Cascading Glia Reactions: a Common Pathomechanism and Its Differentiated Control by Cyclic Nucleotide Signaling

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ABSTRACT: A pathological glia activation, stimulated by inflammatory proteins, β-amyloid, or brain ischemia, is discussed as a common pathogenic factor for progressive nerve cell damage in vascular and Alzheimer dementia. A critical point seems to be reached, if the cytokine-controlled microglial upregulation causes a secondary activation of astrocytes which loose the negative feedback control, are forced to give up their physiological buffering function, and may add to neuronal damage by the release of nitric oxide (NO) and by promoting toxic β-amyloid formation. A strengthening of the cyclic adenosine-5′,3′-monophosphate (cAMP) signaling exerted a differential inhibition of the stimulatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) released from cultured rat microglia, but maintained the negative feedback signal IL-6; cAMP inhibited also the release of free oxygen radicals (OR) but not of NO. Reinforcement of the NO-induced cyclic guanosine monophosphate (cGMP) increase by blockade of the phosphodiesterase (PDE) subtype-5 with propentofylline counterbalanced the toxic NO action that causes with OR neuronal damage by peroxynitrate formation. In rat cultured astrocytes, a prolonged cAMP elevation favored cell differentiation, the expression of a mature ion channel patter, and an improvement of the extracellular glutamate uptake. Cyclic AMP signaling could be strengthened by PDE blockade and by raising extracellular adenosine, which stimulates A_2 receptor-mediated cAMP synthesis. Via an A_1 receptor-mediated effect, elevated adenosine was found to overcome a deficient intracellular calcium mobilization resulting from an impaired muscarinic signaling at pathologically decreased acetylcholine concentrations. We suggest that pharmaca, which elevate extracellular adenosine and/or block the degradation of cyclic nucleotides, may be used to counteract glia-related neuronal damage in dementing processes.

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INTRODUCTION

Glial cells are cells with a Janus face: supportive and potentially destructive. In particular, the astrocytes fulfill a number of physiological tasks prerequisite for the regular function of neurons. But in conjunction with a pathological microglial activation, they may add to nerve cell damage. Thus, astrocytes are an essential element of the blood-brain barrier providing a protective shield, and they significantly add to the maintenance of the extracellular ion homeostasis by buffering neuronally released excitotoxic transmitters and potassium ions. They are able to produce trophic factors, which support neuronal growth and survival. Activated microglia seem to play a role in determining the architecture of the central nervous system during development as well. But it is unclear whether microglia cells in the adult brain exert a physiological function if they are in a resting state. They represent, however, highly sensitive immunocompetent cells, which are in standby position and may adopt powerful weapons upon pathological activation.[1] This, again, reflects a Janus-faced event, which may be part of a meaningful response of the brain-intrinsic immune system that defends against foreign aggressors or helps to clear up cellular debris by the phagocytotic action of microglia-derived macrophages. But if the pathological glia activation escapes its vigorous endogenous control, it may turn into an autoaggressive pathomechanism that contributes to secondary neuronal damage occurring, e.g., after brain trauma, ischemia or during the course of neurodegenerative diseases.

PATHOGENIC MECHANISMS OF A CASCADING GLIAL CELL ACTIVATION

Reactive microglia are able to release glutamate.[2] This increases the risk of excitotoxic neuronal damage, which will further be aggravated if elevated extracellular glutamate leads to an excessive membrane depolarization of astrocytes and to an impairment of their physiological buffer function. The most powerful microglial weapon is presumably the oxidative burst causing the release of extraordinary high amounts of free oxygen radicals.[3] They form, in conjunction with nitric oxide (NO), the highly aggressive peroxynitrites. The probability of glia-related oxidative damage is further increased by the release of tumor necrosis factor-α (TNF-α), a microglial cytokine that has been reported to cause NO-induced nerve cell death *in vitro*.[4] A critical point seems to be reached if the pathological conditions allow a cascading glial cell activation that is not restricted to microglia but also involves the astrocytes. When astrocytes are forced by the microglial cytokine interleukin-1β (IL-1β) to undergo secondary activation, they not only have to give up physiological functions coupled to their mature differentiated state, but may even cooperate with microglia in enhancing oxidative stress. Choi's group has shown that stimulation of astrocytes with the microglial cytokine IL-1β and interferon-γ potentiated neuronal injury by activation of the inducible NO synthase.[5] The damage produced by the pathologically increased NO production of astrocytes resulted from an increased peroxynitrite formation in the presence of oxygen radicals, as can be provided by activated microglia and microglia-derived macrophages. Thus, the neurotoxic potential of a patho-

logical glia activation seems to be determined by functional interactions between microglia and astrocytes.

A pathogenic role of a cascading microglia/astrocyte activation in neurodegenerative diseases is supported by their almost obligatory presence in the periphery of β-amyloid plaques in the brains of Alzheimer disease patients.[6] Only the dense core plaques, associated with dying neurons, but not the diffuse amyloid deposits were apolipoprotein E (ApoE)-positive.[7,8] Reactive astrocytes have been identified to be the brain endogenous producers of those inflammatory chaperones, like ApoE, which promote the formation of toxic β-amyloid.[9,10] An upregulated expression of the amyloid precursor protein (APP) in activated glial cells can be expected to potentiate the pathologically β-amyloid load,[11] particularly since free oxygen radicals, released at large amounts from reactive microglial cells, are known to aggravate the pathological APP processing by C-terminal oxidation.[12] It follows that a cascading glial cell activation, involving microglia and astrocytes, may largely contribute to the formation of toxic β-amyloid, which can be regarded as common pathogenic factor for the deleterious plaque formation in Alzheimer disease as well as for the amyloid angiopathy contributing to vascular dementia.

TRIGGERS OF GLIAL CELL REACTIONS

Besides of the direct cytotoxicity exerted by β-amyloid,[13] its genetically favored generation may be one of the triggers that initiates pathological glia activation. There is evidence that plaque-derived β-amyloid provides a docking site for microglia, which then are stimulated to transform from quiescent to neurotoxic cells. As a consequence, they secrete a lipophilic amine (Ntox), which was found to aggravate glutamate-induced excitotoxic damage of neurons, presumably by modifying the *N*-methyl-D-aspartate (NMDA) receptor function.[14]

Another trigger, which effectively activates microglia, is brain ischemia—the primary pathogenic factor of vascular dementia and also known to intensify the dementing process in Alzheimer disease. An ischemia-induced energy breakdown leads to adenosine triphosphate (ATP) degradation and disturbance of the ion homeostasis. The resulting extracellular potassium rises can be expected to elicit a strong depolarizing inward current through microglia-specific potassium channels,[15] followed by a massive calcium influx that may serve as a trigger for consecutive cell activation.[16] There is evidence that the ATP breakdown product, adenosine, participates in activating resting microglia by stimulating the arachidonic acid pathway via Gs protein-coupled A_2 receptors.[17] Since microglia have complement receptors as well, activation of the classical complement cascade by initial cell damage and reactive brain inflammation could be another possible trigger.[18]

Following experimental brain ischemia, an obvious microglial activation is already seen within the first hours, reflected by the initiation of cell proliferation and by the expression of specific surface antigens.[19] Among those, the expression of the major histocompatibility (MHC)-complexes enables these brain intrinsic glial cells to communicate with the general blood cell-linked immune system. Reactive changes of astrocytes—characterized by a marked cell hypertrophy, increased glial fibrillary acidic protein (GFAP) content, and retraction of processes—were not seen

before the second day after transient brain ischemia.[20] This underlines that the pathogenetically significant activation of astrocytes is a secondary event in a cascade that starts with the activation of microglia.

ENDOGENOUS CONTROL OF GLIAL REACTIONS

There is evidence that astrocytes—via the cytokine transforming growth factor-β (TGF-β)—exert a negative feedback regulation of activated microglial cell functions.[21] This serves presumably as an important endogenous mechanism by which the dangerous peroxynitrite production can be controlled and restricted to the microglia. In order to specify the working parameters of this astrocyte-related control system, we recently studied the NO generation in cultured rat glial cells using lipopolysaccharide (LPS) and interferon-γ as pathological stimulators. The observed powerful inhibitory effect of astrocytes on the massive microglial NO release turned out to be dependent on the astrocytic differentiation state. Conditioned medium, which had been harvested from confluent and low proliferative rat astrocyte cultures, effectively inhibited microglial NO release. But if we used astrocyte-conditioned medium from nondifferentiated and highly proliferative cultures, which resembled pathologically activated astrocytes, the inhibitory effect was largely reduced or missing (unpublished data).

This suggests that a powerful endogenous control of the potentially neurotoxic NO release from activated microglia can be overrun, if the astrocytes are forced to

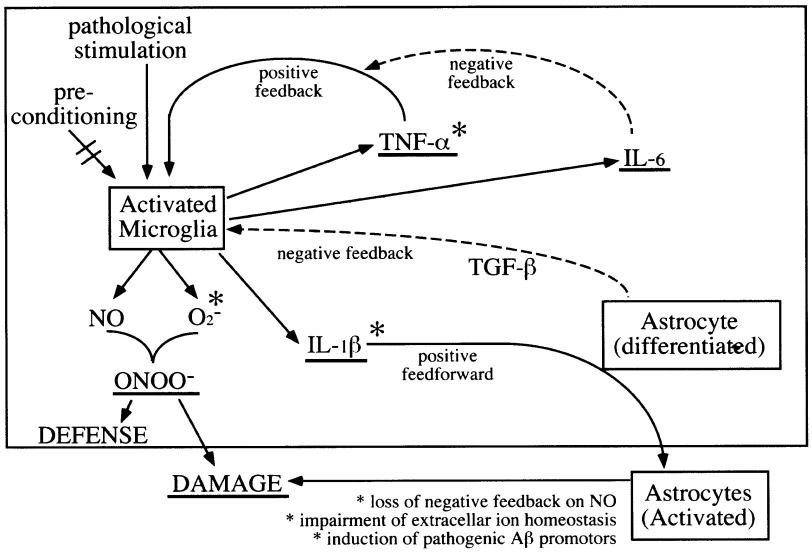

FIGURE 1. Endogenous control of a cascading activation of microglial cells and astrocytes.

undergo dedifferentiation upon pathological activation. Whether or not such a cascading glial activation occurs depends on the level of microglial activation. This is a graded response controlled by the interplay of cytokines that are released from microglia and astrocytes (FIG. 1). Among those, TNF-α acts as a positive feedback signal, which helps to reinforce glial cell activation (see also Ref. 22). As a consequence, the reactive microglia may reach an upgraded level of activation, which enables them to produce IL-1β, the cytokine promoting the feed-forward activation of astrocytes (see FIG. 1). This escalating cascade, however, is also under inhibitory control, e.g., by the cytokine IL-6, which has been shown to exert a negative feed back on TNF-α signaling.[23]

It follows that an inhibition of the stimulatory signals and the maintenance or reinforcement of the inhibitory signal loops may be a possible strategy to prevent an escalating microglial activation that leads to the critical secondary activation of astrocytes. Another chance of interference would be a conditioning of reactive microglial cells that brings them back into a less activated state or the induction of cellular apoptosis. The latter represents a commonly used emergency control by which nonadequately activated and dangerous immune-effective cells can be eliminated.

INDUCTION OF MICROGLIAL APOPTOSIS BY ADENOSINE AND PRECONDITIONING BY REINFORCED cAMP SIGNALING

Immature microglial cells, obtained from the brains of newly born rats, maintain in culture their activated state, which allows them to proliferate and to transform *in vitro* spontaneously into free radical-generating macrophages. They may therefore provide a reliable model to investigate the pathological properties of microglial cells in relation to their activation state and to test possible modes of interference. Similar to immune-activated cells of the general defense system, cultured microglia were sensitive to apoptosis, and adenosine was found to be an effective trigger.[24] The induction of apoptosis, verified by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) staining, DNA ladder formation, and quantitative measurements of intracellular DNA fragmentation, required elevated concentrations of adenosine up to 10 micromolar. This raises the possibility to eliminate potentially neurotoxic microglia by pharmaca, which increase extracellular adenosine by blocking its cellular reuptake.

The sensitivity of microglial cells to adenosine-induced apoptosis is apparently related to the degree of activation and was lost when the microglial cells had been brought into a less proliferative state. This could be achieved by several days' pretreatment with the membrane-permeable dibutyryl-cAMP or with propentofylline (Aventis), a selective phosphodiesterase (PDE) inhibitor. The findings suggest that conditioning of microglial cells by prolonged strengthening of the cAMP-dependent signaling brings them into a less activated and probably less dangerous state, which no longer requires the emergency control by inducible apoptosis. Such a preconditioning also reduced the capacity of cultured microglia to generate NO in response to pathological stimulation (unpublished).

DIFFERENTIAL CONTROL OF CYTOKINE AND OXYGEN RADICAL RELEASE FROM MICROGLIA BY cAMP

Acute strengthening of the cAMP signaling was found to alter the pattern of microglia-released cytokines in a way that can be expected to reduce the neurotoxic power and to add to neuroprotection. Specifically, the LPS-induced release of TNF-α and IL-1β from cultivated rat microglia was markedly inhibited by treatment with dibutyryl-cAMP or propentofylline, whereas the IL-6 release was unaffected or even increased.[25] This means that those cytokines, which tend to stimulate a cascading glial reaction, are suppressed, whereas the negative feedback control remains functioning (see FIG. 1).

Interestingly, such a differential modulation by cAMP was also observed for the stimulated release of NO and oxygen radicals. The latter were significantly depressed, but not the NO release. This should protect against oxidative neuronal damage by preventing the NO-stimulated formation of toxic peroxynitrates, which depends on the availability of oxygen radicals.

GUIDING THE AMBIGUOUS ACTION OF NITRIC OXIDE INTO A cGMP-LINKED PROTECTION

Nitric oxide is an ambivalent molecular signal. It serves as substrate for the formation of toxic peroxynitrate, and it stimulates the cyclic guanosine monophosphate (cGMP) synthesis by guanylcyclase. Mimicking a pathologically increaeased NO load by treatment with nitroprusside caused nerve cell death in microglia-containing

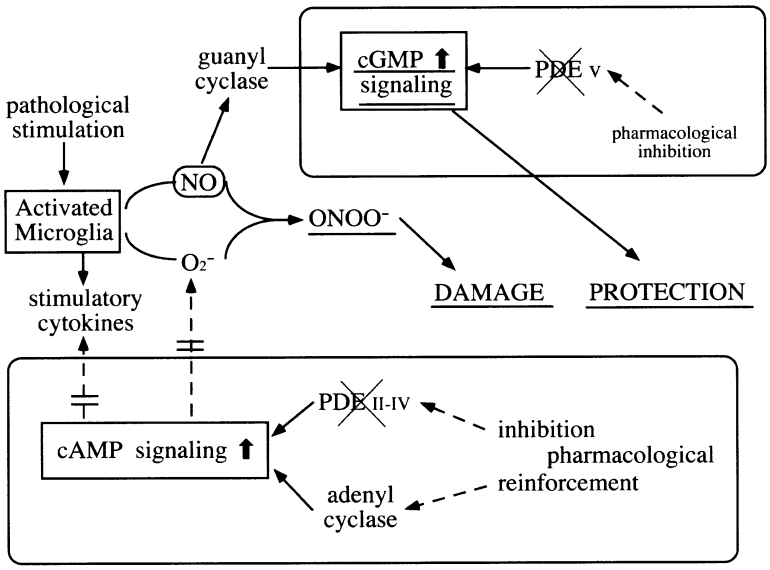

FIGURE 2. Modes of pharmacological interference.

cultures of spinal cord neurons. The damage was prevented in the presence of oxygen radical scavengers indicating that peroxynitrate formation is the NO-induced pathomechanism that can be inhibited by depressing concomitant oxygen radical generation. Protection could further be achieved by strengthening the cGMP-enhancing NO path, i.e., by treatment with the PDE inhibitor propentofylline, which blocks the cGMP degrading subtype-5.[26] Accordingly, protection was seen in the presence of membrane-permeable cGMP analogues.

Taken together, these findings suggest that oxidative neuronal damage resulting from the release of NO and oxygen radicals from pathologically activated glial cells can be counteracted in two ways: the one is to strengthen cAMP signaling, which can be expected to depress microglial activation, their transformation into macrophages, and the related free radical generation; the other is a pharmacologically supported shift of the ambivalent NO action from the peroxynitrate-generating path to a preferential formation of cGMP, which can further be increased by pharmacological blockade of cGMP degradation (see FIG. 2).

MAINTENANCE OF PHYSIOLOGICAL ASTROCYTE FUNCTIONS

A strengthening of cAMP signaling not only reduces the risk of secondary astrocyte activation by microglial IL-1β release, but also seems to influence the astrocytic activation state directly. Cultured astrocytes from the embryonic rat brain resemble pathologically activated astrocytes. This is indicated by a high proliferation rate, a nondifferentiated phenotype lacking cell processes, and an immature ion channel pattern. A prolonged treatment with dibutyryl-cAMP for several days favored the formation of process-rich stellate cells and also induced the new expression of specific potassium and chloride ion channels.[27] These channels belong to the repertoire of mature astrocytes and are required to stabilize the membrane potential and the physiological buffering function of astrocytes. Accordingly, the capacity for the uptake of extracellular glutamate could be improved in astrocyte cultures by a prolonged rise of intracellular cAMP. This was achieved by treatment with an adenosine analogue in conjunction with the PDE-blocker propentofylline (Ogata *et al.*, manuscript in preparation). Treatment with propentofylline has further been reported to increase the release of nerve growth factor from astrocytes.[28]

We conclude from such observations that a prolonged pharmacological strengthening of the cAMP-dependent signaling may help to bring back pathologically activated astrocytes into a more differentiated state. This is supposed to recover physiological astrocyte functions, i.e., the maintenance of the extracellular ion homeostasis, the feedback control of activated microglial functions, and the release of trophic factors, but to reduce the contribution of reactive astrocytes to oxidative damage or to β-amyloid related toxicity.

CONCLUDING REMARKS

Evidence is increasing that the concomitant pathological activation of glial cells plays a pathogenic role for the generation of progressive nerve cell damage in Alzheimer and vascular dementia. Therefore, an interference with the escalating ac-

tivation of microglial cells and with the deleterious secondary involvement of astrocytes could provide a target for the pharmacological treatment. The complex pathomechanism implicating several interwoven vicious circles, which overrun the endogenous control of the glia-related immune system, is not completely clarified. We therefore think, it is a good strategy to focus on a central point of the molecular signaling chains that mediate the alterations of glial cell properties upon pathological stimulation. Such a bottleneck, where the receptor-mediated input from different agonists merges before being transduced to the diverse cellular effectors, is the sophisticated information processing at the second messenger level. There is evidence that the interplay of the second messengers calcium and cAMP is altered in dementing processes. Thus, the Gs-protein coupled synthesis of cyclic nucleotides has been reported to be impaired in Alzheimer disease,[29] and brain ischemia has been found to upregulate those metabotropic glutamate receptors that favor an intracllular calcium mobilization and an inhibition of the cAMP synthesis.

One may speculate that such an imbalance of the second messengers and a reduced cAMP signaling plays a role in executing pathological glial cell functions. This could explain the observed inhibitory effect of a pharmacologically strengthened cAMP signaling on the cytokine-controlled microglial upregulation, on secondary astrocyte reactions, and on related potentially neurotoxic functions. An effective and prolonged intracellular increase of cAMP and cGMP may be achieved by treatment with the respective PDE inhibitors. In addition, an elevation of the extracellular adenosine concentration up to a level that stimulates the A_2 receptor-mediated cAMP synthesis in glial cells can be obtained by pharmaca blocking the uptake of adenosine from the extracellular space. In recent experiments on cultured rat cortical astrocytes, elevated adenosine was also found to overcome a deficient intracellular calcium mobilization that resulted from pathologically reduced acetylcholine concentrations (manuscript in preparation). Since the latter represents a key symptom of Alzheimer disease, the restoration of a deficient muscarinergic signaling by adenosine raising pharmaca may provide a complimentary treatment, in addition to the conventional use of acetylcholinesterase inhibitors (for details, see reviews in Refs. 30 and 31).

REFERENCES

1. BANATI, R.B., J. GEHRMANN, P. SCHUBERT & G.W. KREUTZBERG. 1993. Cytotoxicity of microglia. Glia **7:** 111–118.
2. KLEGERIS, A. & P.L. MCGEER. 1994. Inhibition of respiratory burst in macrophages by complement receptor blockade. J. Neuroimmunol. **260:** 273–277.
3. BANATI, R., P. SCHUBERT, G. ROTHE, J. GEHRMANN, K.A. RUDOLPHI, G. VALET & G.W. KREUTZBERG. 1994. Modulation of intracellular formation of reactive oxygen intermediates in peritoneal macrophages and microglia/brain macrophages by propentofylline. J. Cereb. Blood Flow Metab. **14:** 145–149.
4. MEDA, L., M.A. CASSATELLA & I. SZENDREI. 1995. Activation of microglial cells by β-amyloid protein and interferon-γ. Nature **374:** 647–650.
5. HEWETT, S.J., C.A. CSERNANSKY & D.W. CHOI. 1994. Selective potentiation of NMDA induced neuronal injury following induction of astrocytic iNOS. Neuron **13:** 487–494.
6. MCGEER, P.L., T. KAWAMATA, D. WALKER, H. ABYAMA, I. TOYAMA & E.G. MCGEER. 1993. Microglia in degenerative neurological disease. Glia **7:** 84–92.
7. WISNIEWSKY, T. & B. FRANGIONE.1992. Apolipoprotein E: a pathological chaperone in patients with cerebral amd systemic amyloid. Neurosci. Lett. **135:** 235–238.

8. SHENG, J.G., R.E. MRAK & W.S. GRIFFIN. 1996. Apolipoprotein E distribution among different plaque types in Alzheimer disease: implications for its role in plaque progression. Neuropathol. Appl. Neurobiol. **22:** 334–341.
9. MA, J., A. YEE, H.B. BREWER, S. DAS & H. POTTER. 1994. Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of beta-protein into filaments. Nature **372:** 92–94.
10. BOWMAN, B.H., F. YANG, J.M. BUCHANAN, G.S. ADRIAN & A.O. MARTINEZ. 1996. Human APOE protein localized in brains of transgenic mice. Neurosci. Lett. **219:** 57–59.
11. FORLONI, G., F. DEMICHELI, S. GIORGI, C. BENDOTTI & N. ANGERETTI. 1992. Expression of amyloid precursor protein mRNAs in endothelial, neuronal and glial cells: modulation by interleukin-1. Mol. Brain Res. **16:** 128–134.
12. DYRCKS, T., E. DYRCKS, C.L. MASTERS & K. BEYREUTHER. 1993. Amyloidogenicity of rodent and human β A_4 sequences. FEBS Lett. **324:** 231–236.
13. BEHL, C., J.B. DAVIS, R. LESLEY & D. SCHUBERT. 1994. Hydrogen peroxide mediates amyloid β protein toxicity. Cell **77:** 817–827.
14. GIULLIAN, D. 1998. A strategy for identifying immunosuppressive therapies for Alzheimer disease. Alzheimer Dis. Assoc. Disord. **12**(Suppl. 2)**:** 7–14.
15. KETTENMANN, H., D. HOPPE, K. GOTTMANN, R. BANATI & G.W. KREUTZBERG. 1990. Cultured microglia have a distinct pattern of membrane channels different from peritoneal macrophages. J. Neurosci. Res. **26:** 278–287.
16. ILLES, P., W. NÖRENBERG & P.J. GEBICKE-HAERTER. 1996. Molecular mechanisms of microglial activation. Neurochem. Int. **1:** 13–26.
17. FIEBICH, B.L., K. BIBER, K. LIEB, D. VAN CALKER, M. BERGER, J. BAUER & P.J. GEBICKE-HAERTER. 1996. Cyclooxygenase-2 expression in rat microglia is induced by adenosine A_{2a}-receptors. Glia **18:** 152–160.
18. AKIYAMA, H. & P.L. MCGEER. 1990. Brain microglia constitutively express β-2 integrins. J. Neuroimmunol. **30:** 81–93.
19. MORIOKA, T., A.N. KALEHUA & W.J. STREIT. 1991. The microglial reaction in the rat dorsal hippocampus following transient forebrain ischemia. J. Cereb. Blood Flow Metab. **10:** 850–859.
20. DELEO, J., L. TÓTH, P. SCHUBERT, K. RUDOLPHI & G.W. KREUTZBERG. 1987. Ischemia-induced neuronal cell death, calcium accumulation and glial response in the hippocampus of the gerbil and protection by the xanthine derivative HWA 285. J. Cereb. Blood Flow Metab. **7:** 745–752.
21. VINCENT, V.A.M., F.J.H. TILDERS & A.M. VAN DAM. 1997. Inhibition of endotoxin-induced oxide synthase production in microglial cells by the presence of astroglial cells: a role for transforming growth factor β. Glia **19:** 190–198.
22. WEKERLE, H., C. LININGTON, H. LASSMANN & R. MEYERMANN. 1986. Cellular immune reactivity within the CNS. TINS **9:** 271–277.
23. ADERKA, D., J.M. LE & J. VILEK. 1989. IL-6 inhibits LPS-induced tumor necrosis factor production in cultured human monocytes. J. Immunol. **143:** 3517–3523.
24. OGATA, T. & P. SCHUBERT. 1996. Programmed cell death in microglia is controlled by extracellular adenosine. Neurosci. Lett. **218:** 91–94.
25. SI, Q., Y. NAKAMURA, T. OGATA, K. KATAOKA & P. SCHUBERT. 1998. Differential regulation of microglial activation by propentofylline via cAMP signaling. Brain Res. **812:** 97–104.
26. OGATA, T., S. KOHGAMI, H. OKUMURA, T. SHIBATA, Y. NAKAMURA, K. KATAOKA & P. SCHUBERT. 1998. Nitric oxide-induced neurotoxicity is inhibited by propentofylline via cyclic GMP elevation [Abstract]**.** Neurobiol. Aging **19:** S255.
27. FERRONI, S., C. MARCHINI, P. SCHUBERT & C. RAPISARDA. 1995. Two distinct inward rectifying conductances are expressed in cultured rat cortical astrocytes after long term dibutyryl-cyclic-AMP treatment. FEBS Lett. **267:** 319–325.
28. SHINODA, J., Y. FURUKAWA & S. FURUKAWA. 1990. Stimulation of nerve growth factor synthesis/secretion by propentofylline in cultured mouse astroglial cells. Biochem. Pharmacol. **39:** 1813–1816.
29. O'NEILL, C., C.J. FOWLER, B. WINBLAD & R.F. COWBURN. 1994. G-protein coupled signal transduction systems in the Alzheimer's disease brain. Biochem. Soc. Trans. **22:** 167–171.

30. SCHUBERT, P., K. RUDOLPHI, F. FREDHOLM & Y. NAKAMURA. 1994. Modulation of nerve and glial cell function by adenosine—role in the development of ischemic brain damage. Int. J. Biochem. **26:** 1227–1236.
31. SCHUBERT, P., T. OGATA, H. MIYAZAKI, C. MARCHINI, S. FERRONI & K. RUDOLPHI. 1998. Immuno-reactions of glial cells in Alzheimer's disease and possible sites of interference. J. Neural Transm. Suppl. **54:** 167–174.