable of secreting

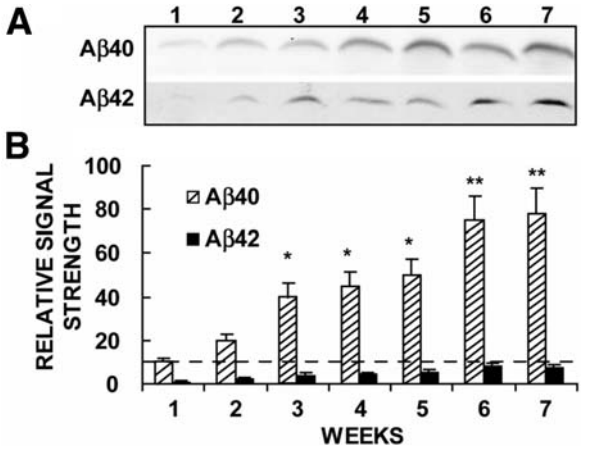

Fig. 3. Release of Aβ40 and Aβ42 peptides from HN cells in primary culture during normal aging of HN cells from 1 to 7 wk. **(A),** Western immunoassay as described in ref. *13* and **(B)**, bar graph of pooled data from **A**. Briefly, HN cells, derived from explanted human fetal brain tissue and cryopreserved in primary passage as "spheroids" were grown for 1 to 7 wk to approx 70% confluence at 37°C and 5% CO_2/20% O_2/75% N_2 in humidified air *(13,43,60,65)*. Growth media were collected, concentrated, and analyzed at weekly intervals as previously described *(13)*. For ease of comparison, dashed horizontal line represents Aβ40 levels at week 1. Both Aβ40 and Aβ42 peptide secretion increases significantly as HN cells age in vitro. (n = 3; significance over controls *$p < 0.05$; ** $p < 0.02$.)

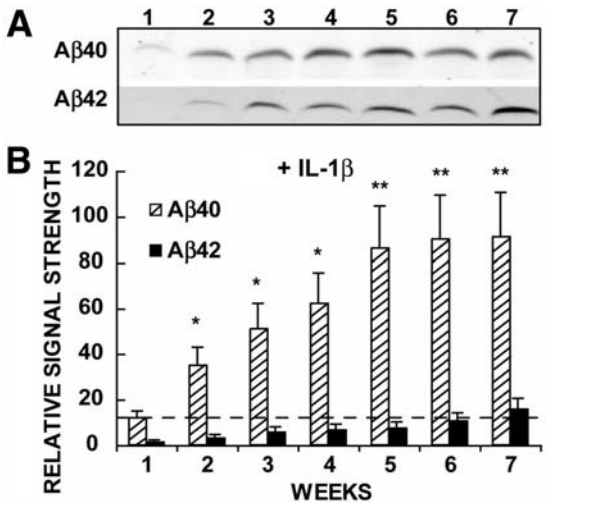

Fig. 4. Release of Aβ40 and Aβ42 peptides from HN cells in primary culture during normal aging of HN cells from 1 to 7 wk in the presence of 10 ng/mL IL-1β) **(A),** Western immunoassay as described in ref. *13* and **(B)**, bar graph of pooled data from **(A)** in HN cells treated with IL-1β, continuously present in the cell growth medium. As in Fig. 3, HN cells were grown for 1 to 7 wk to approx 70% confluence at 37°C and 5% CO_2/20% O_2/75% N_2 in humidified air *(13,43,60,65)*. Growth media were collected, concentrated and analyzed at weekly intervals as previously described *(13)*. For ease of comparison, dashed horizontal line represents Aβ40 levels at week 1. Both Aβ40 and Aβ42 peptide secretion increases significantly as HN cells age in vitro. (n = 3; significance over controls *$p \leq 0.05$; ** $p < 0.02$.)

an array of complex and potentially pathogenic Aβ peptides and other bio-active factors that when misregulated, have potential to alter the homeostatic function in adjacent brain cell types. To further understand how these may interact to induce pathology within the cells of the neurovascular unit**,** the secretory products of human brain microvessel endothelial cells (hBMECs) were examined under conditions of transient hypoxia (5% O_2). Brief periods of hypoxia have previously been shown to induce rapid and dramatic changes in homeostatic gene expression patterns in human primary neural cells in vitro *(43,65)*. Hypoxia-stressed hBMECs were found to secrete significantly more IL-1β than age-matched controls *(47,66)*. In turn, IL-1β secreted at physiological concentrations from hBMECs was found to induce Aβ42 peptide secretion when added at the same concentrations to HN cells, an effect that was dependent on the age of the cell cultures (unpublished observations; Fig. 5). Moreover, 3-wk-old HN cell cultures exhibited a two- to threefold enhancement in these secretory events compared with 1-wk-old HN cells. These data strengthen the concept that non-homeostatic paracrine interactions among neurons, glia, and endothelial cells may contribute to Aβ42-mediated pathogenic processes, and

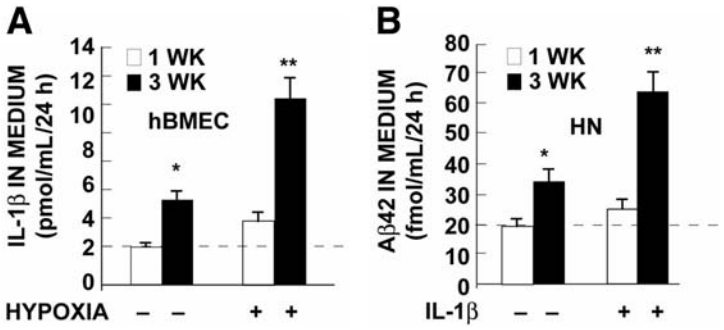

Fig. 5. Dynamics of hBMECs and HN cell secretory products during transient hypoxia. Briefly, hBMEC growth was initiated by the elutriation of dispase-dissociated normal human brain cortical tissue and cells were subsequently cryopreserved serum free in aliquots as previously described *(68)* **(A)**, hBMECs (70% confluent, approx 5×10^4 cells) were cultured for 1 and 3 wk in normoxia (constant 20% O_2), and at each of these time-points cell media were assayed for IL-1β content *(17,65)*. In parallel experiments, 1- and 3-wk hBMECs were grown under conditions of hypoxia (constant 5% O_2), and cell media were again assayed for IL-1β. **(B),** Control or IL-1β-stressed HN cells (10 ng/mL serum-free medium, continuously present) were assayed for Aβ42 in cell media after 1 and 3 wk using enzyme-linked immunosorbent assay *(13)*. Hypoxia-stressed hBMECs secrete physiologically relevant amounts of IL-1β (assuming a constant rate of secretion, accumulating to approx 10 ng/mL after 3 wk). Higher hBMEC confluency and longer periods of hypoxia might be expected to increase the quantity of IL-1β released. Secreted Aβ42 peptides from IL-1β-stressed HN cells may further contribute to senile plaque formation, Aβ42-mediated inflammatory signaling and related pathological mechanisms. Because hBMECs and HN cells are the major cell types of the neurovascular unit, secreted and potentially neurotoxic peptides from one cell type may "prime" non-homeostatic signaling and gene expression in adjacent cells. Therefore, this paracrine effect within stressed cells of the neurovascular unit may further propagate pathogenic cycles within brain parenchyma. For purposes of comparison, dashed horizontal line represents in **(A)** control (normoxia) levels of IL-1β in medium after 1 wk of culture of hBMECs or in **(B)** control (no added IL-1β) levels of Aβ42 after 1 wk of culture of HN cells. ($n = 3$; significance over controls $^*p < 0.05$, $^{**}p < 0.02$.)

these may be important in interneural cell signaling and cell-to-cell "spreading effects" characteristic of chronic, inflammatory, and degenerative brain disease *(69–71)*.

Conclusions

Neurons, glia, and endothelial cells lining cerebral microvessels are the primary cell types of the neurovascular unit that collectively form the neuronal-astrocytic-endothelial syncytium. Primary cultures of human microvessel endothelial and neural cells remain useful as models to study the molecular biology and mechanism of cerebral vasculature contribution to neurological disorders such as AD and related disorders with a neurovascular component. Neurons, glia, and endothelial cells secrete various peptides and bio-active factors that may affect adjacent cells of the neurovascular unit via paracrine effects and under nonhomeostatic conditions, one brain cell type has considerable potential to contribute to coordinated dysfunction of the neurovascular unit. For example, hypoxia-stressed hBMEC cells secrete IL-1β at physiological concentrations that are known to induce significant release of Aβ42 peptides from HN cells in primary culture. Paracrine signaling among brain cells that constitute the neurovascular unit should be considered not only for their potential contribution to the "spreading effects" characteristic of common neurodegenerative diseases but also for the strategic design of pharmaceuticals useful for

rational drug treatment of chronic and progressive neurological disorders.

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