MINIREVIEW

Prostaglandins as Putative Neurotoxins in Alzheimer's Disease (44323)

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Abstract. **Chronic inflammatory reactions in the brain appear to be one of the primary etiological factors in the pathogenesis of Alzheimer's disease (AD). This is supported by the fact that the secretory products of inflammatory reactions, which include cytokines, complement proteins, adhesion molecules, and free radicals, are neurotoxic. We have recently reported that prostaglandins (PGs), which are also released during inflammatory reactions, cause rapid degenerative changes in differentiated murine neuroblastoma cells (NB) in culture. PGA_1 is more effective than PGE_1. Similar observations were made in a primary culture of fetal rat hippocampal cells. Epidemiological and clinical studies on AD also support the involvement of PGs in neuronal degeneration. Thus, we propose a hypothesis that PGs are one of the major extracellular signals that initiate neuronal degeneration, which is mediated by intracellular signals such as the β-amyloid peptide (Aβ) and ubiquitin, since the levels of these proteins are increased by PG treatment. We further suggest that adenosine 3′, 5′-cyclic monophosphate (cAMP) is one of the factors that regulate the levels of both Aβ and ubiquitin in NB cells. Increases in the level of Aβ in NB cells following an elevation of intracellular cAMP levels appear to be due to an increase in the rate of processing of the amyloid precursor protein (APP) rather than an increase in the expression of APP. The mechanisms underlying Aβ-induced neuronal degeneration have been under intense investigation, and several mechanisms of action have been proposed. We postulate that PG-induced elevation of Aβ may lead to an increased binding of Aβ to the 20S proteasome, resulting in a reduction of 20S proteasome-mediated degradation of ubiquitin-conjugated proteins. This is predicted to lead to an increase in an accumulation of abnormal proteins, which ultimately contribute to neuronal degeneration and death. Based on our hypothesis and on studies published by others, we propose that a combination of nonsteroidal anti-inflammatory drugs, which inhibit the synthesis of PGs, and antioxidant vitamins, which quench free radicals and both of which have been recently reported to be of some value in AD treatment when used individually, may be much more effective in the prevention and treatment of AD than the individual agents alone.** [P.S.E.B.M. 1998, Vol 219]

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It is estimated that 5%–11% of the U.S. population over the age of 65 and approximately 47 percent of the population over the age of 85 suffer from Alzheimer's Disease (AD) (1–3). Approximately 2–4 million Americans have been diagnosed with AD, many of whom require institutional care at a cost that is estimated to exceed 50 billion dollars.

The involvement of inflammatory reactions in the pathogenesis of AD was first noted by Alois Alzheimer himself. Later, it was shown that the products of inflamma-

tory reactions, such as cytokines (4–7), complement proteins (8–16), free radicals (17–24), and adhesion molecules (14, 25–28), are neurotoxic. Therefore, these may represent extracellular signals that initiate neuronal degeneration in AD through several intracellular signals. Among the intracellular signals, β-amyloid (Aβ) (29–33), ubiquitin (34, 35), and presenilins (PS) I and II (36, 37) are considered to be important in initiating neuronal degeneration. Several reviews on the involvement of Aβ, in particular, have been published (29–33). We have recently reported that prostaglandins (PGs), which are also produced and secreted during inflammatory reactions, induce rapid degeneration in differentiated murine neuroblastoma (NB) cells in culture (38, 39). The purpose of this minireview is to discuss experimental, clinical, and epidemiological data that support our hypothesis that PGs may also be one of the critical external signals that initiate degeneration of nerve cells in AD.

Prostaglandins E_1 and A_1 Induce Differentiation and Degeneration in Cultured Nerve Cells

In the early 1970s, we were the first to discover that PGE_1, a stimulator of adenylate cyclase, induces terminal differentiation in NB cells in culture by increasing the intracellular levels of cAMP (40). Subsequently, it was found that both the PGE and PGA series are effective inducers of differentiation, whereas other PGs, such as PGF2α, are not (41). Other agents that elevate intracellular levels of cAMP such as 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO20-1724), an inhibitor of cyclic nucleotide phosphodiesterase, and dibutyryl cAMP, an analog of cAMP, produce similar effects on differentiation of NB cells (41). In addition to inducing NB cell differentiation, PGE_1 was found to inhibit the growth of certain non-neuronal tumor cells in culture (42–44). The neurotoxic effect of PGE_1 was discovered accidentally. In an effort to generate a 100% terminally differentiated NB cell culture for neural transplant studies, we used either a polar carotenoid, initially referred to as β-carotene (45), or α-tocoperol succinate (α-TS, vitamin E) in combination with either R020-1724 or PGE_1. The number of terminally differentiated cells increased from 40%–60% to over 95% at 3 days after each of the above treatments (46). In order to assess the viability of differentiated NB cells *in vitro* after achieving a maximal level of differentiation, the cultures treated with R020-1724 plus a polar carotenoid or PGE_1 plus a polar carotenoid were observed for an additional 3 days (Table I). The results showed that almost all of the differentiated NB cells treated with PGE_1 were either dead or degenerated. In contrast, most of the differentiated cells treated with R020-1724 were still viable (38–46). This suggested that PGE_1 could act as a neurotoxin for differentiated NB cells. To substantiate this finding, NB cells were maximally differentiated by treatment with either R020-1724 plus the polar carotenoid or dibutyryl cAMP plus the polar carotenoid for a period of 3 days prior to treatment with PGE_1. Marked degenerative changes were seen in the differentiated NB cells treated with PGE_1 as early as 1 day after treatment. At 3 days after PGE_1 treatment, most of the differentiated cells were either dead or degenerated (39). When similar experiments were performed with PGE_2, PGA_1, or PGA_2, it was found that the PGA series was more effective in causing neuronal degeneration than the PGE series (39). In order to establish whether PGE_1 and PGA_1 induce degeneration in hippocampal neurons, which are the primary targets of degeneration in AD, the effect of these PGs on neuronal degeneration in these cells was studied. Results showed that PGE_1 and PGA_1 induce apoptosis in a primary culture of fetal rat hippocampal cells in a manner similar to that observed in differentiated NB cells. Similarly, PGA_1 was also found to be more effective than PGE_1 in this primary culture (Clarkson ED, La Rosa FG, and Prasad KN, unpublished observations).

Table I. Effects of Prostaglandins (PG) on Differentiation and Degeneration of Neuroblastoma (NB) Cells in Culture

Treatment	Time	Percentage of differentiation	Percentage of degeneration
RO20-1724 + PC	4 days	92 ± 2	5 ± 2
PGE_1 + PC	4 days	95 ± 3	41 ± 3
PGA_1 + PC	4 days	77 ± 4	72 ± 3
RO20-1724 + PC	6 days	91 ± 2	12 ± 2
PGE_1 + PC	6 days	90 ± 3	60 ± 3
PGA_1 + PC	6 days	0	100

Note. RO20-1724 (200 μg/ml); Polar Carotenoid (PC, 20 μg/ml); Prostaglandin E_1 (PGE_1, 10 μg/ml); Prostaglandin A_1 (PGA_1, 2.5 μg/ml) (39).

Elevation of β-Amyloid and Ubiquitin Protein Levels by PGE_1 in NB Cells

The levels of both Aβ (29–33) and ubiquitin (34, 35) appear to be elevated in the AD brain. Therefore, a role for each in neurodegeneration has been proposed. In addition, a role for the mutated presenilin genes (PS), PSI, and PSII, has been proposed for familial AD (36, 37). However, the mechanisms by which these proteins either individually or in combination cause degeneration of nerve cells are not well understood. Currently, one of the major areas of investigation for AD research is to determine the mechanisms underlying the generation and secretion of elevated levels of the amyloidogenic Aβ peptide. A recent hypothesis is that the mutant PSI and PSII proteins form a complex with APP (33). This PS-APP complex, in turn, interacts with the intracellular organelles containing proteolytic enzymes. The result is increased proteolysis of APP to form Aβ which, subsequently, is secreted into the extracellular spaces. Indeed, PS-APP complexes have been found to form Aβ by cleavage that is facilitated by the γ secretase enzyme (47). This enzyme is located primarily in the endoplasmic reticulum (48), suggesting the possibility that Aβ is secreted through the endoplasmic reticulum to the extracellular

spaces of the AD brain. The mechanisms underlying Aβ-induced neuronal degeneration are as yet unknown; however, some indirect mechanisms have been proposed. For example, Aβ upregulates the synthesis of IL1 and other cytokines (49, 50). Conversely, IL1β enhances the production of APP, which then becomes available for generating increased amounts of Aβ (51–54). Similarly, aggregates of Aβ activate C1q, a complement protein, and C1q enhances Aβ aggregation (16). Thus, two products of inflammatory reactions, cytokines and complement proteins, are known to interact with Aβ in a way that enhances their respective levels and/or activity.

The relationship between PGs, which are also major secretory products of inflammatory reactions, and Aβ in nerve cells has never been studied. Recently, we have found that PGE_1 markedly increases the level of Aβ as early as 24 hr after treatment (39). Since this was initially determined by immunostaining the NB cells with a primary antibody whose epitope lies within the N-terminal 14 amino acids of the Aβ peptide that is common to full-length APP (Fig. 1), it was not clear whether the enhancement in immunostaining was due to an increase in the levels of APP or Aβ. Therefore, an additional study was done with an antibody whose epitope lies within amino acids 44–63 of APP and, thus, is specific only for APP. Results showed that PGE_1 failed to yield a significant increase in the level of APP in NB cells (La Rosa FG and Prasad KN, unpublished observations). These data suggested that the increase in intensity of the fluorescent signal in NB cells following treatment with PGE_1 was primarily due to an increase in the processing of APP that yields the Aβ peptide rather than an increase in APP levels. We also showed that R020-1724 increases Aβ levels by a mechanism similar to that observed with PGE_1. Since both of these agents increase the intracellular level of cAMP within 15 min after treatment (41), we propose that cAMP may be one of the factors involved in the regulation of APP processing that leads to increased levels of Aβ production in NB cells. This is in contrast to observations made in astrocytes, glioma, and neuroblastoma X glioma hybrid cells, in which cAMP upregulates the level of APP mRNA (55, 56). However, it remains uncertain whether an elevation of cAMP in these cells leads to either increased levels of APP or an increase in production of Aβ from APP, since corresponding protein levels were not measured. These results suggest that an elevation of cAMP increases the level of APP in glia cells, whereas we show that cAMP increases the level of Aβ, but not APP, in NB cells. The increased accumulation of Aβ is associated with PG-induced degeneration of NB cells; however, the degenerated cells do not produce Aβ (39). Therefore, we argue that Aβ may be the cause of cell death rather than a byproduct of cell death. However, the fact that the degenerative changes seen with PGA_1/PGE_1 are much more rapid than those that result from R020-1724 treatment indicates that PG-specific factors other than cAMP are involved. Thus, PGs released during inflammatory reactions may represent another external signal for neuronal degeneration that initiates a series of alterations in intracellular signals such as Aβ.

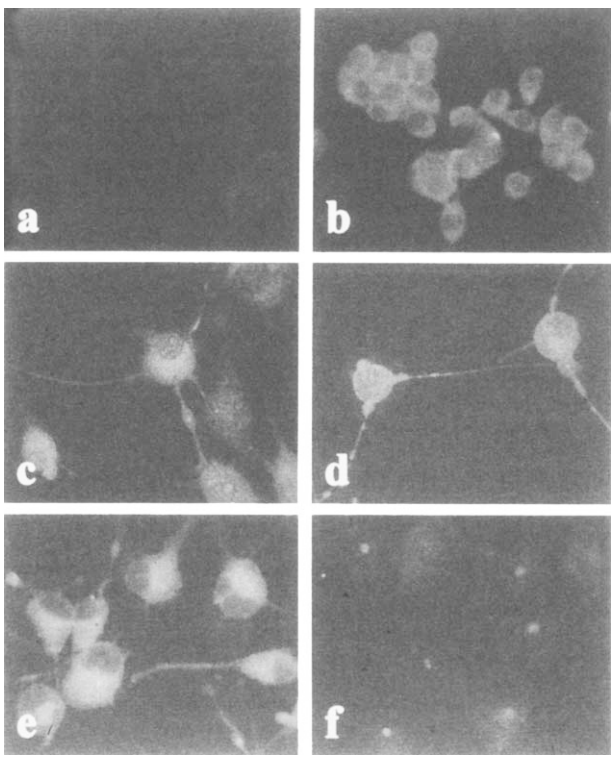

Figure 1. Photomicrographs of immunostained mouse neuroblastoma (NB) cells with a polyclonal antibody against the first 14 amino acids of the β-amyloid peptide (A^{β}) (Chemicon, Temecula, CA) at 4 days after treatment with prostaglandins (PGs). Control undifferentiated NB cells exhibited cytoplasmic staining (Panel b). Cells not treated with the primary antibody did not stain (Panel a). Most of the differentiated NB cells induced by RO20-1724 (200 μg/ml) plus a polar carotenoid (PC) (20 μg/ml), originally referred to as β-carotene (45), showed increased cytoplasmic staining, and neurites were also stained (Panels c and d). Differentiated NB cells induced by PGE_1 (10 μg/ml) plus a PC exhibited more cytoplasmic staining with intense fluorescent spots in some cells (Panel e) than that found in RO20-1724 treated cells (Panels c and d). Most of the degenerated NB cells in cultures treated with PGA_1 (2.5 μg/ml) plus a PC showed a marked decline in cytoplasmic fluorescent staining (Panel f). Magnification 400×. (39)

Another protein proposed to have a role in neuronal degeneration in AD is ubiquitin (34, 35), a protein involved in the normal processing and turnover of proteins. However, its mechanism of action in neurodegeneration is not known. In addition, the relationship between ubiquitin and Aβ, if any, has not been defined adequately. Finally, the regulation of intracellular ubiquitin levels in nerve cells is not known. Recently, we have observed that both PGA_1 and PGE_1 increase the levels of ubiquitin in NB cells as determined by immunostaining of the cells with both monoclonal and polyclonal antibodies against ubiquitin (Fig. 2). The increase in ubiquitin levels coincides with the observed increase in Aβ levels. R020-1724 also increases the level of ubiquitin in a manner similar to that observed with PGE_1 (57). Therefore, we suggest that cAMP may be one of the primary factors involved in the regulation of ubiquitin levels in NB cells as well. Since the levels of both Aβ and ubiquitin increase at

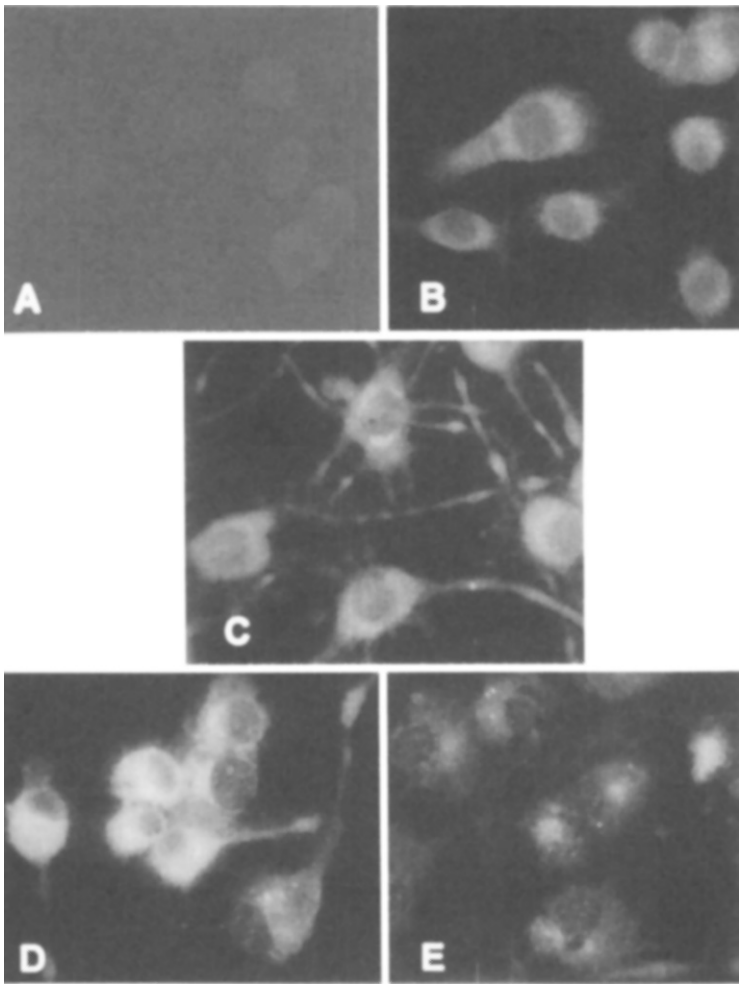

Figure 2. Photomicrographs of immunostained mouse neuroblastoma (NB) cells with a primary antibody to ubiquitin at 4 days after treatment with prostaglandins (PGs). Immunostaining was done with a polyvalent rabbit anti-ubiquitin antibody (Sigma, St. Louis, MO). Control cells showed primarily cytoplasmic staining (Panel B), whereas cells without the primary antibody did not stain (Panel A). All differentiated NB cells induced by RO20-1724, an inhibitor of cyclic nucleotide phosphodiesterase, showed increased cytoplasmic fluorescent staining and contained fluorescent neurites. Differentiated NB cells induced by PGE_1 (10 μg/ml) plus a polar carotenoid (PC) (20 μg/ml), originally referred to as β-carotene (45), exhibited a more intense cytoplasmic fluorescent staining (Panel D) than that found in RO20-1724 treated cells (Panel C). The degenerated NB cells induced by PGA_1 (2.5 μg/ml) plus PC showed a marked decline in cytoplasmic staining (Panel E). Magnification 400×. (39)

about the same time following elevation of intracellular levels of cAMP, we also propose that their regulation may be coupled (39).

Interestingly, the level of ubiquitin expression appears to be higher than that of Aβ in differentiated NB cells (39). This observation taken together with the fact that ubiquitin binds to the 20S proteasome *in vitro* (58), an enzyme that degrades ubiquitin-conjugated proteins (59), has led us to propose a hypothesis regarding the relationship between ubiquitin and Aβ in causing neuronal degeneration. We suggest here that PG-induced elevation of Aβ may cause increased binding of Aβ with the 20S proteasome and thereby reduce degradation of ubiquitin-conjugated proteins. This is predicted to lead to an increased accumulation of ubiquitin-conjugated proteins which, ultimately, leads to neuronal death. Indeed, increased levels of ubiquitin-conjugated proteins have been observed in AD brains (60–63). A recent study on non-neuronal cells demonstrated that apoptosis coincided with an accumulation of ubiquitinated proteins in these cells (64). The following evidence further supports our hypothesis and could explain neuronal degeneration in AD: a) increases in intracellular deposition of Aβ correlates with cell death in AD brains (65); b) Aβ peptides are associated with intracellular neurofibrillary tangles (66, 67); c) using transient transfection assays in COS cell intracellular expression of Aβ results in the formation of amyloid-like fibrils leading to cell death (68); and d) increased accumulation of intracellular Aβ occurs in neurons before the appearance of extracellular deposits of Aβ in aged monkey (69) and dog (70) brains.

Epidemiological and Clinical Studies

The involvement of PGs in AD is supported by clinical and epidemiological studies. For example, nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit PG synthesis, reduce the rate of deterioration of cognitive behavior in AD patients (71–77). In addition, rheumatoid arthritis patients who are on high doses of anti-inflammatory drugs have a reduced incidence of AD (78–81). Thus, NSAIDs or any other drugs that inhibit PG synthesis in the CNS may represent agents that have a sound experimental, clinical, and epidemiological basis for use in the prevention of AD among high risk groups and in the treatment of AD. Since PGs increase the levels of Aβ in NB cells and since increased binding of Aβ with the 20S proteasome can occur possibly leading to increased levels of ubiquitin-conjugated proteins, an agent that can prevent the binding of Aβ with