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Modulation of Glial Cell Signaling by Adenosine and Pharmacological Reinforcement

A Neuroprotective Strategy?

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ABSTRACT

In view of the increasing evidence that a pathological glial activation plays a significant role in the development of neurodegenerative diseases, we investigated the underlying molecular signaling as a possible target for a pharmacological therapy. Here, we are particularly focusing on the endogenous modulation of the Ca^{2+} and cyclic nucleotide -dependent signaling by the nucleoside adenosine and its reinforcement by the xanthine derivative propentofylline (PPF). As an experimental model, we used cultured rat microglial cells and astrocytes that are immature, show a high proliferation rate, and resemble in several aspects pathologically activated glial cells. A prolonged increase of the cellular cAMP level favored the differentiation of cultured astrocytes and associated properties required for the physiological nerve cell function. On the other hand, a strengthening of the cyclic nucleotide-dependent signaling inhibited potentially neurotoxic properties of cultured microglial cells. Similar effects were

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obtained by treatment with propentofylline, which mimicked modulatory adenosine effects and increased the intracellular level of cAMP and cGMP. Such a pharmacological glial cell conditioning, obtained by modifying the strength and the timing of these second messengers, may provide a therapy of neurodegenerative diseases in which a pathological activation of microglial cells and astrocytes is discussed to play a pathogenic role.

Index Entries: Adenosine, propentofylline; neuroprotection; cyclic AMP, pathological activation astrocytes; microglia; Alzheimer disease.

INTRODUCTION

The development of dementia during the course of neurodegenerative diseases results from a progressive nerve cell damage that is accompanied by a marked glial cell reaction. Since nerve- and glial cell functions are intimately related, an alteration of the physiological glial cell properties owing to their pathological activation may significantly add to neuronal damage. Reactive microglial cells, so called activated microglia, are the immunecompetent cells of the brain, and they produce potentially neurotoxic substances, such as NO, free oxygen radicals, and various cytokines (reviewed by Banati et al., 1993). On the other hand, regulatory functions of differentiated astrocytes are required to maintain the physiological nerve-cell activity. For example, astrocytes limit membrane depolarizations by the uptake of glutamate and potassium, released into the extracellular space upon nerve cell firing. An excessive depolarization would reduce the buffering capacity of astrocytes, favor seizure generation, and a pathological Ca^{2+} loading of neurons owing to an uncontrolled Ca^{2+} influx through voltage and NMDA receptor-operated ion channels. The delivery of various trophic factors from astrocytes may further add to the protection of neurons against structural and functional impairment. Therefore, maintaining or reinforcing the properties of differentiated astrocytes and preventing a hyperergic activation of microglial cells can be expected to protect neurons under pathological conditions.

Cell activation and differentiation are initiated and controlled by a complex network of molecular signals that, in turn, are influenced by endogenous cell modulators. A pathologically increased extracellular glutamate concentration leads to the stimulation of different metabotropic receptors that inhibit the cellular cAMP formation and/or favor the mobilization of Ca^{2+} from intracellular stores. This stimulates the Ca^{2+}-dependent molecular signaling cascade and may cause cell activation, whereas a critical elevation of the cellular cAMP content seems to exert a negative feed back favoring cell differentiation (Nishizuka, 1986).

EFFECTS ON ASTROCYTES

The endogenous cell modulator adenosine influences both second messengers, Ca^{2+} and cAMP in nerve- and glial cells (for a review, *see* Schubert et al., 1994). We investigated the influence of adenosine on these molecular signals in cultured hippocampal astrocytes from the embryonic rat hippocampus. The so-called type 1 astrocytes, which nearly developed any cell processes during prolonged cultivation, were immature, showed a high proliferation rate, and resembled pathologically activated astrocytes in this respect. In our experiments, a pathological activation of metabotropic glutamate receptors was mimicked by stimulating the astrocyte cultures with the selective glutamate receptor against agonist t-ACPD. Under these conditions, adenosine initiated a large intracellular Ca^{2+} mobilization via an A_1 receptor-mediated action (Ogata et al., 1994). Surprisingly, this was accompanied by a Ca^{2+}-dependent potentiation of the cellular cAMP content (Ogata et al., 1996). A sustained intracellular cAMP elevation (by prolonged treatment of cortical astrocyte cultures with the membrane-permeable dibutyryl-cAMP) induced the development of far extending cell processes rich in glial fibrillary acidic protein. The formation of such stellate cells, which resemble resting astrocytes in the mature brain, is taken as a morphological correlate of astrocyte differentiation. Patch clamp experiments revealed that these stellate cells had acquired newly formed K^+ and Cl^- channels that opened at resting potential (Ferroni et al., 1995). They were absent in nontreated cultures, but are also present in the differentiated astrocytes of the normal brain. The availability of such Cl^- channels allows a cellular influx of negatively charged ions, which supports the physiological uptake of extracellular K^+ by astrocytes and may also protect against a depolarization-induced blockade of the glutamate uptake. It follows that the maintenance of the physiological nerve cell activity by the homeostatic function of astrocytes largely depends on their state of differentiation. Our findings show that a strengthening of the cAMP-dependent signaling favored the differentiation of the immature cultured astrocytes, which resemble pathologically activated astrocytes in several aspects. Therefore, a pharmacological reinforcement of the intracellular cAMP signaling may allow regaining or even improvement of the neuroprotective properties in pathologically activated astrocytes by leading them back to differentiation.

A pharmacon that elevates the cellular cAMP level is the xanthine derivative propentofylline (PPF), which has previously been shown to protect neurons against ischemic damage (DeLeo et al., 1987). By blocking specific membrane transporters, PPF raises the extracellular concentration of adenosine, which favors the A_2 receptor-mediated cAMP formation (Parkinson and Fredholm, 1991), and inhibits the breakdown of cAMP by an apparently highly selective blockade of phosphodiesterases (Stefanovich, 1985; Meskini et al., 1994). When cultured astrocytes were treated with PPF

for 10 d, they showed a similar but less pronounced transition into differentiated stellate cells as observed after the treatment with dibutyryl-cAMP (unpublished). PPF has been reported to increase the release of the nerve growth factor (NGF) in cultured astrocytes (Shinoda et al., 1990), and we have emerging evidence that it stimulates the mRNA formation of several trophic factors. In addition, PPF seems to maintain differentiated astrocyte functions also by inhibiting the initiation of a pathological astrocyte activation. In vivo experiments revealed that pretreatment with PPF prevented the ischemia-induced astrocyte reaction (DeLeo et al., 1987). This could be an indirect effect that results from a primary inhibition of the pathological activation of microglial cells known to trigger astrocyte reaction by the release of interleukin-1 (Giulian et al., 1986).

EFFECTS ON MICROGLIA

We tested the possibility that adenosine and PPF also exert an analog modulatory influence (as observed on astrocytes) on microglial cells. The experiments were performed on cultivated rat microglial cells. They showed strong immunostaining with antibodies against the major histocompatibility complex 1, continued to proliferate, and transformed into full blown macrophages during prolonged culturing. This indicates that the cultivated microglial cells were in an activated state. Their proliferation rate could be considerably increased by the addition of tumor-promoting phorbol esters, suggesting that a pathological stimulation of the Ca^{2+}-dependent protein kinase-C activation increases the microglia reaction. If tested under these conditions, low micromolar concentrations of adenosine or PPF inhibited the proliferation of microglial cells by more than 60% (in cooperation with Prof. Kataoka's group; Si et al., 1995). Although changes in intracellular Ca^{2+} and cAMP have so far not been determined in microglial cells, it seems likely that the observed effects of adenosine and PPF are mediated by the signaling cascade controlled by these second messengers. Acute treatment with PPF inhibited the particularly high production of free oxygen radicals in cultured microglia-derived macrophages and reduced the amount of lipid peroxidation in rat hippocampal slices (Schubert et al., 1992; Banati et al., 1994). This could exert a neuroprotective effect, which may imply an inhibition of the C-terminal oxidation of the β-amyloid precursor protein, shown to be induced in vitro by reactive oxygen intermediates (Dyrcks et al., 1993). The supposed pathogenic role of activated microglial cells in Alzheimer disease is supported by the finding that cerebrospinal fluids from several Alzheimer patients showed a positive immune reaction with activated microglial cells (McRae et al., 1993, 1995). It should be noted that this reaction was even seen in microglial cells from another species (gerbil) upon pathological activation by transient brain ischemia. Daily posttreatment with PPF, started 24 h after the initiation of the ischemia-induced microglial reaction, almost

prevented the immunostaining with cerebrospinal fluids from Alzheimer patients (McRae et al., 1994). This suggests that a prolonged PPF treatment also changes the properties of microglial cells, which have been activated during the course of an ongoing pathological process. The latter is supported by our observation that a chronic treatment of primarily activated cultured microglial cells with PPF prevented their further transformation into full blown macrophages.

CONCLUSION

The findings indicate that pathological glial cell reactions are influenced by the power of their cAMP-dependent signaling, which tends to inhibit potentially neurotoxic effects of activated microglial cells and maintain or regain neuroprotective properties linked to astrocyte differentiation. A strengthening of the cAMP-dependent signaling can apparently be achieved by pharmaca, which reinforce or mimick the action of adenosine. Such a pharmacological conditioning of glial cells, using the evolutionary know-how of this endogenous cell modulator, may provide a neuroprotective therapy of neurodegenerative disorders with a minor risk of side effects.

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