# The Nineteenth Day of Neuropsychopharmacology

Krynica Zdrój, September 17, 2010

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# Symposium

## Neuropsychopharmacology

# Histaminergic activity in adult rats with neonatally lesioned central noradrenergic, serotoninergic and dopaminergic (rodent model of Parkinson's disease) systems

Ryszard Brus[1], Przemysław Nowak[1], Ryszard Szkilnik[1], Jerzy Jochem[2], Richard M. Kostrzewa[3]

[1]Department of Pharmacology, [2]Department of Physiology, Medical University of Silesia, Jordana 38, 41-808 Zabrze, Poland;
[3]Department of Pharmacology, J. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614, USA

Histamine (H) is a neuromodulator in brain, regulating motor activity, awareness, wakefulness, pain and other activities. Histaminergic cell bodies in brain are located in the hypothalamus, sending diffuse projections to all brain regions and interacting with other neurotransmitter systems. In an effort to study the latter process, monoaminergic neurotoxins DSP-4, 5,7-dihydroxytryptamine (5,7-DHT) and 6-hydroxydopamine (6-OHDA) were administered to rats to destroy respectively, noradrenergic, serotoninergic and dopaminergic systems in brain.

Each neurotoxin was injected to newborn male Wistar rats as follow: DSP-4 (50.0 mg/kg, *sc*) on the 1st and 3rd days after birth; 5,7-DHT (75 mg, base, *icv*) or 6-OHDA (134 mg, base, *icv*) on the 3rd day after birth. Controls were injected with saline *sc* or *icv* respectively. Adulthood levels of biogenic amines were estimated in brain by HPLC/ED; and histamine levels, by immunoenzymatic methods. In addition, behavioral observations of oral activity and stereotyped behavior induced by SKF 38393 and apomorphine (respective D1 and D1/2 agonists) were assessed before and after S(+)chlorpheniramine (10.0 mg/kg, *ip*), cimetidine (5.0 mg/kg, *ip*), or thioperamide (5.0 mg/kg, *ip*), respective H1, H2 and H3 histamine receptors.

DSP-4 reduced the adulthood level of norepinephrine and histamine in brain, while 5,7-DHT reduced the adulthood level of serotonin and histamine in brain. 6-OHDA-lesioned rats had a reduced DA but elevated histamine level in brain. The H3 receptor antagonist thioperamide only attenuated SKF 38393-induced oral activity as well as apomorphine-induced stereotyped behavior in 6-OHDA-lesioned adult rats, while DSP-4 and 5,7-DHT intensified it.

Findings indicate that the histaminergic system, *via* H3 receptors, exerts a modulatory role in the brain of 6-OHDA-lesioned rats – a model of severe Parkinson's disease.

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# The therapeutic role of natural polyphenolic compounds in vascular endothelial cell dysfunction

Bernhard Hennig, Michal Toborek

Molecular Neuroscience and Vascular Biology Laboratory, Department of Neurosurgery, University of Kentucky Medical Center, Superfund Research Program, Lexington, KY 40536, USA

Complications of vascular diseases at the systemic and cerebral level are a major cause of death in most parts of the world. Dysfunction of endothelial cells is a critical underlying cause of the pathology of vascular diseases. An imbalance in oxidative stress and antioxidant potential can lead to chronic vascular inflammation, which could be a result of exposure to environmental toxicants. We have evidence that diet-derived polyphenolic compounds, can modulate toxicant-induced endothelial cell dysfunction and associated inflammatory parameters. For example, dietary flavonoids can down-regulate the inflammatory potential of persistent organic toxicants. Specifically, both EGCG and quercetin reduced polychlorinated biphenyl (PCB)-induced oxidative stress, CYP1A1 induction, and AhR-DNA binding activity in endothelial cells. Our data also suggest that membrane domains abundant in endothelial cells, called caveolae, provide a regulatory platform for proinflammatory signaling pathways, which can be modulated by organic pollutants and dietary polyphenolics with therapeutic potential. PCBs induced caveolin-1 and accumulated mainly in the caveolae fraction of endothelial cells. Silencing of caveolin-1 significantly attenuated the PCB-mediated induction of CYP1A1 and oxidative stress, events which were also observed in caveolin-1-deficient mice. Most importantly, certain flavonoids blocked caveolae-dependent proinflammatory responses induced by persistent organic toxicants. Our data suggest that diet-derived polyphenolic compounds may play a therapeutic role by reducing endothelial activation and inflammation associated with systemic and cerebral vascular diseases.

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# Evolution of understanding of the mechanism of antidepressants (AD) and pharmacological treatment of major depression (MAJ)

Zbigniew S. Herman

Professor and Chairman Emeritus of the Chair of Pharmacology, Internal Medicine and Clinical Pharmacology, Silesian Medical University, Medyków 18, 40-752 Katowice, Poland

The depressive illness are still the problem of greatest importance. 340 million persons suffer from major depression, among them 1/3 are females. Dramatically increases the morbidity of this illness of youngsters. Roughly estimating in Poland 20% of the population (1/to 20 persons) have the symptoms of depressive disease (DD). WHO estimates, that on the year 2020 unipolar depression will be the second case of global diseases behind only ischemic heart disease.

It is dramatic that 30% population has no benefit from existing drugs. In untreatable patients with major depression (MAJ), electroshock (in modern sense of meaning) usually brings, a great benefit. But still is not very popular because, especially in intellectuals it attenuates the highest psychic functions. On this best world where we live the greatest individuals suffered a depression.

Among others were: Dickens Charles, Dostoyevsky Fyodor, Goya de Francisco, Hemingway Ernest, Huxley Sir Julian, (1887–1975 – English evolutionary biologist), Kurosava Akira, Mozart Wolfgang Amadeus, Newton Isaac, Nietzsche Friedrich, Poe Edgar, Spears

* The lecture dedicated to my masters in psychopharmacology: Tadeusz Chruściel, Bernard Beryl Brodie and Julius Axelrod

Britney (pop music) Tchaikovsky Peter Ilyich, Wilson Brian (Beach Boys – rock).

Polish, great persons suffering MAJ were e.g. Dąbrowska Maria – writer, candidate to Nobel Price; completely forgotten Haiman Mieczysław (1888–1849, polish-american poet and novelist, "Herodot of Polish emigrants" in USA; Herbert Zbigniew – poet; Mickiewicz Adam – poet and bard; Piłsudski Józef – marshal, founder the Poland in November 1918 after 1772–1918 partition of Poland by Austria, Prussia and Russia, had one episode of severe depression; Słowacki Juliusz – poet and bard (and tuberculosis); Radziwiłłówna Barbara (1520 or 1523–1551), most tragic Queen of Poland, the second wife of the King Sigismund Augustus; Jan III Sobieski, king of Poland. hero of great victory on Turks on Suburbs of Vienna.

Presenting the problem in nut shell I would like to remind you that all was born after discovery and studies on the mechanism of action of three biogenic amines 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA), respectively ( B.B Brodie, J. Axelrod – Nobel Prize; A. Carlsson – Nobel Prize).

Oleg Hornykiewicz discovered and proved that the cause of Parkinson disease is a deficiency of dopamine in the nigro-striatal pathway. It is very peculiar that his name is obsolete.

Actually psychiatrists can prescribe one of the drug from 4 groups of antidepressants: classical, selective serotonin reuptake inhibitors (SSRI), balanced inhibitors of reuptake of 5-HT and NA and AD acting on several another targets.

I neglect the problem of mood stabilizers: lithium (actually used not frequently, since it evoke very dangerous side effects) and antiepileptics, e.g. valproic acid, lamotrigine indicated in patients suffering especially with severe MAJ. These drugs are prescribed in small doses, compared with the doses used in epileptics.

## The neurotransmitters or neuromodulators other than NA, DA and 5-HT

### Nitric oxide (NO)

A few clinical and several pre-clinical studies, strongly suggest involvement of NO signalling pathways in MAJ.

Several of the conventional neurotransmitters including 5-HT, GABA and excitatory aminoacids are regulated by NO, and some groups of antidepressants modulate the hippocampal concentration of NO *in vivo*. Therefore the NO pathways are a potential target for AD.

NO synthase inhibitors have antidepressant effect.

### GABA (γ-aminobutyric acid)

GABA agonist, valproate is effective as a mood stabilizer. $GABA_A$ and $GABA_B$ agonists represent a novel therapeutic target for the depression treatment.

In patients with major depression the GABA level in cerebro-spinal fluid, blood and also in *post-mortem* brain was diminished.

### Excitatory aminoacids

Competitive NMDA (N-methyl-D-aspartate) antagonists (2-aminophosphonoheptanoic acid) or dizolcipine reduce immobility time in forced swimming test (FST).

### Opioids and cholecystokinin (CCK)

Morphine at one time was the first-line antidepressant, quickly abandoned, since it elicited severe, clinically unacceptable effects.

SSRIs and TCA increase enkephalins level in the brain, fluoxetine increases μ-opioid receptor population in the rat forebrain.

CCK is a neuropeptide colocalized with opioids

In a number of brain regions $CCK_B$ receptors but not $CCK_A$ receptors enhance antidepressive effects in opioid-like manner.

### Neurokinins

Substance P acts on neurokin NK receptors, mainly on $NK_1$. Substance P antagonist (300 mg) MK-0869 acts antidepressively very similarly to 20 mg of paroxetine. However MK0869 does not enhance NA pathways and function.

### cAMP response element binding protein (CREB) and brain derived neurotrophic factor (BDNF)

Prolonged treatment of experimental animals by almost all of groups of antidepressants elevated CREB concentration and its active phosphorylated form (pCREB).

pCREB induces BDNF expression, which lead to neurogenesis neuronal survival and neuronal plasticity. Therefore it is possible that concentration of these proteins may contribute to the therapeutic mechanisms of antidepressants.

Both chronic antidepressants and electric shock (ECS) increase BDNF level in rats brain and its transmembrane receptor tyrosine kinase B.

Drugs which can lead to the up regulation of any of the factors in the CREB and BDNF cascade have the potential antidepressive activity.

### Potassium ($K^+$) channel blockers

$K^+$ channel blockers: tetraethylammonium, apamin, charydotoxin and gliquidone have antidepressant-like properties.

$K^+$ channel activators prolonged time of immobility in FST (cromacalin, minoxidil, pinacidil). Fluoxetine inhibits voltage-activated $K^+$ channels.

### Imidazoline receptor ligands

Imidazoline binding site attaches to clonidine and idazoxan.

Clonidine has affinity to $I_1$-receptors and induce the increase of 5-HT and NA level. On the other hand idazoxan has an affinition to $I_2$ receptors.

$I_2$ sites present on MAO are located at a ligand binding domain, distinct from the catalytic binding domain of the enzyme, which might explain why $I_2$ selective compounds elevate 5-HT and NA concentration.

Since MAO-I act antidepressively $I_2$ selective compounds may represent a novel class of drugs to modulate monoamine levels is brain; therefore they can display antidepressant potential.

$I_2$ ligands 2-BFI, BU 224. In platelets of patients with depression there is an increase $I_1$binding sites.

### Histamine

Release of monoamines and acetylcholine act on histaminergic receptor of type 3 ($H_3$ receptors).

Compounds that affect negatively $H_3$ receptors e.g. thioperamide and more selective $H_3$ receptors antagonist – dobenpropit are active antidepressants.

### Cannabinoid $CB_1$ receptor antagonists

Cannabinoid $CB_1$ receptors blockade might be associated with antidepressant and anti-stress effects.

Several substances studied experimentally have these effect (e.g. AM 251; AVE 1625; SLV 319 and several others).

### Nicotinic cholinergic receptors in the brain

In some smoking individuals it was observed that the quitting smoking was one of early symptoms of depression. This symptoms disappears after recommencing smoking or antidepressant treatment.

Patients with minor depression, which moderately smoked before, fall into MAJ.

This finding make rational to search nicotine or the other cholinergic nicotine receptor agonists as a eventual antidepressants.

The additional argument is an experimental fact, that nicotine increases the NA, 5-HT as well as DA concentration in different structures in the brain.

Using animal models of depression antidepressive effects were observed. In one of this model in which nicotine was implanted subcutaneously (1,5 mg/kg/24 h), corresponds the plasma level, after smoking 1 package of cigarettes daily, the antidepressant effects were observed. Different genetically several lines of rats, react with different efficacy on nicotine effects.

The substances which stimulated cholinergic nicotine receptors are worth to undertake the effects to study the rat of nicotine and nicotine-like agents.

Very recently the e-cigarettes were introduced into the market. As a matter of fact they are inhalators of the pure nicotine, which satisfied the demand of reflexes during cigarettes smoking.

This device enables to quit smoking quickly, even in person suffering major depression, since they get only pure nicotine inhaled into the respiratory system.

### Glia and cytokines

A two decades ago the role of the glia in the central nervous system (CNS) was defined as a supportive for neurons and as a source of vascularisation.

The neurotransmitters between glial cells are glutamates and adenotriphosphate (ATP), and perhaps D-serine.

Neurotransmitters between neurons and glial cells are NA, acetylcholine and GABA. In glial cells cytoki-

nes TNF-α and IL-β are synthesised. Neuroglia comprise more than 50% of brain. The components of it are e.g. astrocytes, microglia, oligodendrocytes, ependymal cells and satellite cells.

Microglia are a specific macrophages constitutes 15% of brain mass. In these cells the synthesis of IL-1β; IL-6 transforming growth factor-β (TGF-β) take part.

The astrocytes regulates growth, differentiation, maturation of embryonal and new-born cells of the CNS. Not very long ago it was stated firmly that neurogenesis in adults does not exist. Actually this axiom is not true. The neurogenesis in adult brain take place in under ventricle region, granular hippocampal region, still no neurogenesis in adult spinal cord was discovered. Astrocytes has also regulatory functions in generations of new synapses.

These cells induce growth or neurotrophic functions by four factors. Astrocytes induces protective efficiency of glia.

If sudden transformation of the limits of this protective border of astrocytes and microglia is in action, the severe brain damage appear.

Pathological changes of neuroglia causes ischemia, inflammatory processes in the brain and degenerative disorders of the brain, e.g., Parkinson disease, Huntington chorea, multiple sclerosis, Alzheimer disease, aging.

### Cytokines – depression

In MAJ increased levels of pro-inflammatory cytokines was observed.

On the other hand in patients with hepatitis C and some cancers treated by interleukin-2 (IL-2) and interferon-α (IFN-α) depressive symptoms were observed.

Postnatal depression may be involved in some women by twice increase of the cytokines concentrations.

In depression it was described: oversecretion from macrophages of interleukin-1 (IL-1), TNF-α and interferon γ (IFN-γ).

Il-1 increases 5-HT transporter activity, which effectively reduce the concentration of 5-HT in the synaptic cleft.

It was shown that imipramine, fluvoxamine and maprotiline significantly increased the production of IL-1 receptor antagonist (IL-1 ra) mRNA in hypothalamus, hippocampus, frontal cortex and brain stem.

The treatment with IL-1 receptor antagonist (IL-1ra) of rats prevented the development of learned helplessness.

Therefore IL-1ra may provide novel therapeutic strategy of depression.

## How to improve shortcomings of today antidepressants

**A.** Combination antidepressant therapy.

Use of tetracyclic antidepressant mirtazapine combined with fluoxetine, paroxetine or buproprion is more effective and as well-tolerated in the treatment of the major depression as mentioned drugs used alone. There are some evidences that after combination therapy remissions of the depression are longer comparing with monotherapy.

Among all patients who had marked response a blind discontinuation of 1 agent produced a relapse in about 40% of the cases.

**B.** Fast antidepressants.

As you now perfectly the clinical effect of the mostly used antidepressants is delayed between 2–4–6 weeks.

Actually it was shown that the combination of unspecific β blocker pindolol used concomitantly with fluoxetine, in comparison with the antidepressant prescribed alone in some patients are effective after few days.

**C.** Efficacy of atypical antipsychotics in bipolar disorder.

Atypical antipsychotics (olanzapine, aripiprazol, quetiapine, ziprasidone, risperidone, clozapine, amisulpiride) have emerged as both an alternative and adjunct to conventional mood stabilizers. Actually it is known that they are really effective in manic phase, and most data refers to olanzapine. Their role in acute depressive episodes is less proved, controversial, and requires further studies.

**D.** Social phobia.

Described few years ago as specific entity.

The main symptoms of major depression is a deep grief and especially very low mood and very dangerous suicidal ideas and attempts.

The main symptoms of social phobia are shyness and timidity, treated by paroxetine or fluoxetine, fluvoxamine, venlafaxine, tricyclic antidepressants and β-adrenolytics; propranolol is the best, penetrating blood-brain barrier.

## News 2010

**1.** Antipsychotics (typical and atypical) increases risk of sudden cardiac death in proportion 17.9 per 10,000 patients risk.

**2.** Variant potassium-channel gene has the role in a core feature of schizophrenia.

**3.** Which newer antidepressants is a best?
**a.** most effective: escitalopram, mirtazapine, sertraline and venlafaxine;
**b.** best tolerated: bupropion, citalopram, escitalopram, sertraline.

## Tomorrow antidepressants

There is suggestion of Institute of Mental Health that enzyme called glycogen synthase kinase β (GSK 3β) may be closer to the cause of depression than are diminished 5-HT levels.

GSK 3 might be better and faster target for new antidepressants.

Blocking GSK 3β in mice with induced low level serotonin, normalizes concentration of 5-HT. Recently it is supposed that also tryptophan hydroxylase 2 (Tph2) gene play role in some mental disorders. The influence on pathological variants may be possible.

# Induction of ribosomal stress as a novel mouse model of neurodegeneration

Grzegorz Kreiner[1,3], Claus Rieker[1], David Engblom[1], Holger Bierhoff[2], Jan Rodriguez Parkitna[1,4], Ingrid Grummt[2], Günther Schütz[1], Rosanna Parlato[1]

[1]Department of Molecular Biology of the Cell I and [2]Department of Molecular Biology of the Cell II, German Cancer Research Center, Heidelberg, Germany; [3]Department of Brain Biochemistry and [4]Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland

Neurodegenerative diseases are associated with mitochondrial and proteasomal dysfunction, alteration of cellular defense mechanisms and extensive evidence of oxidative stress. The inhibition of protein synthesis represents a basic response to cope with stress conditions. The nucleolus being a center of ribosomal RNA (rRNA) synthesis is an essential stress sensor maintaining cell homeostasis. However, the possible function of nucleolus in neurodegeneration has not yet been explored. We propose a novel approach to generate mouse models based on the genetic ablation of the transcription initiation factor IA (TIF-IA) that blocks the synthesis of rRNA. Encouraged by increasing evidences of a crucial role of both cellular stress and p53 protein in the pathophysiology of several human neurodegenerative diseases, we used the conditional inactivation of the gene encoding TIF-IA by the Cre-loxP system to inhibit rRNA synthesis and, consequently, induce overexpression of p53 in different types of neurons in mice. Deletion of the TIF-IA gene leads to rapid loss of neural progenitors and, more interestingly, to progressive loss of postmitotic neurons. Disruption of the nucleoli in dopaminergic neurons and striatal dopaminoceptive neurons results in the generation of mutants showing respectively the typical phenotype of Parkinson's disease (degeneration of dopaminergic neurons in substantia nigra and ventral tegmental area, depletion of dopamine in the striatum and typical motor dysfunctions) or Huntington's disease (loss of medium spiny neurons in striatum, impairment of motion control and clasping behavior). In addition, our study indicates that cellular changes associated with nucleolar disruption may recapitulate some events associated with neurodegeneration. Furthermore, these mutant mice represent valuable tools to gain understanding of the neuronal response to ribosomal stress and underscore a new role for nucleolar activity in neurodegenerative processes.

# The role of NCAM protein in psychiatric disorders

Marzena Maćkowiak

Laboratory of Pharmacology and Brian Biostructure, Pharmacology Department, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland

The neural cell adhesion molecule (NCAM) is involved in normal brain development, including axonal/dendritic growth and branching, synaptogenesis and synaptic plasticity. Several findings have implicated NCAM protein as a susceptibility risk for neuropsychiatric disorders such as schizophrenia and depression. Moreover, it has been also found that either neuroleptics or antidepressants administration is capable of affecting expression of NCAM and its polysialylated form, PSA-NCAM protein in the medial prefrontal cortex. The last evidence might have an important meaning for therapeutic effects of antipsychotics, especially in the context of disruption of prefrontal cortex function observed in schizophrenia or depression. In addition, it has been reported that NCAM protein modulates dopaminergic signaling *via* D2 receptor internalization. On the other hand, some evidence indicates that PSA-NCAM protein expression is regulated by D2 receptor activation in the medial prefrontal cortex. Recently, we have also found that cocaine, which is an inhibitor of dopamine reuptake, increases the expression of PSA-NCAM protein in the medial prefrontal cortex enhancing mRNA level of the polysialyltransferase ST8SiaII, but not ST8SiaIV, the enzymes involved in the polysialylation of NCAM. The above effect is abolished by D1 receptor antagonist (SCH23390) or D2 receptor antagonist (raclopride) administration. The obtained results indicate that NCAM/PSA-NCAM expression in the medial prefrontal cortex is regulated by dopaminergic signaling, which might be an important mechanism of the proper control of synaptic activity disrupted in the medial prefrontal cortex in the schizophrenia or depression.

# Age-related macular degeneration, an incurable ophthalmological disease – unraveling a complex pathogenesis and searching satisfying therapies

Jerzy Z. Nowak

Department of Pharmacology, Chair of Pharmacology and Clinical Pharmacology, Medical University of Lodz, Kościuszki 4, 90-419 Łódź, Poland

Age-related macular degeneration (AMD) is a disease with a progressive course, leading to severe deteriorations and loss of vision in the elderly population. Clinically, the disease can manifest as nonexudative-atrophic (dry) form AMD seen in 85–90% of patients, and as exudative-neovascular (wet) form AMD (10–15% of patients), the latter displaying usually dramatic course. AMD pathogenesis is multifactorial, involving a combination of genetic predisposition, the impact of ageing, metabolic and environmental risk factors. Although major abnormalities are seen in four functionally interrelated tissues of the central retina, i.e. photoreceptors, retinal pigment epithelium (RPE), Bruch's membrane and choriocapilaries, the impairment of RPE cell function is an early and crucial event in molecular pathways leading to clinically relevant AMD changes. RPE cells progressively degenerate which results in a progressive irreversible degeneration of photoreceptors. Four processes: lipofuscinogenesis with related oxidative stress, drusogenesis, inflammation and neovascularization (in the case of exudative AMD) specifically contribute to the development of the disease. Lipofuscinogenesis refers to two phenomena: 1. physiological activity of RPE

cells consisting of phagocytic uptake and lysosomal digestion of the constantly shed photoreceptor outer segments and 2. age-dependent lysosomal insufficiency, finally leading to accumulation of lipofuscin aggregates containing mainly an undigested photoreceptor-derived material rich in various retinoids (including photocytotoxic bis-retinoid A2E) and polyunsaturated fatty acids and their peroxidation-derived fragments. Drusogenesis refers to the formation of extracellular deposits of insoluble metarial (called drusen – a hall-mark AMD symptom) accumulating between RPE cells and Bruch's membrane. Proteomic and immunohistochemical analysis of drusen revealed the presence of an array of constituents, including protein elements belonging to the immune system. These discoveries, together with recent genetic studies (showing specific AMD-related polymorphisms in several genes encoding elements of the complement system, e.g. CFH, C3, CFB/C2), reinforced the hypothesis emphasizing the role of a local inflammatory process in the development of AMD. Despite tremendous progress in our understanding of AMD pathogenesis, clinically relevant therapeutic approaches are at present poor, especially in the case of widely occurring dry form AMD, as they do not effectively counteract the progression of the disease. Although the uses of visudine-based photodynamic therapy (PDT) and particularly anti-VEGF antibodies (Lucentis, Avastin for intravitreoues application) in patients with exudative AMD rapidly became more optimistic therapeutic options, these treatments – even when used repeatedly – appear to be only partially effective; they do not prevent the appearance of new neovascular episodes and do not restore pathologically driven disequilibrium between pro- and antiangiogenic factors. In conclusion, more details concerning molecular bases of AMD pathology are urgently needed in order to establish both useful biomarkers enabling early diagnosis of the developing pathology, and clinically effective agents endowed with preventive and therapeutic potency.

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# Statin-mediated protection against amyloid and HIV-1-induced disruption of the blood-brain barrier

Michal Toborek, Bernhard Hennig

Molecular Neuroscience and Vascular Biology Laboratory, Department of Neurosurgery, University of Kentucky Medical Center, Lexington, KY 40536, USA

An increase in the older population infected with HIV-1 is an emerging development in HIV-1 epidemiology. Aging is connected with increased deposition of amyloid beta peptide in the brain. Therefore, we studied the hypothesis that amyloid beta and HIV-1 can potentiate their toxic effects at the blood-brain barrier (BBB) level. Exposure of human brain microvascular endothelial cells (HBMEC) to HIV-1 resulted in a markedly increased amyloid beta accumulation in HBMEC. Simvastatin, the HMG-CoA reductase inhibitor, blocked these effects. We next evaluated the effects of HIV-1 and/or simvastatin on expression of the receptor for lipoprotein related protein (LRP1) and the receptor for advanced glycation end products (RAGE), known to regulate amyloid beta transport across the BBB. LRP1 expression was not affected by HIV-1; however, it was increased by simvastatin. Importantly, simvastatin effectively attenuated HIV-1-induced RAGE expression and protected against HIV-1-induced amyloid accumulation in HBMEC.

We also studied proinflammatory reactions in HBMEC co-cultured with a human astrocyte cell line producing HIV-1 specific protein Tat (SVGA-Tat cells) and exposed to amyloid beta. Such a treatment resulted in a significant upregulation of E-selectin, CCL-2, and IL-6 promoter activities and protein levels as compared to the individual effects of amyloid beta or Tat. However, simvastatin blocked these proinflammatory responses.

These results indicate that HIV-1 can predispose the brain to increased amyloid accumulation. Such effects are pathologically relevant because a combined

exposure to amyloid beta and HIV-1 can synergistically potentiate expression of inflammatory genes in brain endothelial cells. Importantly, statins can provide a beneficial influence by reducing amyloid levels in HBMEC and protect against proinflammatory influence of amyloid beta and HIV-1 at the BBB level.

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