MINIREVIEW

The Relation of Oxidative Stress and Hyperexcitation to Neurological Disease

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Abstract. **The expression of a considerable range of neurological diseases is thought to involve two pathological states, namely, the production of excessive amounts of reactive oxygen species and neuronal hyperactivity. These events may represent an outcome of general vulnerable features of nervous tissue. Since they are associated with many unrelated diseases, the phenomena may be considered the final common pathways of a range of metabolic defects. Superimposed on these general signs of cellular impairment are the more specific features of each disorder. This review describes some of the evidence for the presence of hyperactivity and oxidative stress in various neurological disorders. Mechanisms whereby the states may interact and modulate each other are discussed. The manner in which such understanding has implications for the development of novel therapeutic approaches is also described.**

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The central nervous system (CNS) has a very high metabolic rate and needs for this rate to be continuously maintained. This requirement can only be filled by an uninterrupted supply of both glucose and oxygen. Transient deficits in such materials can rapidly compromise brain function in a profound manner. Underlying this unusual energy demand is the requirement for the maintenance of appropriate ionic gradients across the neuronal plasma membrane (1). While all cells have such gradients, nerve tissue is distinctive in that these gradients must transiently be allowed to be attenuated in order to enable normal neuronal activity. The temporary relaxation of the normal sodium gradient is needed for the passage of the axonal action potential, and the very steep calcium gradient must be reduced in order to allow the presynaptic exocytotic release of neurotransmitters into the synaptic cleft. It has been proposed that the initial response to acute insufficiency of energy may involve sodium influx into axons while more delayed neurodegenerative events may be enabled by the prolonged presence of excess levels of cytosolic calcium (2).

The influx of these cations occurs by the opening of ion-selective gates within the external neuronal membrane, which increases entropy and this means that cation influx requires no energy. However, removing excess ions from the cytosol by pumping them out into the extracellular space or, in the case of calcium, by sequestering mechanisms, is an energy-demanding process. The ability to maintain an adequate glucose supply to nerve cells is especially critical in view of the very limited intraneuronal glycogen

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stores and the relative inability to use other potential sources of energy such as fatty acids. The supply of oxygen to the brain is equally vital because of the rather low capacity of brain to carry out anaerobic glycolysis.

These features of the CNS, while they may basically be only exaggerations of processes common to many cells, result in the brain possessing a very distinctive set of susceptibilities to impaired energy supply or utilization. Many neurological conditions involve either suboptimal nutrient supply (e.g., stroke) or an excessive energy demand (e.g., epilepsy). It is for this reason that, in addition to the specific and definitive characteristics of each disorder, certain recurring pathological features are found common to a wide range of unrelated neurological disorders.

This review is intended to both highlight two of these features and to illustrate their interrelatedness. These features are (i) excessive neuronal activity and (ii) the production of deleterious amounts of reactive oxygen species (ROS), also (less appropriately) named, free radicals.

Evidence for Oxidative and Excitotoxic Events Associated with Neurological Disorders

Epilepsy and Other Seizure Disorders. It is widely accepted that seizures alter many chemical and biophysical processes in the CNS. While the hallmark of these disorders obviously is hyperexcitability, there are several reports indicating that ROS may play a role in seizure phenomena. The generation of superoxide anion in brain increases during seizure activity in newborn pigs (3). Accumulation of arachidonate metabolites (4) and free fatty acids (5) with ROS promoting potential has been reported in drug-induced seizures. The cyclooxygenase metabolism of arachidonate leading to ROS generation may be an integral component of seizure activity.

Increased superoxide dismutase activity has been reported in brains isolated from kindled rats, and the administration of exogenous superoxide dismutase affords some protection against these seizures (6, 7). Alterations in antioxidant enzyme activities follow chemical-induced seizures (8). The induction of such enzymes involved in the dispersal of ROS is generally taken as indicative of the presence of excess ROS. A brief (1 sec) electroconvulsive shock is sufficient to cause elevated rates of ROS formation in rat cerebral cortex (LeBel and Bondy, unpublished data).

The attenuation of convulsive activity by phenytoin or corticosteroids simultaneously reduces cerebral levels of lipid peroxidation (9). A reciprocal relation between seizures and ROS appears to exist, since free radicals induced in the brain by hyperbaric oxygen can precede, and may provoke, convulsions (10).

Huntington's Disease. The genetic disorder known as Huntington's disease (HD) can be acquired as a sporadic mutation, inherited as an autosomal dominant, or result from the spontaneous degeneration of neurons primarily localized in the striatum. While the etiology of HD remains unresolved, it is generally accepted that the corticostriatal afferents that innervate striatal neurons employ glutamate as an excitatory transmitter. Excessive glutamate activation of excitatory receptors may be involved in the pathogenesis of HD. Evidence for the role that excitotoxic stress plays in HD has been increasing for three decades. Glutamate has been shown to destroy striatal neurons following direct intracerebral application (11). Decreased glutamate receptor content in the caudate and putamen regions of HD patients have been described (12). This probably reflects the loss of cells in the same brain regions that typically occur in HD. Bioenergetic defects, involving mitochondrial metabolic dysfunction, have also been reported in HD brain (13–15). It has also been shown that direct injection of the mitochondrial energy-impairing agent, aminooxyacetate, into the striatum produced HD type lesions (16). Energy impairment may be a result of excess demand arising from excitatory stress in the HD brain.

Reference to a direct association between oxidative stress and HD has not been reported. Perry and Hansen (17) found no statistically significant differences between total glutathione content in the caudate nucleus isolated from patients with HD compared with tissue from neurologic or non-neurologic diseased patients. However, accurate assessment of brain glutathione content in humans is complicated by the artifactual oxidation of glutathione in isolated tissues (18).

Alzheimer's Disease. A combination of oxidative and excitatory stress has been proposed to underlie some of the behavioral deficits associated with aging (19). Both normal cerebral aging and Alzheimer's disease (AD) involve a degree of cell loss. The "free radical theory of aging" proposes that the accumulation of free radical-induced damage is a main contributor to the aging process. The brain may go through intermittent periods of excess oxidative pro-oxidant conditions (20). The progressive accumulation of damage from these transient stressor conditions over a long period can contribute to the aging process. It is likely that cumulative oxidative stress incurred over many years plays a major role in aging.

For several years, theories on the etiology of Alzheimer's disease have drawn an association between aging and AD. However, it is clear that the pathology of AD is complex and involves multiple abnormal fibrous protein deposits. AD is typically characterized by the presence of neuritic plaques and neurofibrillary

tangles. β-amyloid, the abnormal protein that is widely dispersed in AD brain, has been shown not only to enhance glutamate toxicity in culture (21) but also to exert a degree of neurotoxicity when injected directly into rat brain (22). Additionally, and parallel to Huntington's disease, AD is associated with bioenergetic deficits (1, 14, 23, 24), and this may lead to induction of ROS (25). Lipid peroxidation levels are reported elevated in the cortex of AD patients (26).

Superoxide dismutase and catalase levels are elevated in AD (27, 28). Since these are inducible enzymes, a rise in levels implies a compensatory response to excess levels of ROS. In parallel, potentially adaptive changes have been reported in levels of α-tocopherol and glutathione in brains from AD patients (18).

Perhaps the most convincing evidence of a role for ROS in AD is the finding that those areas of the brain most affected by AD, such as the frontal cortex, show a significantly greater degree of oxidative damage to proteins and to the activity of glutamine synthetase, an enzyme especially susceptible to oxidative damage (29). In contrast, the corresponding areas that are less affected, such as the occipital cortex, show a lesser degree of such changes but, nevertheless, are significantly more affected than parallel areas from adult, but not aged, humans.

Parkinson's Disease. A role for oxidative stress in the processes underlying 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity has been proposed. This compound, a contaminant of an illicitly manufactured meperidine analog, has been the subject of much interest since the neurological damage that it can cause closely resembles Parkinson's disease. MPTP is a very specific dopaminergic neurotoxin. Dopaminergic circuitry is especially vulnerable to neurotoxic damage, and this is probably due to the readiness with which dopamine is auto-oxidized in the presence of trace amounts of metals with multivalence potential. In addition, dopamine can be enzymically oxidized by monoamine oxidases to 3,4-dihydroxyphenyl acetaldehyde and H_2O_2.

There is evidence for the "mitochondrial theory" of MPTP toxicity, which postulates that 1-methyl-4-phenylpyridinium (MPP^+), the ultimate oxidation product of MPTP, blocks the reoxidation of NADH dehydrogenase by coenzyme Q_{10} and eventually leads to ATP depletion in a rotenone-like fashion (30). However, there are also several studies that suggest oxygen radicals may play a role in the MPTP-induced neuronal damage (31–33). These concepts may be reconciled by the finding that the metabolic inhibitors rotenone and antimycin can increase the generation rate of oxygen radicals in crude rat synaptosomes and mitochondria (34, 35). Emerging information concerning MPTP has led to several new ideas relating to Parkinson's disease. These concepts include both the possibility of an environmental agent contributing to the pathogenesis of this disease (36) and the potential for antioxidant therapy of this disorder (37). Parkinsonism has been associated with abnormally high levels of superoxide dismutase within the substantia nigra (38), implying an induced response to oxidative stress. Abnormally low levels of ferritin are associated with Parkinson's disease (39, 40). This diminution of iron-sequestering capacity could also enhance ROS generation (41). The hypothesis that free radicals might play a role in drug-induced toxic effects in dopaminergic neurons is supported by the finding that the neurotoxicity of MPTP is attenuated in transgenic mice that overexpress superoxide dismutase (42).

The involvement of excitatory mechanisms in MPTP toxicity is suggested by the finding that the calcium channel antagonist, MK-801, is able to protect against MPTP-induced Parkinsonism in primates (43). Overall, these data support the concept of a dual involvement of oxidative and excitotoxic stress in the pathogenesis of Parkinson's disease.

Stroke and Cerebral Ischemia. Stroke is associated with cerebral ischemia, which can be of varying duration since the vascular supply has the potential be subsequently restored. In addition to hemorrhage caused by the extravasating type of stroke, all transient interruptions of vascularity including traumatic injury to the brain or spinal cord have the potential to lead to severe postischemic damage. Acute prenatal ischemia is a major component in the pathogenesis of cerebral palsy. The presence of free hemoglobin in nerve tissue can exacerbate potential for ROS-effected damage, and that this is due to the appearance of free iron salts is suggested by protection afforded by desferrioxamine (44, 45). Such events have been proposed to account for post-traumatic epileptogenesis (46).

Free radical generation during cerebral ischemia may underlie delayed neuronal death (47, 48). Ischemic injury and edema within the CNS have frequently been found to involve excessive oxidative activity as judged by lipid peroxidation, induction of superoxide dismutase, and the protection afforded by antioxidant chemicals, such as α-tocopherol, iron chelators, or 21-aminosteroids (49–54). The potential for therapy of spinal cord injury by use of novel pharmacological antioxidants is already very promising (55).

The pathological changes consequent to restoration of the normal blood supply may be initially related to excessively high levels of cytosolic calcium (56), and this appears to be the basis for subsequent elevations of ROS (57–59). The high metabolic rates associated with reperfusion injury can lead to excessive ROS production (60). At the onset of ischemia, there is

an accumulation of hypoxanthine due to breakdown of adenine nucleotides. Upon reperfusion, a combination of oxidative and proteolytic events converts xanthine dehydrogenase to the direct oxygen-acceptor, xanthine oxidase. The final combination of elevated enzyme and substrate leads to superoxide production and consequent oxidative stress. It is significant that focal cerebral ischemic injury is attenuated in transgenic mice constitutively overexpressing superoxide dismutase (61).

The complex relation between ROS-induced events and anoxia is illustrated by the finding that morphological damage to the penumbra region around an ischemic region is generally more severe than at the core (62). The presence of excess oxygen free radicals in postischemic tissue has been directly demonstrated (63). Even a brief period of ischemia can lead to ROS generation and, hence, to delayed neuronal death (47). Hypoxia has been directly shown to stimulate ROS production by astroglia (64).

It has been proposed that during ischemia, ROS and excitatory amino acids may cooperate in effecting neuronal damage (65–67). Transient ischemia elevates cerebral levels of both excitatory amino acids and rates of hydroxyl radical formation (68). Whether one of these events gives rise to the second is not well established. Excitatory events may stimulate ROS, but there is also evidence that ROS can lead to release of excitatory amino acids and thus a bidirectional relationship is an attractive hypothesis (69).

The most likely course of events following ischemia may be as follows. The resultant low oxygen supply (ischemia) can lead to reduced energy supply. This anabolic deficit may then result in diminution of the ionic gradients across the plasma membrane. The influx of extracellular calcium will then stimulate neurotransmitter release. In addition, the capacity of the energy-requiring high-affinity reuptake systems will be diminished, and thus extracellular levels of glutamate will rise. A hyperexcitable state will ensue, resulting from both a reduced axonal membrane potential and increased calcium-stimulated neurotransmitter release. Subsequent reperfusion will lead to an abrupt return of glucose and oxygen to neurons, which disrupts mitochondrial function. Such uncoupling will then increase rates of generation of ROS. Elevated intracellular calcium will also exacerbate levels of ROS by way of phospholipase activation. This will give rise to a cycle leading to increasingly severe neuronal damage. This may explain why protection is afforded against ischemic states by both ROS scavengers and calcium channel antagonists.

The role of nitric oxide (NO) in hypoxic neuronal injury is ambiguous. Influx of calcium into the cell can activate NO synthetase and increase levels of NO. This radical can interact with superoxide radical to form the intensely reactive nitroperoxyl radical (70). The inhibition of NO synthetase can be protective to spinal motor neurons (71). However, nitric oxide has also been found to be neuroprotective, perhaps by reducing the toxicity of hydrogen peroxide (72). Thus, under some circumstances, inhibition of NO synthesis can potentiate excitotoxicity (73). The dual nature of nitric oxide may best be accounted for in terms of its ability either to exacerbate the harmfulness of some oxidant species such as superoxide or to form less reactive compounds with other oxidants such as hydrogen peroxide. The effect of NO may then depend on the precise chemical nature of ROS species that are coexisting with NO in a tissue.

Amyotrophic Lateral Sclerosis. Recently, a familial type of amyotrophic lateral sclerosis (ALS) has been described with a genetic inheritance pattern. Another subset occurs within about 20% of this ALS variant in which the disease is associated with abnormal relatively inactive forms of cytosolic superoxide dismutase (74). This implies that ALS may have an association with inadequate means of detoxifying excess ROS. Some symptoms of ALS can be induced in experimental animals by injection of antibodies to motor neurons, and another variant of ALS is suspected to involve an autoimmune attack on calcium gating channels (75). Thus, this disorder may also involve excitotoxic/oxidotoxic mechanisms.

Chemically Induced Neurological Disorders. Several neurotoxic chemicals have been shown to elevate cerebral rates of ROS production in experimental animals. These include methyl mercuric chloride, trimethyltin, toluene, and other organic solvents (76, 77). All of these agents are also capable of increasing intracellular levels of calcium ions in isolated systems (78, 79). The presence of excess levels of ROS has been described both for schizophrenia and for iatrogenically induced tardive dyskinesia (80). There are several reports from double-blind clinical studies that tardive dyskinesia can be ameliorated by treatment with α-tocopherol (81). In view of the toxicity of excitatory amino acids under some circumstances, and their potential for facilitating free radical generation, the safety of some dietary constituents such as monosodium glutamate and the synthetic sweetener, aspartame (which is catabolized to aspartic acid and phenylalanine) has been questioned.

Multiple Sclerosis. The iron chelator, desferrioxamine, has been reported to be of therapeutic value in the treatment of multiple sclerosis, implying an oxidant component in the etiology of this disease (82). The disorder is characterized by bouts of reversible demyelination of axons, and, while this process can ultimately block axonal transmission, an excitatory

phase initially characterizes a partially demyelinated axon. The mobilization of lipid during demyelination may provide a focus for elevated rates of lipid peroxidation.

Interrelations Between Excitatory and Pro-oxidant Events

Many common neurological diseases are suspected to involve a combination of interacting excitatory and oxidative processes (44). In addition, several neurotoxic agents may trigger both of these events simultaneously (83). This does not, however, illuminate the precise relation between, and degree of interdependence of, these phenomena.

Several studies provide evidence that the two phenomena are interrelated. Elevated levels of calcium can enhance free radical-induced damage to cerebral morphological fractions (84), and antioxidants can protect against cytotoxicity induced by high intracellular levels of calcium in the CNS (85). Furthermore, reduction of cytosolic levels of ionic calcium may reduce damage to DNA effected by hydrogen peroxide (86), and calcium channel blockers can prevent cyanide-induced lipid peroxidation and cell death (87). Activation of the NMDA receptor site has been implicated in the postischemic elevation of lipid peroxidation in the hippocampus (88). There is a report of exacerbation of NMDA toxicity in glutathione-deficient cortical cultures (89).

Various mechanisms could underlie these interactions. Glutamate toxicity in a neuronal cell line has been attributed to inhibition of cystine transport and consequent oxidative stress (57). There are several reports implicating elevation of cytosolic levels of ionic calcium as an important link between oxidative stress and excitotoxicity. Increased levels of cytosolic free calcium, may result from either breakdown of the steep concentration gradient of calcium across the plasma membrane consequent to repetitive neuronal activity, or by liberation of the large amounts of calcium bound intracellularly within mitochondria or endoplasmic reticulum. Calcium stimulation of phospholipase A_2, and hence the arachidonic acid cascade, can lead to the release of arachidonic acid. The enzymic conversion of this compound to prostaglandins, leukotrienes, and thromboxanes by cyclooxygenases and lipoxygenases, which directly utilize molecular oxygen, leads to considerable ROS generation (90–92). This may be a means by which excitatory events promote excess generation of ROS (93). Reciprocally, phospholipase A_2-generated oxidative activity has also been shown to inhibit functioning of the GABA receptor gated channel, the major inhibitory receptor complex of the cell (94). Calcium can also activate the respiratory burst of polymorphonuclear leukocytes leading to production of, and extracellular appearance of, superoxide anion. Such leukocytes are known to accumulate in postischemic cerebral tissues (95).

NMDA agonists are especially potent in the stimulation of nitric oxide synthetase (96), and nitric oxide can interact with superoxide to form the intensely oxidant nitroperoxyl radical. There is evidence suggesting that the free radical nitric oxide mediates the neurotoxicity of glutamate (97).

Kainate-induced damage to cerebellar neurons may also be mediated, at least in part, by induction of superoxide (58). In the study of Dykens *et al.*, allopurinol (a specific inhibitor of the superoxide-forming enzyme), xanthine oxidase, and hydroxyl radical scavengers such as mannitol were protective against kainic acid-initiated tissue injury. A possible mechanism underlying this may be through the induction of nerve growth factor (98). However, kainate has been shown to directly promote ROS generation in an isolated subcellular system (99).

The relation between calcium and free radicals seems complex in that extracellular calcium may be an important defence against lipid peroxidation (100, 101). In this context, it may be relevant that the influx of calcium into the cell seems less critical that the liberation of calcium from intracellular storage sites (102).

Excitatory events may stimulate ROS, but there is also evidence for a reciprocal relation between these phenomena. Thus, ROS can lead to extracellular release of excitatory amino acids (69, 103), or can release mitochondrial calcium into the cytosol (104, 105). Peroxidative damage can also impair inhibitory processes as judged by inhibition of GABA-stimulated chloride uptake (94). Glutamine synthetase appears to be especially sensitive to ROS-induced damage, and this may increase intracellular glutamate concentrations (106). A bidirectional cooperation between excess neuronal activity and excessive ROS formation may therefore be relatively common (107). Regardless of whether or not they can quantitatively intensify each other, ROS and increased levels of calcium within the cell can act in concert—for example, by bringing about deleterious cross-linking reactions between proteins (108).

There are, however, conflicting data suggesting that pro-oxidant conditions reduce the ionic gating of the NMDA receptor site (88, 109) and that lipid peroxides act pharmacologically as depressants (110). Some of these apparent contradictions may be due to the key role of iron in ROS production. The presence of ionic iron is essential in the promotion of postischemic lipid peroxidation (111), and iron liberation from sequestration sites within proteins is closely related to tissue pH (56). The extent to which free Fe cations are

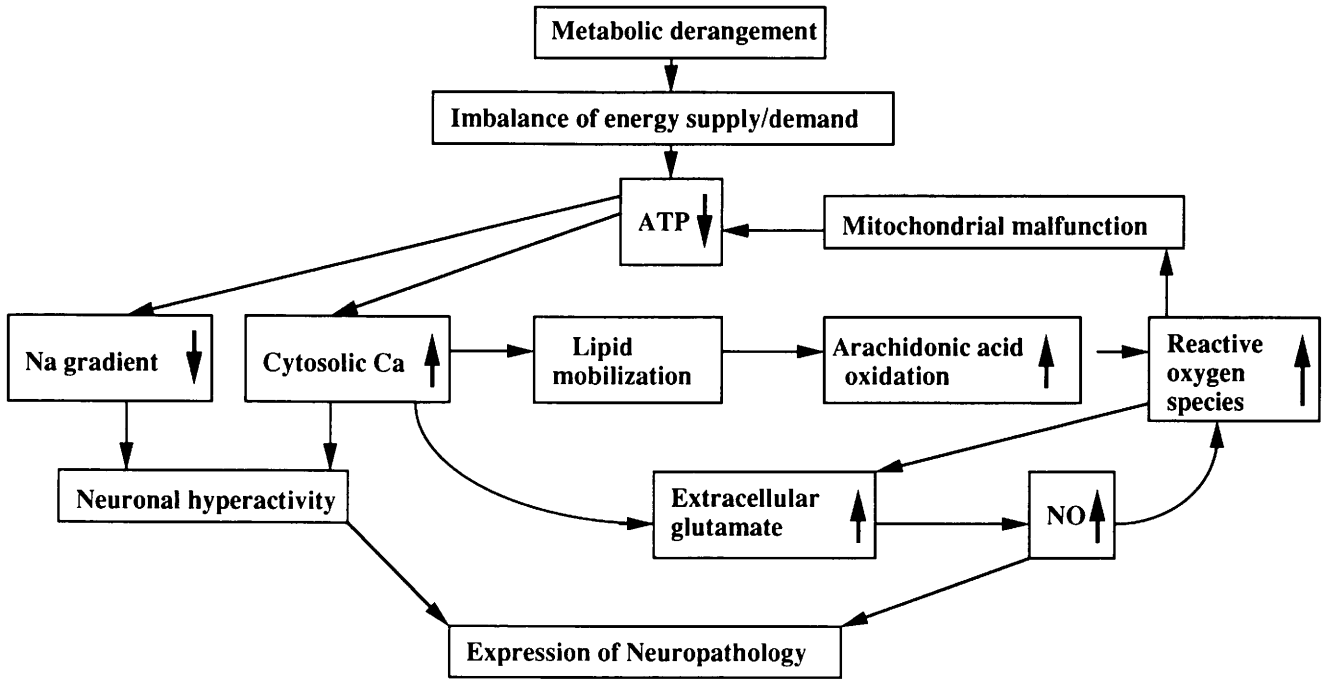

Figure 1. Potential interconnections between hyperexcitation and generation of excessive levels of reactive oxygen species.

present intracellularly, is likely to strongly influence the potency of pro-oxidant agents.

Conclusion

Normally, ROS production is maintained at non-deleterious levels by a wide range of antioxidants, metal chelators, and detoxifying enzymes. Extracellular levels of excitatory amino acids are regulated by potent high-affinity transmitter reuptake mechanisms found in both neurons and glia. Failure of cell energy generation processes to keep up with demand can lead to both the hyperexcited state and the appearance of excessive amounts of ROS. Disease almost invariably involves increased cellular entropy, and so it is reasonable to expect that oxidative stress and excitotoxicity can be contributory factors to many superficially unrelated neurological diseases. In addition to the definitive characteristics of a given disease, a less specific feature involving anabolic failure is likely to be present, and this may account for phenomena under discussion being common to several neurodegenerative diseases.

There is now evidence that oxidative/excitatory interactions may also play a role in the regulation of orderly, programmed neuronal death. Chronic inhibition of superoxide dismutase produces apoptic death of spinal neurons, and this can be blocked by concurrent administration of glutamate receptor agonists (112).

Major surges in intracellular levels of free ionic calcium occur normally, while basal rates of ROS production are not known to flux as dramatically. In pathological states, abnormally high resting calcium levels are thus perhaps more likely to initially precede extended changes in ROS. Some of the potential interactions between excitatory and ROS-enhancing events are illustrated in Figure 1. These interactions can be described as both convergent and interactive.

An unequivocal causal relation between these two broad classes of events, oxidative and excitatory, remains to be uncovered. To a degree, the phenomena may be regarded as parallel and independent. While the general outlines of the co-occurrence and partial interdependence of these events is clear, the precise cause-and-effect relationships taking place in the living brain are unknown. Many correlations have been made in isolated systems, but the quantitative role played by these events in the intact animal awaits elucidation. However, the potential therapeutic validation of antioxidants and calcium channel blockers, need not await final clarification of the interrelation of these processes that are initiated by failure of intracellular generation of energy and by collapse of ion gradients.

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