Free-Radical Mechanisms in Cerebral Pathologies (Review)

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The current status of knowledge about the roles played by radical reactions in the pathogenesis of cerebral pathologies is reviewed, and it is shown that these reactions are of key importance to the damage sustained by cellular structures of the central nervous system. Novel approaches to the management of cerebral disorders, based on the use of antioxidants, are considered.

Key Words: *reactive oxygen species; lipid peroxidation; central nervous system; cerebral disorders*

The importance of research on cerebral pathologies hardly needs emphasizing. Suffice it to say that the World Health Organization has defined human death as the death of the brain, which controls all vital functions during life. Moreover, the 1990s have been declared a Brain Research Decade, which means that pathological conditions of the brain are now accorded the highest priority in the field of biomedical research.

The research focus in the late 1970s on elucidating the contribution of free-radical reactions to cerebral disorders would probably have been impossible but for the recognition of two basic facts. First, the evidence amassed by that time on the functional biochemistry, biophysics, and cellular physiology of neurons left no doubt but that neuronal membranes are the sites of all major processes in the central nervous system (CNS) involving the reception, processing, and transmission of information derived from the external environment and the internal milieu of the body. Indeed, both the conduction of action potentials (i.e., the relay of signals along nerve fibers) and the transmission of signals resulting from the interaction of neurotransmitters with neuronal membrane receptors and the consequent initiation of intracellular processes (i.e., transmembrane information transfer) were found to proceed on neuronal membranes. This is not only because the major entities mediating information processes (receptors, ion channels, enzymes) are associated with neuronal membranes but also because their smoothly coordinated activity, integrating all physical and chemical processes involved in information transfer in the CNS, is made possible through the functioning of neuronal membranes as integral cellular structures. It is important to stress in this context that the functional state of those entities (functional proteins) as well as their concerted action are largely determined by structural and functional properties of the neuronal membrane's lipid bilayer, which ensures both the selective permeability of neuronal membranes and the regulation of functional proteins.

Second, it became clear that neuronal membranes function under "harsh" conditions: although the brain has a small weight, it consumes up to 20% of all oxygen utilized by the body; its membranes contain very unsaturated lipids [10,18] and the levels of protective antioxidant lipids are low, so that the reactive oxygen species generated in bioenergetic and specific neurochemical processes favor the oxidation both of membrane lipids and

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of proteins and nucleic acids [25]. These considerations stimulated research into the roles free-radical reactions play in CNS disorders. We have tried to provide an overview of the major results from these studies and to assess trends in this research area.

Such an overview is not possible without addressing the following three issues, which have been the main focus of research: what are the nature and extent of the modifying (injurious) effects exerted by free radicals on the CNS; are free-radical processes activated in cerebral abnormalities and, if so, can their activation be incriminated as a factor in the pathogenesis of such abnormalities; and, if free-radical processes are activated and implicated in the origin and development of disease processes, how can this be exploited for treatment purposes.

EFFECTS OF FREE RADICALS ON BRAIN CELLS

The evidence available today leaves no doubt that free radicals can exert modifying, including injurious, effects on brain cells. *In vitro* experiments with nerve cell cultures have proved that activation of molecular oxygen may result in cell death. Morphologically this is manifested by lysis of all cellular structures with the exception of nuclei, which, however, become pyknotic. Probably, the key event in the death of neurons is activation of lipid peroxidation (LPO), given that neurons are destroyed appreciably less following the addition of lipophilic antioxidants to the culture [9,24]. Since neurons in culture can be effectively destroyed by activated oxygen only in the presence of exogenous Ca^{2+}, the most likely mechanism of their death involves accumulation of LPO products with a consequent increase of membrane permeability for ions, followed by massive entry of Ca^{2+} into the cytosol, leading to activation of Ca-dependent intracellular proteases and lipases which kill the cell [9]. Free radicals also appear to damage glial cells, but this phenomenon has been studied quite inadequately and the only thing one can state with a fair amount of certainty at present is that glial cells are more resistant to free radicals than are neurons.

Less intensive exposure to free radicals modifies neuronal membranes without damaging them to a significant degree. The phenomenology of modifying effects produced by free radicals still has many gaps, and these will undoubtedly be bridged in the course of time. However, the experimental results already in hand permit the conclusion that the modification affects key properties of neuronal membranes, both structural and functional. Thus, LPO activation in these membranes has been shown: 1) to cause their depolarization (which results in an altered sensitivity threshold of the neurons) [8]; 2) to change the physical state (microviscosity) of the neuronal membrane lipid bilayer (which eliminates optimal conditions for the interaction of functional membrane proteins such as receptors, channels, enzymes, and modulators) [9,18]; 3) to change the number of membrane receptors and their affinity for neurotransmitters and drugs [18]; 4) to inhibit the operation of ion pumps [18]; and 5) to impair the coupling of receptors to the enzyme systems mediating cellular responses [9]. This conclusion is based on what is known about the effects of free radicals on a limited range of receptors (adrenergic, serotonin, and benzodiazepine receptors) [9,18], channels (mainly Na,K-ATPase, which is also an enzyme) [18], and enzymes (mainly adenylate cyclase) [9]. That the modifications listed above are caused by LPO activation is attested by their suppression in the presence of lipophilic antioxidants [9]. Further phenomenological studies will almost certainly demonstrate that all functional proteins are subject to modification by free radicals and that the extent of this modification is determined solely by a protein's resistance to oxidative destruction.

The effects of free radicals are not confined to modification of neuronal membranes. Activation of molecular oxygen has also been shown to result in damage to the nuclear DNA and cytosolic proteins of brain neurons [9,21,30]. This means that errors may accumulate in the genetic code, with all of their deleterious consequences. Moreover, when cytosolic proteins are damaged, the normal course of intracellular metabolic events is disrupted and the cell is unable to function properly.

It follows from the above that the modifying effects of free-radical reactions in the brain may be implicated as potential determinants of both neurodegenerative disorders involving particular parts of the CNS and those disorders which affect higher CNS functions without causing appreciable morphological lesions.

ACTIVATION OF RADICAL PROCESSES IN CNS DISORDERS

That LPO is activated in a variety of cerebral disorders is now beyond doubt. This statement is based on the demonstration of LPO activation in emotional pain stress, ischemia (hypoxia, anoxia), cranial trauma, stroke, brain tumors, Alzheimer's disease, Parkinson's disease, Down's syndrome,

toxicoses, hyperkineses, neurological disorders, dementias, demyelinating viral diseases, lipofuscinoses, endogenous psychoses, and a number of other pathological conditions [17,18,25,27,31]. Even this incomplete listing gives sufficient grounds for stating that cerebral disorders involve activation of radical processes in neuronal membranes. Although their activation per se is not sufficient proof of their contribution to the origin or progression of particular brain abnormalities, this phenomenon is clearly of great interest in studies of the pathogenesis, clinical course, management, or prevention of brain disorders. Below, we will illustrate this with special reference to emotional pain stress and epilepsy because the availability of adequate animal models of these conditions has enabled investigators to gain the greatest amounts of pertinent information.

In 1979 it was discovered that emotional pain stress leads to LPO activation in certain organs, including the brain, and this activation was interpreted as being the major membrane-damaging factor [14]. This discovery gave rise to the hypothesis (whose validity was soon confirmed) that antioxidants can protect against stress. Subsequently, both LPO activation and the stress-mitigating properties of antioxidants have been repeatedly demonstrated on various animal models of stress.

Detailed examination of the time course of free-radical reactions in the brain of stressed animals showed phasic changes distinct from the changes taking place elsewhere in the body [3]. On an animal model of emotional pain stress it was found that the first response of the brain to such stress was inhibition rather than activation of LPO [5]. Shortly afterward, this was also shown to be the case in an animal model of immobilization-induced stress [15]. Systematic studies of temporal variations in LPO, membrane lipids, and antiradical systems in the brains of animals with acute and chronic stress led to the conclusion that inhibition of free-radical oxidation in the brain is a link in the chain of events resulting in adaptation to stressors [4].

The parameters of LPO and antiradical defense and the lipid composition of neuronal membranes in the brain of stressed animals have been shown to change in a phasic and synchronous manner. During the first few minutes of acute stress, antioxidant activity in the brain rises and LPO is inhibited. Phospholipids in the brain membranes also rise and become more readily oxidizable, while the level of cholesterol declines. With continued exposure to the stressor, all parameters return to normal, but later LPO activation occurs as cholesterol accumulates and the readily oxidizable phospholipids are depleted [3]; this pattern of coincident changes in free-radical reactions and the lipid composition of cell membranes corresponds to that described earlier [2]. The phasic changes in LPO (its decrease followed by a rise) that take place in the brain during acute stress can probably be accounted for by the simultaneous occurrence of two processes - inhibition (e.g., as a result of the secretion of biologically active compounds possessing properties of radical scavengers) and LPO activation (as a result of increased radical formation), with the former process dominating in the initial period and the latter process subsequently [3].

The initial period, during which LPO is inhibited, reflects the stage of short-term adaptation that is initiated immediately on the basis of preexisting mechanisms [13]. The duration and degree of expression of this stage depend on the type of stress and on the age, sex, and general condition of the animal. It lasts longer in females, when the stress is mild, and in spring and autumn. In males, when the stress is severe, and in the summer and winter months this stage is shorter but more strongly marked. Animals differing in their behavioral responses in stressful tests (and hence in their resistance to stress) also differ in the degree of initial LPO inhibition and of associated changes in the lipid composition of neuronal membranes. In addition, lateralization (interhemispheric asymmetry) of such changes has been observed in the same animals [3,6].

The stage of long-term adaptation (compensation) is associated with a long-lasting depression of peroxidation processes. Chronic emotional pain stress is characterized by phasic changes in free-radical reactions corresponding to those in physiological (behavioral and vegetative) parameters and reflecting the evolution of the total adaptational syndrome (short-term adaptation, a transitional phase, long-term adaptation, a transitional phase, and finally the breakdown of adaptation) [3]. With the use of an animal model of chronic emotional pain stress ("experimental neurosis") it has been shown that the pathological (in terms of physiological parameters) character of long-term adaptation is manifested at the brain membrane level in a lack of coordination between the regulation of LPO and that of the lipid composition of brain membranes. Also of an adaptive/compensatory nature are those phases of sustained LPO inhibition during which the level of cholesterol remains low and readily oxidizable phospholipids accumulate; such a situation obtains a long time after brain injury and also

when fetal brain tissue has been grafted into the damaged brain area [3].

In *in vivo* studies [3,7,12], natural or synthetic antioxidants or free-radical scavengers administered to animals produced adaptogenic effects which were detected at the physiological level (by observing the stabilization or disappearance of stress-induced abnormalities in the behavior of animals and in their vegetative functions) and which were accompanied by brain changes characteristic of stress in its initial phase, i.e., such changes mimicked the stage of short-term adaptation at the brain membrane level. A single injection of adaptogenic neuropeptides (analogs of ACTH, vasopressin, or substance P) that lacked antioxidant properties *in vitro* was followed by sustained (for up to 7 days) depression of LPO against the background of a stably increased capacity to scavenge superoxide radicals in the brain and blood [3, 26]. Interestingly, the time course and pattern of changes in the lipid composition of brain membranes were specific for each neuropeptide.

Thus, both the adaptation to stressors and the effects of adaptogenic (including pharmacological) agents involve reduction of LPO and activation of radical scavenging in the brain. The inhibition of LPO, the elevation of antioxidant activity, and the lowering of the cholesterol content in brain membranes may be regarded as nonspecific processes of brain adaptation. Antiradical activity of the brain is a major stress-limiting factor. At the stage of short-term adaptation, the enhanced antiradical protection is mainly afforded, directly or indirectly, by low-molecular compounds (catecholamines, corticosteroids, neuropeptides), some of which convert from radical scavengers at this initial stage into radical generators later on. At the stage of long-term adaptation, a major role in antiradical protection is played by antioxidant enzymes whose activity rises [3]. The adaptive inhibition of LPO leads to the mobilization of other molecular mechanisms in short-term adaptation and to the effective functioning of adaptational systems that come into play later in the process of long-term adaptation.

In 1980 it was shown for the first time that when focal or primarily generalized epileptic activity is developing in an animal's brain, the regulation of LPO in the area(s) of hyperactivity becomes impaired, which results in uncompensated activation of LPO and in elevated levels of its products not only in the CNS but also in the blood [11]. Further detailed studies of this phenomenon led to the conclusion that the impairment of LPO regulation is an important determinant of epileptic activity [16,17]. This conclusion was chiefly based on the following findings made with various animal models of epilepsy and in clinical investigations [16,17].

Preventing uncompensated LPO activation by administration of antioxidants to animals was found to delay the development of epileptic activity and to reduce its magnitude. Antioxidants exhibit marked anticonvulsive activity, and anticonvulsants are capable of inhibiting LPO in brain membranes *in vitro*. In the test animals, epileptic activity of acute onset was accompanied by the development of a functional deficit in the antiradical system, but when this system had been made more powerful through administration of antioxidants, epileptic activity set in later and was less pronounced. LPO regulation was also shown to be impaired in patients with various forms of epilepsy and in children with a convulsive syndrome, as was indicated by considerably elevated levels of LPO products in their blood. LPO products were present at highest levels in patients with long-standing epilepsy, generalized convulsive seizures, and pronounced personality changes. Epileptic patients with impaired LPO regulation were also found to have reduced activity of the antioxidant (antiradical) system, as was attested, in particular, by lowered blood levels of superoxide dismutase and glutathione peroxidase.

The above-mentioned and other studies on animals with emotional pain stress and on animals and patients with epileptic activity have provided convincing evidence that LPO plays a key role in the pathogenesis of cerebral disorders, including probably those occurring in Parkinson's disease, ischemic and traumatic brain injuries, and in neuroses [23,28,31]. This view is supported by the observation that various CNS disorders involving LPO activation may accompany and aggravate each other. For example, reactive states and affective disorders may have an ischemic component, while Down's syndrome may be accompanied by Parkinson-like symptomatology [31].

As regards many other cerebral pathologies, the question of how LPO activation is linked with their pathogenesis remains open and will undoubtedly be addressed in future studies. Nevertheless, the evidence already obtained in studies of various brain diseases (as well as of diseases affecting other organs [29,31]) in which LPO is activated gives sufficient grounds for regarding LPO activation as a universal mechanism of damage to membrane structures [2,29]. Here, an analogy may be drawn with other ubiquitous cellular processes - for example regulatory ones (phosphorylation/dephosphorylation, systems of cyclic nucleotides, the phosphoinositol cycle, etc.), which can each respond in similar ways to a diversity of stimuli. The potential ability of LPO to exert powerful modifying (injurious)

effects on brain membranes leaves little doubt about the desirability of inhibiting radical reactions in patients being treated for cerebral disorders.

ANTIOXIDANTS IN THE TREATMENT OF CNS DISORDERS

The history of antioxidant use in the treatment of cerebral disorders is still very short, and most of the data have come from preclinical (experimental) rather than clinical studies. Clinical benefits have been reported from the use of antioxidants in patients with traumatic or ischemic CNS injuries, epilepsy, reactive states, or a cardiocerebral syndrome following myocardial infarction [3,16,18,31]. It must be stressed that antioxidants cannot replace psychotropic agents in the treatment of neurological or psychiatric disorders. In neurodegenerative diseases (e.g., parkinsonism, dementia, multiple sclerosis) antioxidants can weaken or delay the pathological process [31]. Their use in mental disorders (schizophrenia, affective disorders) increases the efficacy of psychopharmacotherapy, in particular by diminishing its side-effects and reducing the body's resistance to it, especially in old age [1,20,28,31]. Combination therapies that include antioxidants substantially mitigate the adverse effects of radicals on the genome which are strongly marked in a variety of pathological conditions [30].

In recent years, the range of both naturally occurring and synthetic antioxidants applied in clinical settings has appreciably expanded. The currently used antioxidants include tocopherols, ascorbic acid, carotenoids, nicotinamide, glutathione and its precursors, superoxide dismutase and its low-molecular analogs, derivatives of hindered phenols and of oxypyridines, probucol, thiolic compounds, xanthine oxidase inhibitors, iron-chelating agents, and dimethyl sulfoxide [27]. However, only a few of them (mainly tocopherol, ascorbate, dimethyl sulfoxide, and their combinations) have been employed in the treatment of CNS disorders [1,3,7,16,18,28,31], which can probably be explained by the low bioavailability of most antioxidant agents to the brain because of the blood-brain barrier.

Research to explore the possibilities for utilizing antioxidants in the management of cerebral disorders promises to develop at a rapid pace. Efforts are likely to be directed at finding more substances with antioxidant activity, designing new dosage forms, and optimizing their application, particularly in combination therapies. There can be no doubt but that antioxidants will be increasingly used in the areas of brain traumatology, neurosurgery, neurology, and psychiatry.

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