Antioxidants, Oxidative Stress, and Degenerative Neurological Disorders (44448)

ROBERT A. FLOYD[1]

Free Radical Biology and Aging Research Program, Oklahoma Medical Research Foundation and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104

Abstract. **Recently, clinical trials of several neurodegenerative diseases have increasingly targeted the evaluation of the effectiveness of various antioxidants. The results so far are encouraging but variable and thus confusing. Rationale for the possible clinical effectiveness of antioxidants in several degenerative conditions has arisen out of the many years of basic science generally showing that reactive oxygen species (ROS) and oxidative damage are important factors in the processes involved. Aging is one of the most significant risk factors for degenerative neurological disorders. Basic science efforts in our laboratory have centered on exploring the role of ROS and oxidative stress in neurodegenerative processes. The present review brings together some of the basic concepts we have learned by following this approach for the last 20 years and specifically the results we have obtained by following up on our serendipitous findings that a nitrone-based free radical trap, α-phenyl-*tert*-butylnitrone (PBN), has neuroprotective activity in several experimental neurodegenerative models. The mechanistic basis of the neuroprotective activity of PBN does not appear to rely on its general free radical trapping or antioxidant activity *per se*, but its activity in mediating the suppression of genes induced by pro-inflammatory cytokines and other mediators associated with enhanced neuroinflammatory processes. Neuroinflammatory processes, induced in part by pro-inflammatory cytokines, yield enhanced ROS and reactive nitric oxide species (RNS) as well as other unknown components that have neurotoxic properties. Neurotoxic amounts of RNS are formed by the activity of inducible nitric oxide synthase (iNOS). The demonstration of enhanced 3-nitro-tyrosine formation in affected regions of the Alzheimer's brain, in comparison to age-matched controls, reinforces the importance of neuroinflammatory processes. iNOS induction involves activation by phosphorylation of the MAP kinase p38 and can be induced in cultured astrocytes by IL-1β or H2O2. The action of PBN and N-acetyl cysteine to suppress the activation of p38 was demonstrated in cultured astrocytes. The demonstration of activated p38 in neurons surrounding amyloid plaques in affected regions of the Alzheimer's brain attest to enhanced signal transduction processes in this neurodegenerative condition. The major themes of ROS and RNS formation associated with neuroinflammation processes and the suppression of these processes by antioxidants and PBN continue to yield promising leads for new therapies. Outcomes of clinical trials on antioxidants will become less confusing as more knowledge is amassed on the basic processes involved.** [P.S.E.B.M. 1999, Vol 222]

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[1] To whom requests for reprints should be addressed at Free Radical Biology and Aging Research Program, Oklahoma Medical Research Foundation and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104. E-mail: Robert-Floyd@omrf.ouhsc.edu

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Brain is Highly Susceptible to Oxidative Damage

All aerobic organisms are susceptible to oxidative stress simply because semireduced oxygen species, superoxide and hydrogen peroxide, are produced by mitochondria during respiration (1). The exact amount of ROS produced is considered to be about 2% of the total oxygen consumed during respiration, but it may vary depending on several parameters. Brain is considered abnormally sensi-

tive to oxidative damage (2) and in fact early studies demonstrating the ease of peroxidation of brain membranes (3) supported this notion. Figure 1 presents in simplified form the rationale of why we considered brain to be susceptible to oxidative stress (2). Brain is enriched in the more easily peroxidizable fatty acids (20:4 and 22:6), consumes an inordinate fraction (20%) of the total oxygen consumption for its relatively small weight (2%), and is not particularly enriched in antioxidant defenses (2). In fact, brain is lower in catalase activity, about 10% of liver (4). Additionally, human brain has higher levels of iron (Fe) in certain regions and in general has high levels of ascorbate. Thus, if tissue organizational disruption occurs, the Fe/ascorbate mixture is expected to be an abnormally potent pro-oxidant for brain membranes (5).

Rigorous measurement of H2O2 production from isolated brain mitochondria shows that it amounts to about 2% of the total oxygen consumed when NADH supplies the reducing equivalents (6). In addition to mitochondria, additional sources of ROS include mixed function oxidases as well as other oxidative processes. Of particular importance to brain is the H2O2 produced by oxidative deamination of catecholamines. Relative to this point, the DATATOP clinical trial for Parkinson's disease, which included deprenyl, a monamine oxidase B inhibitor, along with vitamin E, was designed in part to arrest oxidative stress on two fronts (7). Deprenyl itself showed efficacy, but vitamin E alone did not (7). It is not known if deprenyl suppressed oxidative damage in the Parkinson's subjects or alternatively if vitamin E suppressed oxidative stress and yet was not effective. Lack of real time *in situ* assessment of oxidative damage to specific targets makes it more difficult to evaluate these critical questions rigorously.

Quantitation of Free Radical Formation *in vivo* and Serendipitous Discovery of the Neuroprotective Activity of PBN

The lack of rigorous methodology to assess ROS formation *in vivo* was the driving force to develop the use of salicylate as an exogenous trap for hydroxyl free radical formation (8–10). Salicylate permeates all tissues in a relatively short time (20–30 min), and reacts at nearly a diffusion-limited rate with hydroxyl free radicals to form 2,3- and 2,5-dihydroxybenzoic acid (8). These hydroxylated products can be extracted effectively from tissue (9), and quantitated at very low levels, relative to the salicylate present, using HPLC with tandem optical/fluorescence, in series with and prior to, electrochemical detection methods (9–11). First use of this approach to study hydroxyl radical formation in experimental brain stroke was done in the Mongolian gerbil (9). The data obtained convincingly demonstrated that during the reperfusion phase, brain produced enhanced amounts of hydroxylated salicylate reflecting enhanced hydroxyl free radical flux during these events (9). Subsequent studies where brain regions were analyzed and where oxidized protein levels were also obtained revealed very convincingly that enhanced oxidative processes occur after ischemia during the reperfusion phase (12). Figure 2 presents a summary of the data obtained. Since blood flow in the brain stem and cerebellum is not altered in gerbils when the common carotid arteries are ligated, due to the unique anatomical features of these animals, these regions are good reference "control" tissue in the same animal. The results clearly show that cortex, and even more so hippocampus, experiences enhanced oxidative stress in reperfusion but not in the ischemia period. No change occurred in the brain stem or cerebellum as expected since blood flow to

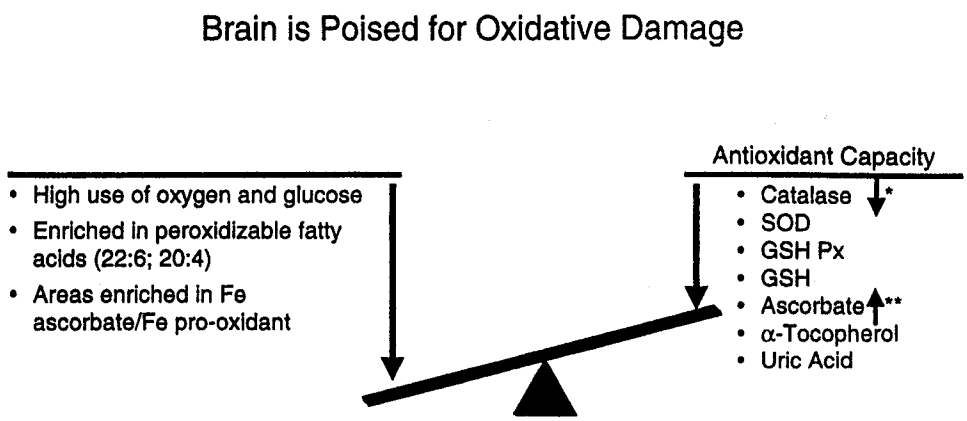

Figure 1. General summary of why the brain is poised to undergo oxidative damage. Under normal conditions, oxidative stress is held in check; however, specific insults such as a stroke or general aging will induce oxidative damage.

*Human brain has 10–20% of liver and heart
**Human brain has 1.1 mM, Plasma 62.4 μM

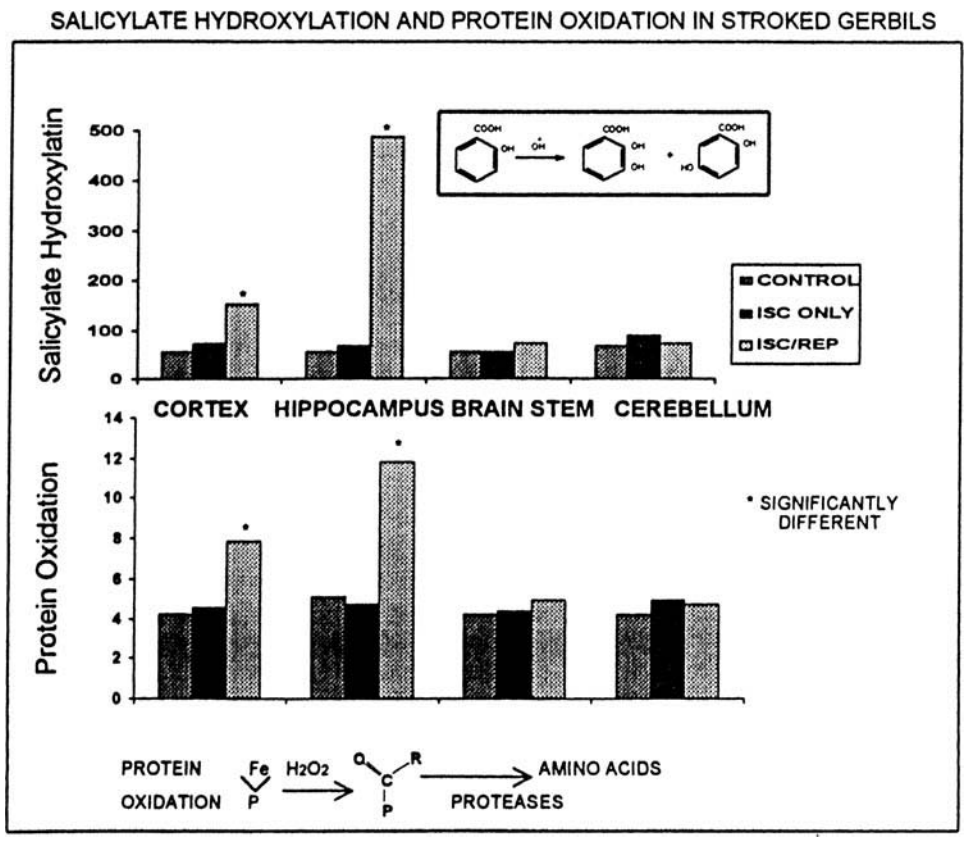

Figure 2. Summary of data collected showing that experimental stroke in the gerbil (12) caused increased formation of protein oxidation products and increased hydroxylation of salicylate as an index of hydroxyl free radical formation in affected brain regions but not in brain stem and cerebellum.

these regions was not affected by constriction of the common carotids to induce ischemia in the fore brain.

Success with salicylate and protein oxidation methods spurred us to attempt to determine if there were secondary, perhaps membrane lipid derived, radicals formed in the stroked gerbil brain. PBN, α-phenyl-*tert*-butyl nitrone, was used to obtain answers. The trapping experiments with PBN were relatively unsuccessful (13), but we discovered serendipitously that PBN exhibited remarkable neuroprotection against stroke (14). This discovery was rapidly confirmed by Phyllis *et al.* (15, 16), and was shown to be effective even if administered up to a few hours after the ischemic period in the Mongolian gerbil (17), as well as in the rat middle cerebral artery occlusion stroke model (18). Chronic administration of PBN (32 mg/kg/day) to older gerbils for 14 days demonstrated that the oxidized proteins in their brains decreased back down to younger levels and that removal from PBN then allowed protein oxidation levels to slowly rise again to where it rebounded to the old higher levels 14 days after PBN cessation (19).

The fact that PBN offered protection when given hours after the ischemia strongly implicated that its neuroprotective action was not dependent on its ability to trap free radicals directly. Another observation led to the rejection of the tacit assumption that the neuroprotective activity of PBN was directly related to its ability to trap free radicals *per se*. It was demonstrated that older gerbils were much more susceptible to a stroke than were younger gerbils (14, 20). However, we showed that lethality of older gerbils to a stroke was reduced markedly if they were given chronic (32 mg/kg) daily administration of PBN for 2 weeks (20). This is a striking fact in its own right, but the protective effect of PBN lasted for several days following cessation of its administration (20), long after it was expected to be present in the animals (i.e., its half-life in rat is 134 min (21, 22). The results are shown in summary form in Figure 3. These results clearly implicate that PBN at low doses altered the older brain such that it was much more resistant to a stroke, much like the younger brain, and that this condition existed in animals for some time even though PBN was not present. Data collected but not shown, demonstrated that age-dependent enhanced protein oxidative damage was mostly reversed by as little as 10 mg/kg/day PBN, and that even 3.2 mg/kg/day had a significant effect when administered for 2 weeks (20). The questions that remain include, Why does age alter the gerbil brain such that it is more vulnerable to a stroke and what mechanism is involved in the action of PBN in reversing this age effect? As discussed later, we postulate that the age effect can be explained partly by the increased smoldering neuroinflammatory state of the old brain.

Pharmacology, Antioxidant Properties of PBN, and Comparisons to Brain α-Tocopherol

The neuroprotective activities of PBN noted above stimulated studies to understand the pharmacology and antioxidant properties of this compound. Some important data are summarized in Figure 4. Incomplete as the studies are,

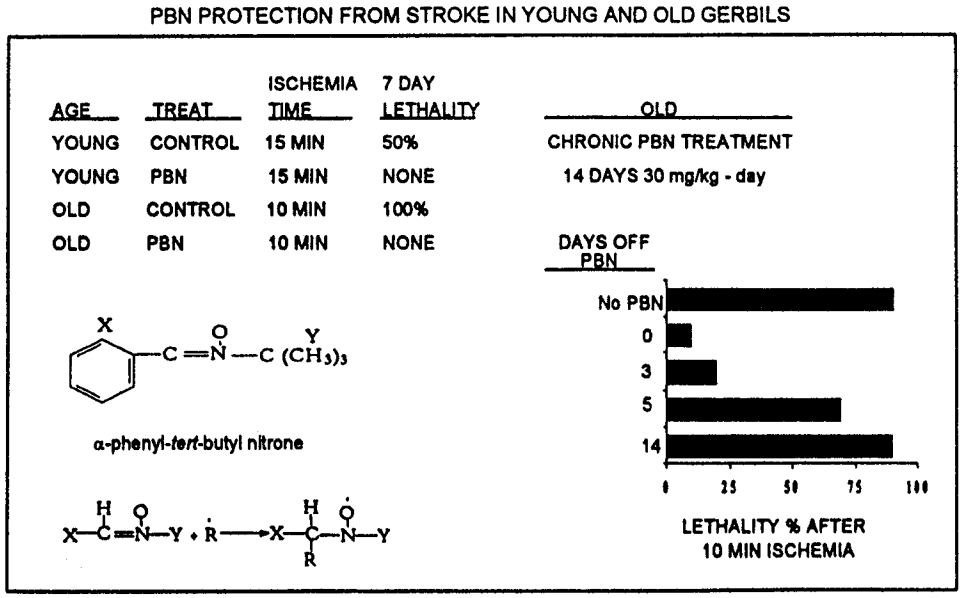

Figure 3. Summary of data illustrating that PBN is neuroprotective in a gerbil global stroke model (14) and that older animals are much more susceptible to stroke than are younger animals (14). Additionally presented is a summary of data demonstrating that the increased susceptibility of older animals to stroke can be reversed by chronic administration of PBN for 14 days and that the protective effect lasts for several days after cessation of PBN administration (20). Also presented is the chemical structure of PBN and a general illustration of a free radical trapping reaction by PBN.

it is clear that PBN when administered to rats interparietally rapidly penetrated all tissues and was subsequently excreted mostly in the urine with a half-life of about 134 min (21). PBN did penetrate the brain and reached over 500 μM within 20 min after a 150-mg/kg dose when its content was assessed by microdialysis (22). Microdialysis probably measures only extracellular PBN whereas if the total brain tissue is measured, then it reached 39 μM after 20 min following a 75-mg/kg dose (21). Comparisons of PBN content with natural α-tocopherol levels (23), shows that within 20-60 min after dosing with the nitrone, the brain content of the two are expected to be essentially the same (see Fig. 4). When a tracer dose of radioactive α-tocopherol was administered into the femoral vein, Vatassery *et al.* (23) showed that exchange in the brain was quite slow (i.e., ≈ 1.6% of the dose in 30 min). In contrast, 226% of the dose was exchanged with liver in 30 min (23). Even though α-tocopherol exchange with brain was slow, the highest rate of exchange was in the cerebellum, which had the lowest total content of this natural antioxidant of the brain regions examined (23). Studies on the changes noted in antioxidant and energy metabolites in brain after an experimental stroke showed that α-tocopherol levels dropped 7% during ischemia and an additional decrease to 13% below normal levels after 15 min of recirculation (24). In this same study, they also noted free fatty acids, including arachidonate and docosohexaenoic acid, increased markedly during ischemia but fell rapidly during recirculation (24).

The general antioxidant properties of PBN are certainly not very impressive. When compared to butylated hydroxy toluene or trolox in three different rat liver microsomal lipid peroxidation systems, Janzen *et al.* (25), demonstrated that PBN was in general about 1000-fold less effective than these widely used powerful antioxidants in preventing lipid peroxidation. So it appears that the neuroprotective activity of PBN can not be ascribed to its direct antioxidant properties *per se.*

PBN Inhibition of Neuroinflammation: A New Model of Neurodegeneration and the Mechanistic Basis of its Neuroprotective Action

The experimental data rule out direct free radical trapping as the mechanistic basis of the neuroprotective action of PBN (26, 27). Newer developments in the understanding of neurodegenerative processes allow the emergence of a more rational view of the field and the mechanistic basis of the neuroprotective activity of PBN. Results from five scientific research areas provided a foundation to the notion that PBN protects by suppressing neuroinflammatory processes that produce neurotoxic products. First, it was discovered that PBN suppresses gene induction following an experimental stroke (28). Second, it was shown that its protective activity in LPS-mediated septic shock is associated with prevention of nitric oxide formation in liver and that this is due to inhibition of the induction of inducible nitric oxide synthase (iNOS), but that PBN itself did not act as a competitive inhibitor of the iNOS enzyme *per se* (29). Third, it was demonstrated that glial cells are capable of producing large amounts of nitric oxide due to their increased iNOS expression and that they are relatively resistant to higher levels of nitric oxide but that neurons are much more susceptible (30). Fourth, it was clearly demonstrated that experimental stroke caused the induction of many genes including pro-inflammatory cytokines (31) as well as iNOS (32). And fifth, it was clearly demonstrated that glial cells (microglia and astrocytes) are capable of being activated to mediate iNOS induction (33, 34), by several factors including pro-inflammatory cytokines (35). These facts led us to propose a working hypothesis that the action of PBN involves the prevention of the induction of genes, such as iNOS, in glia by pro-inflammatory cytokines and other factors, thereby preventing the production of products that are toxic to neurons. This concept is presented in simplified form in Figure 5. Data we have collected thus

Brain α-Tocopherol and PBN

α-Tocopherol*

- rat cortex – 45.5 nmole/gm, cerebellum – 27.9 nmole/gm[23]
- percentage uptake (exchange) after 30 min of radioactive I.V. dose[23]

	Uptake	Exchange (dpm x 10^{-3}/mg)
Cortex	1.61%	7.96
Cerebellum	1.64%	12.62
Liver	226%	—

PBN*

- content by microdialysis after 150 mg/kg IP dose in rat[22]
  brain peak height at 20 min, ~ 560 μM
  blood peak height at 20 min, ~ 480 μM
  steady state after 120 min,
  blood 224 μM, brain 331 μM
- content by tissue analysis after 75 mg/kg IP dose in rat[21]

	PBN (μM)			
	15 min	30 min	60 min	
Brain	39	51	38	
Liver	56	69	53	half life ~ 134 min
Kidney	45	64	45	

Figure 4. Summary of data comparing brain α-tocopherol content (23) and its exchange from a tracer dose in rats (23). Also presented is a summary of results of brain PBN levels after a bolus dose (21, 22).

*data from references 21 – 23

far support this notion. It will be summarized in the remaining portion of this review.

Analysis of Neuroinflammatory Products Using HPLC: Electrochemical Array Methods

Nitric oxide is formed at higher levels by iNOS. This is an enzyme that does not require calcium for its activity. This is in contrast to the two constitutive NOS enzymes, endothelial and brain- or neuron-specific enzymes. The formation of larger levels of nitric oxide in a sustained fashion by glia is expected to be neurotoxic because of the enhanced sensitivity of neurons to it. The neurotoxic action of nitric oxide is probably due to the formation of peroxynitrite, which is formed by the rapid reaction of nitric oxide with superoxide. Peroxynitrite will react with tyrosine to form 3-nitro-tyrosine. Additionally, free radical reactions are ex-

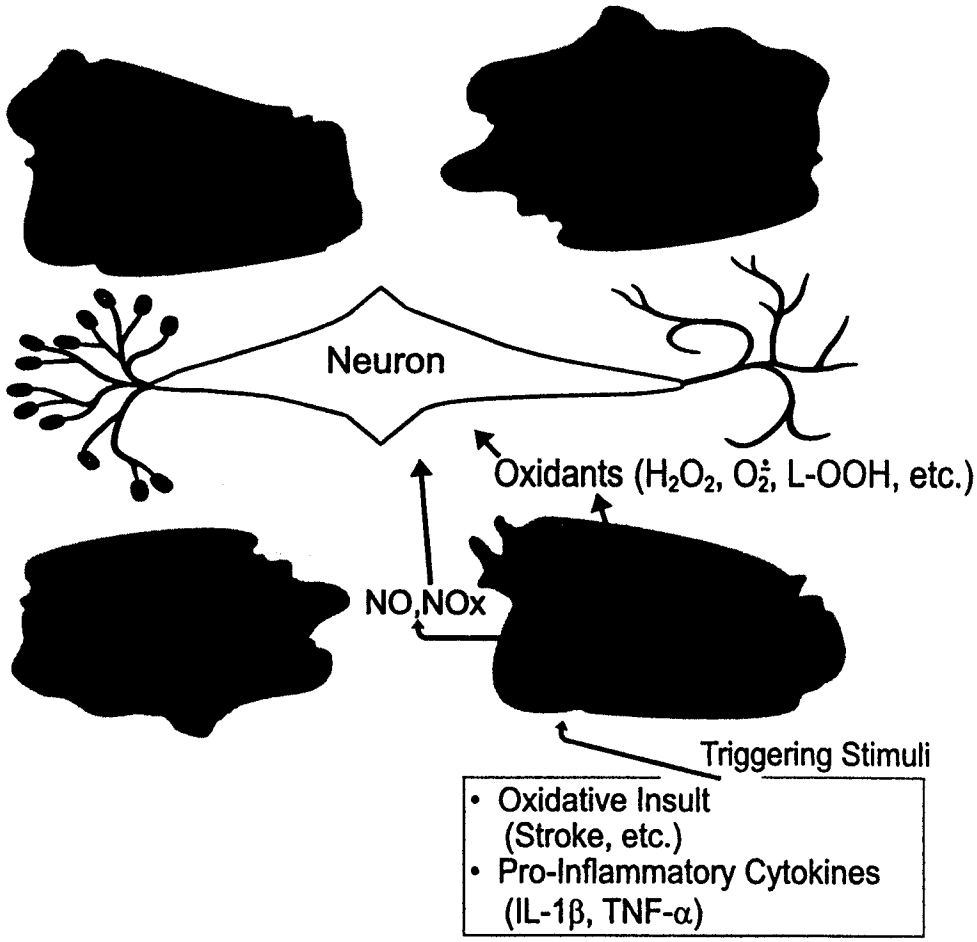

Figure 5. Illustration presenting the basic concepts of neuroinflammation where glia cells are activated to produce oxidants and nitric oxide and its oxidation products, which are toxic to neurons. Glia are activated by various triggering stimuli resulting in the induction of iNOS and other genes. PBN inhibits induction of iNOS and other genes and also the production of oxidants that cause death or dysfunction of neurons.

pected to form dityrosine adducts. For these reasons and also because it is possible to reference tyrosine using the HPLC-electrochemical array detection approach, we developed a method to digest protein into its component amino acids and quantitate the tyrosine adducts (3-nitro-tyrosine and dityrosine) present and reference them to the total tyrosine present (36). Using cultured primary astrocytes, we demonstrated that stimulation with IL-1β caused increased formation of 3-nitro-tyrosine (36). Additionally, we demonstrated that PBN at low levels(100 μ*M*) prevented formation of IL-1β induced formation of nitrotyrosine (36). These data collected in cultured cells provided proof in principle that this approach would be valuable to assess if products of neuroinflammatory processes were present in brain regions of Alzheimer's subjects.

We have conducted an analysis of specific regions of Alzheimer's disease (AD) subjects and compared them with age-matched control subjects. The postmortem time was less than 3.5 hr. Table 1 presents a summary of the results obtained (37). There are several important points, which the data demonstrate. First the dityrosine (diTry) and 3-nitro-tyrosine(3-NO2-Try) content are significantly higher in the severely affected regions of the AD brain, and it is much higher than in the same regions of the normal subjects. Second, the cerebellum, which is considerably less affected in AD, contains nearly the same amount of the tyrosine oxidation products as the normal brains. Third, the uric acid content of the AD brains, in all regions, is significantly lower than in the normal brains (37). It is not sure the reason why this is the case. These data clearly demonstrate that products of neuroinflammatory processes are significantly enhanced in the affected regions of the AD brain as compared to the normal brain. The data support the concept that neuroinflammatory processes are important in AD.

Table I. Analysis of Alzheimer's Versus Age-Matched Control Brains

Subjects	Number	Age	Sex
Normals	5	78 ± 6 yrs	3 M, 2 F
Alzheimer's (AD)	11	78 ± 8 yrs	7 M, 4 F

Protein Oxidation Products			
	Relative Change in AD (mean value of AD/mean value of normals)		
Brain region	di-Try	3-NO_2-Try	Uric Acid
Hippocampus	4.6	7.8	0.52
Inferior parietal lobule	4.5	6.5	0.50
Superior & middle temporal gyri	2.7	5.2	0.42
Cerebellum	1.3	0.17	0.25

Note. Data from Ref (37).

Enhanced Signal Transduction Processes, p38 Activation in Astrocytes and Alzheimer's Brain

If neuroinflammatory events are active in the Alzheimer's brain, then enhanced signal transduction processes should be occuring. Cells respond to stimuli, whether they are environmental agents, mitogens, pro-inflammatory cytokines, or other unknown agents by receptor-mediated enhanced protein phosphorylation (MAP) kinase cascades. Protein MAP kinases mediate phosphorylation of sequential proteins, and in many cases the subsequent phosphorylated protein then becomes a protein kinase itself. Figure 6 presents a simplified diagram of the several MAP kinase cascades known to exit presently and their action in mediating the induction of genes such as iNOS and cycloxygenase II (COX II) as well as several cytokines.

We have focused on the p38 activation pathway because it has been shown to be involved in the activation of COX II and iNOS and is activated in stroke (38, 39). Activation of p38 involves the dual phosphorylation of threonine and tyrosine amino acids on a specific domain (Thr180-Gly181-Try182) of the unactivated protein, which resides in the cytosol. Upon activation, the phosphorylated protein then moves to the nucleus where it catalyzes phosphorylation and the activation of specific target transcription factors including cyclic AMP-responsive element binding protein (CREB) and DNA damage-inducible gene *CHOPP/GADD153* and monocyte enhancement factor 2C (MEF2C). We have investigated p38 activation in cultured astrocytes (38). The results can be summarized as follows: 1) p38 is activated by IL-1β, H2O2 and several other factors; 2) p38 activation occurs rapidly peaking within 5 min and then subsequently becomes deactivated with a return to basal levels after about 1 hr; and 3) the thiol antioxidant N-acetyl-cysteine (NAC) as well as PBN are very effective in suppressing IL-1β as well as H2O2-mediated p38 activation in astrocytes (38). Data illustrating some of these facts are presented in Table II. The data show that addition of NAC as well as PBN even suppressed basal levels of p38 activation. NAC as well as PBN are effective in suppressing p38 activation at the 5-min time as well as at other times after stimulation (data not shown). The fact that H2O2 activates p38 is a novel observation. Data presented in Table III show that treatment of astrocytes with IL-1β caused formation of H2O2 by these cells, and PBN suppressed the production of H2O2 (38). These observations imply that treatment of astrocytes with IL-1β sets in motion events such as production of second messengers that activate mitochondria to produce H2O2 and that PBN acts to suppress these events. One simple interpretation is that PBN acts on the mitochondria to prevent enhanced production of H2O2. PBN has been shown to be capable of suppressing H2O2 production at Site 1 in isolated brain mitochondria (6).

Success with the p38 studies in astrocytes and the availability of both a highly specific antibody for activated p38 and high quality brain samples of Alzheimer's subjects and age-matched normal subjects made it possible to determine if p38 is activated in the AD brain (39). The results showed that p38 is significantly activated in affected regions of the AD brain but is not in age-matched normal brains (39). The results showed that there was widespread p38 activation in the neurons near the neuritic plaques in the AD brain (39). Very few, if any, neurons showed p38 activation in the normal brain. Close examination of the immediate plaque regions demonstrated that activated p38 was localized also

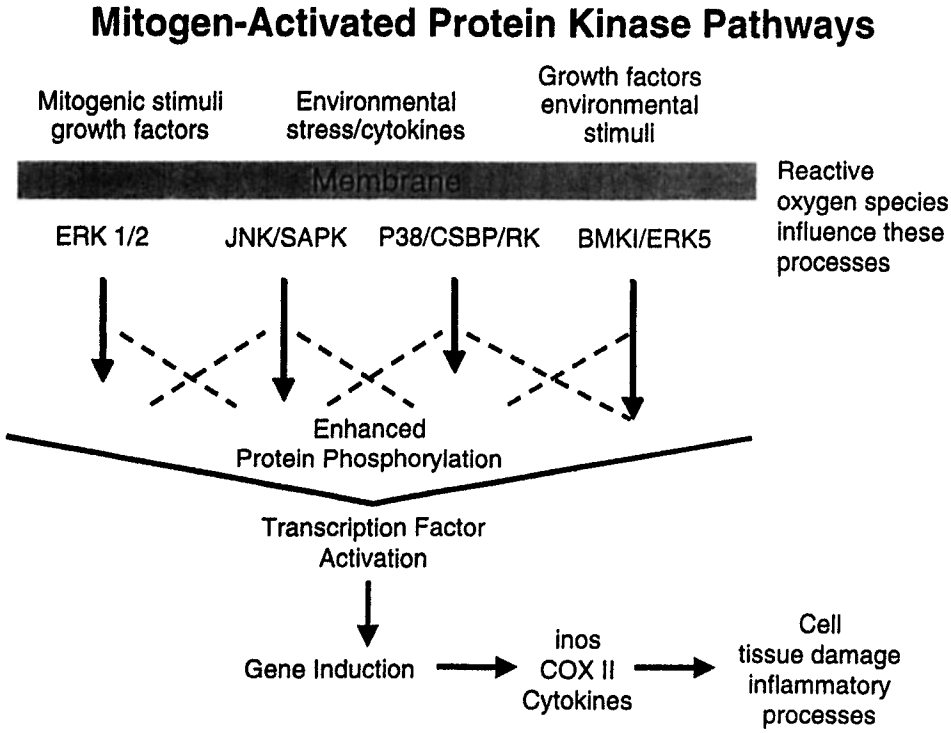

Figure 6. Illustration presenting the concept of the activation of genes by various stimuli brought about by various MAP kinase cascades.

Table II. p38 Activation in Astrocytes

		Effect of PBN and N-Acetyl-Cysteine (NAC) (change in density of Western band)	
	Additions	0	Time 5 Min
Unstimulated	None	0	
	PBN	−61.2 ± 17.8[a]	
	NAC	−62.3 ± 9.6[a]	
IL-1β	None		922.1 ± 267.8
	PBN		108.3 ± 92.1[a]
	NAC		14.5 ± 60.0[a]
H_2O_2	None		1279.7 ± 58.1
	PBN		239.9 ± 266.6[a]
	NAC		293.3 ± 80.0[a]

[a] Significantly, $P < 0.05$, different from no additions.
PBN and NAC = 1m*M*, IL-1β = 10 ng/ml H_2O_2 = 0.5 m*M*.

Table III. Effect of PBN on IL-1β Induced H_2O_2 Production in Astrocytes

	H_2O_2 Efflux[a] (pmole/min/well)
None-stimulated	0 ± 0.48
IL-1β (20 ng/ml)	2.42 ± 0.69
IL-1β + PBN (1m*M*)	1.18 ± 0.72

[a] Data from (38).

in cells that had the appearance of astrocytes. Control studies were done to demonstrate that the activated p38 antibody did not react with hyper- phosphorylated *tau* (39), which is also prominent in neurons in the plaque region of the AD brain.

Antioxidants as Therapeutics and the Mechanistic Basis of PBN Action

Since results showed that several neurodegenerative conditions are associated with enhanced ROS and RNS formation, it is quite likely that more antioxidant-based potential therapeutics will be forthcoming. As noted earlier, even though there is compelling evidence showing the enhancement of oxidative damage under neurodegenerative conditions, results of clinical trials thus far clearly indicate that much more sophisticated knowledge is needed to understand why certain antioxidant-based trials are successful, and some are apparent failures. The failure of vitamin E in Parkinson's disease is one such example (7). On the other hand, vitamin E clearly had a beneficial effect in an Alzheimer's disease clinical trial (40). It has been demonstrated recently that the antioxidant thioctic acid (α-lipoic acid) was not effective, but deprenyl was effective in improving cognitive function in a clinical trial of dementia associated with advanced HIV infection (41). Although these three pathologies are certainly different, detailed knowledge as to why vitamin E failed in one and had some success in another and why thioctic acid failed is still an unsolved problem.

In light of the neuroinflammatory processes ongoing in some (if not all) neurodegenerative conditions, it is clear regarding the basic science aspects that therapeutics based on PBN and its action may be effective. Table IV presents a summary of pertinent processes where it has been shown to be active. The various processes listed are all interconnected. So to say that its action in one particular process is its key mechanism of action is not possible with absolute certainty at this point. Certainly there is a hierarchy in the broader sense vs. the more basic aspects of the processes listed. The action of PBN in suppressing ROS production by mitochondria may be very important in linking its effect in the many different processes together. Lack of knowledge about the certainty regarding mitochondrial-based ROS-mediated signaling and uncertainty of ROS and PBN action in mitochondrial processes, other than in the isolated ones, has held back more definitive conclusions. The fact that enhanced signal transduction processes are significantly elevated in brains of Alzheimer's subjects implies that suppression of these events would certainly be beneficial. It is not known exactly how exacerbated neuroinflammatory processes are linked to processes responsible for dementia. Based on the magnitude of the small magnetic fluxes arising from neuroneal events brought about by touch-mediated impulses, simultaneous firing of about 1000 neurons is required. So to the extent that neuroneal network firing is influenced by dysfunction and/or loss of neurons brought about by enhanced neuroinflammatory processes it is possible that AD dementia may be diminished by neuroinflammatory-suppressing therapeutics. This remains to be explored.

The foregoing rationale must be reconciled with the age-associated increase of AD and the experimental evidence showing stroke outcome is much more severe in older animals and that this is reversed in a gradual fashion by chronic PBN administration. To rationalize these facts, we postulate that events, which resemble smoldering neuroinflammatory processes, increase gradually in magnitude with age. This logic would suggest that these age-associated processes may influence a network of neurons and/or a specific defined region of neuroneal tissue and that the lesions may be at a very basic level perhaps associated with diminution of the capacity to produce enough energy to mount an effective challenge to a large oxidative insult (i.e., such as a stroke). Thus, the old brain functions well on most normal maintenance demands, but when a large oxidative challenge is presented, it is incapable of responding effectively. Our demonstration that phosphate energy charge restoration to an old brain, after a stroke, was much slower than that of a younger brain (42) is in keeping with these ideas. PBN has been shown to make it possible for a stroked brain to more

Table IV. Mechanistic Basis of PBN Neuroprotection

Suppresses Oxidative Stress
–Suppresses ROS and RNS formation in neuroinflammation
–Suppresses signal transduction processes
–Suppresses ROS production in mitochondria

rapidly restore its ATP levels and suppress lactate accumulation (43). This provides support for a mechanism acting on energy-based processes. A gradual increase in levels of a compound (a neurotoxin) that acts to suppress the ability of mitochondria to respond to a challenge might explain some of the observed results. Smoldering neuroinflammatory processes may cause the gradual buildup of such a neurotoxin.

Slight shifts in rate processes associated with normal equilibrium-based events could, when applied over a very long time, have manifestations that could account for most, if not all, of the observed facts. In this case, age-associated and smoldering are the key operative factors. Increased oxidative damage with age may reflect altered equilibrium processes. The fact that brain oxidative damage is reversed by chronic PBN administration provides a strong coupling of altered equilibrium processes, oxidative damage, and loss of energetic capacity to meet an oxidative challenge (44). Viewed in this manner, slight alterations in the flux and/or repair processes with age may be contributing factors to the age-associated lack of capacity to meet an oxidative challenge. PBN action could rapidly readjust the equilibrium processes put off balance by a smoldering neuroinflammatory state. In this case, the action of PBN to suppress overall oxidative stress and allow equilibrium readjustment is in a broad sense its mechanism of action.

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