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*Hypothesis Paper*

# NEUROPROTECTION BY THE METABOLIC ANTIOXIDANT α-LIPOIC ACID

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**Abstract**—Reactive oxygen species are thought to be involved in a number of types of acute and chronic pathologic conditions in the brain and neural tissue. The metabolic antioxidant α-lipoate (thioctic acid, 1, 2-dithiolane-3-pentanoic acid; 1, 2-dithiolane-3 valeric acid; and 6,8-dithiooctanoic acid) is a low molecular weight substance that is absorbed from the diet and crosses the blood–brain barrier. α-Lipoate is taken up and reduced in cells and tissues to dihydrolipoate, which is also exported to the extracellular medium; hence, protection is afforded to both intracellular and extracellular environments. Both α-lipoate and especially dihydrolipoate have been shown to be potent antioxidants, to regenerate through redox cycling other antioxidants like vitamin C and vitamin E, and to raise intracellular glutathione levels. Thus, it would seem an ideal substance in the treatment of oxidative brain and neural disorders involving free radical processes. Examination of current research reveals protective effects of these compounds in cerebral ischemia-reperfusion, excitotoxic amino acid brain injury, mitochondrial dysfunction, diabetes and diabetic neuropathy, inborn errors of metabolism, and other causes of acute or chronic damage to brain or neural tissue. Very few neuropharmacological intervention strategies are currently available for the treatment of stroke and numerous other brain disorders involving free radical injury. We propose that the various metabolic antioxidant properties of α-lipoate relate to its possible therapeutic roles in a variety of brain and neuronal tissue pathologies: thiols are central to antioxidant defense in brain and other tissues. The most important thiol antioxidant, glutathione, cannot be directly administered, whereas α-lipoic acid can. In vitro, animal, and preliminary human studies indicate that α-lipoate may be effective in numerous neurodegenerative disorders. *Copyright © 1996 Elsevier Science Inc.*

**Keywords**—α-Lipoate, α-Lipoic acid, Thioctic acid, Dihydrolipoic acid, 1, 2-Dithiolane-3-pentanoic acid, 6,8-Dithiooctanoic acid, Antioxidant, Neuroprotection, Ischemia, Free radicals

## HYPOTHESIS

Both the metabolic role and the antioxidant properties of the dithiol antioxidant α-lipoate (thioctic acid, chemically designated 1, 2-dithiolane-3-pentanoic acid; 1, 2-dithiolane-3 valeric acid; and 6,8-dithiooctanoic acid) may be clinically useful in treating or preventing brain and nerve disorders. Several factors make it likely that brain and neural tissue would be especially subject to free radical-mediated disorders. The brain is one of the most metabolically active organs in the body, with an oxygen consumption of 35 ml/min•kg (compare to 44, 59, and 96 ml/min•kg for liver, heart, and kidney, respectively).[1,2] The membranes of the brain and nervous tissue are rich in polyunsaturated fatty acids, and their antioxidant status is in some ways lower than in other tissues. Disorders that may be related to free radical damage include ischemia-reperfusion, neurodegenerative diseases, diabetic neuropathy, and inborn errors of metabolism. Very few neuropharmacological intervention strategies are currently available for the treatment of stroke and numerous other brain disorders involving free radical injury. In this article we will relate the various metabolic antioxidant properties of α-lipoate to its possible therapeutic role in a variety of brain and neuronal tissue pathologies. Thiols are central to antioxidant defense in brain and other tissues. The most important thiol antioxidant, glutathione, cannot be directly administered, whereas α-lipoic acid can. In vi-

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tro, animal and preliminary human studies indicate that α-lipoate may be effective in preventing or treating numerous neurodegenerative disorders.

## OXIDATIVE DAMAGE IN BRAIN AND NEURAL TISSUE

The brain is exposed throughout life to oxidative stress, and a number of diseases of brain and the nervous system have been hypothesized to involve free radical processes and oxidative damage, either as a cause or a consequence of the disease process (Table 1). The brain and neural tissue contain sources of oxidative stress unique to these tissues: excitatory amino acids and neurotransmitters whose metabolism produces reactive oxygen species. In addition, other sources of oxidative stress occur during the high and constant use of oxygen (accompanied by superoxide production) in mitochondria to supply the energy needs of these tissues. Cytochrome P450 electron transport, monoamine oxidase activity of the outer mitochondrial membrane, and endogenous guanidino compounds like guanidinoglutaric acid are other sources of free radicals that arise from brain metabolism.

### *Excitatory amino acids*

The amino acid neurotransmitters, glutamate and aspartate, and many of their derivatives, are known as excitatory amino acids. When present in excess, excitatory amino acids can trigger a series of events leading to neuronal damage and death (Fig. 1). Oxidative processes are part of these events.[3] One event is an increase in intracellular $Ca^{2+}$, which can lead to production of free radicals from many sources, including the respiratory burst of leukocytes, arachidonic acid release and subsequent release of reactive oxygen species, and conversion of xanthine dehydrogenase to xanthine oxidase.[3] In addition, influx of calcium leads to activation of nitric oxide synthase,[4] and high levels of intracellular calcium are taken up by mitochondria, where elevated calcium levels lead to production of free radicals; when mitochondria are exposed to calcium at the same concentration as that seen in neurons exposed to excitotoxins, they generate hydroxyl and carbon-centered radicals.[5] Activation of the *N*-methyl-D-aspartate (NMDA) receptor, considered one of the most sensitive receptors to excitatory amino acids, leads to elevation of lipid peroxidation in the hippocampus.[6] Stimulation of NMDA receptors can also lead to increases in intracellular nitric oxide, which rapidly reacts with superoxide to form peroxynitrite, which can be cytotoxic, and which is involved in tyrosine nitration and formation of hydroxyl radical.[7,8] However, recent studies also indicate that NO and its donor compounds (excluding sodium nitroprusside) are potent neuroprotectants because NO suppresses brain lipid peroxidation and inhibits brain injury caused by ferrous citrate.[9,10]

Table 1. Pathological Conditions of Brain and Neural Tissue in Which Oxidative Stress is Thought to Play a Role, Either as a Cause or a Consequence of the Disease Process

| | |
|---|---|
| Alcoholism | Oxygen inhalation therapy |
| Alzheimer's disease | Parkinson's disease |
| AIDS (HIV infection) | Progeria |
| Amyotrophic lateral sclerosis | Retinal damage |
| Cerebral edema | Schizophrenia |
| Cocaine syndrome | Shock |
| Dementia | Shock-brain damage by hyperoxia and hyperbaric oxygen |
| Demyelination (multiple sclerosis) | Spinal cord damage |
| Down's syndrome | Ischemia and stroke |
| Epileptic seizures | Tardive dyskinesia |
| Head trauma (brain) | Vitamin E deficiency |
| Inflammatory diseases | |

Hence, excitotoxins can trigger the formation of a variety of oxidant species in acute conditions such as ischemia-reperfusion and head trauma. Radical scavengers prevent neuronal damage by excitotoxins, further supporting the view that their damage is mediated at least in part by free radicals.[11] It has recently been proposed that milder, continuous excitotoxic stress may play a role in chronic neurological diseases such as amyotrophic lateral sclerosis, Alzheimer's disease, Huntington's disease, and Parkinson's disease.[12]

### *Metabolism of neurotransmitters and endogenous compounds*

The metabolism of dopamine and other neurotransmitters can produce a variety of toxic products, many

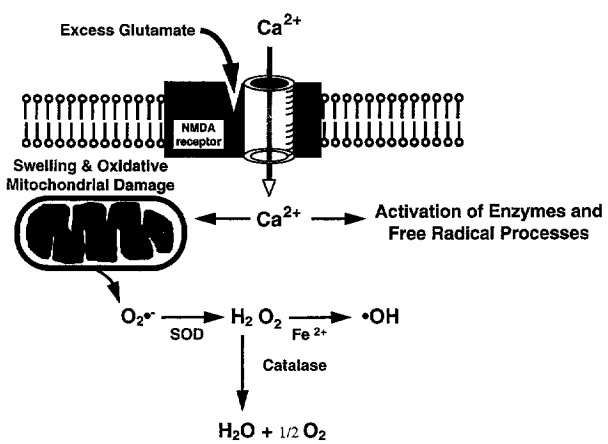


Fig. 1. Mechanism of pathologic consequences of excitatory amino acids. NMDA receptor is shown as an example; excess extracellular glutamate triggers influx of $Ca^{2+}$, with consequent activation of free radical processes and mitochondrial dysfunction.

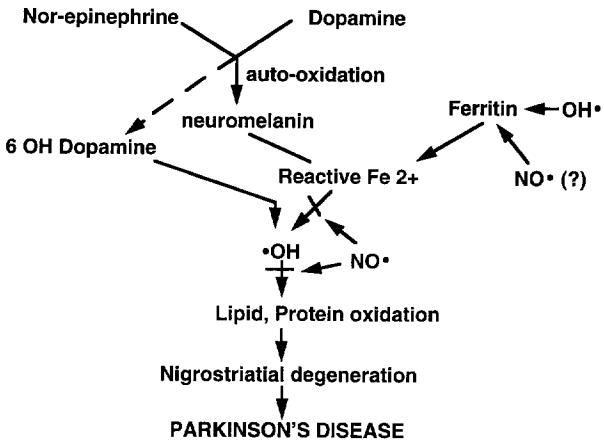


Fig. 2. Degeneration-aging in the substantia nigra compacta neurons in the basal ganglia and Parkinson's disease.

of which are reactive oxygen species (Fig. 2). Monoamine oxidase catabolizes dopamine and produces hydrogen peroxide as a product,[13] and nonenzymatic reactions of dopamine also produce hydrogen peroxide and other reactive oxygen species;[14] because dopamine concentrations in neurons in the substantia nigra are in the millimolar range,[15] this represents a substantial potential source of reactive oxygen species. Auto-oxidation of dopamine produces neuromelanin, and hydrogen peroxide and oxyradicals are formed in the process.[16] In addition, neuromelanin binds iron, and it has been hypothesized that neuromelanin enhances hydroxyl radical formation by reducing bound $Fe^{3+}$ to $Fe^{2+}$.[17] Epinephrine may also be a source of damage during oxidation processes. The auto-oxidation of epinephrine and norepinephrine produces superoxide radicals[18] and neurotoxic products,[19] and epinephrine–copper complexes have been shown to degrade DNA in a hydrogen peroxide-dependent process.[20] In addition, reaction of serotonin with hydroxyl radical results in the formation of the neurotoxin tryptamine-4,5-dione.[21,22]

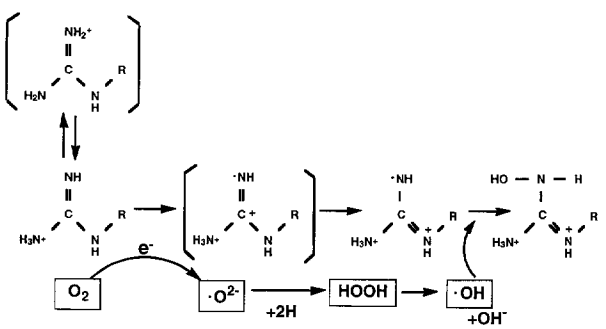


Fig. 3. Proposed mechanism for oxidation of α-guanidinoglutaric acid producing superoxide and hydroxyl radicals. (Adapted from Mori et al.[24])

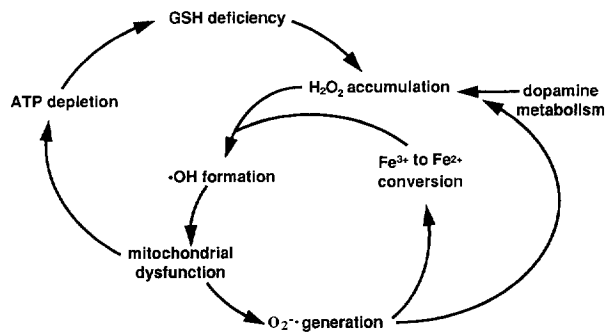


Fig. 4. Vicious cycle of oxidative damage to mitochondria, depletion of ATP and glutathione, and greater production of oxidants such as superoxide and hydroxyl radicals. (Modified from Tritscher et al.[36])

α-Guanidinoglutaric acid, an endogenous guanidino compound associated with epileptic foci in the cat cerebral cortex,[23] has been shown to generate reactive oxygen species such as superoxide and hydroxyl radical, in aqueous solution[24] (Fig. 3). Other guanidino compounds such as methylguanidine, guanidinoacetic acid, guanidinosuccinic acid, or arginine also generate reactive oxygen species, in a manner similar to α-guanidinoglutaric acid (A. Mori, personal communication). These guanidino compounds are known to be endogenous convulsants[25,26] and also to be toxic metabolites in patients with renal failure.[27]

*Mitochondrial dysfunction*

The constant generation of $O_2^{\bullet -}$ by mitochondria is a further source of continuous oxidative stress in neurons that may lead to chronic degenerative neurological diseases. The brain relies on oxidative metabolism for a constant source of energy, but it is estimated that approximately 2% of the oxygen used is converted to oxygen radicals.[28] These free radicals produced during normal oxidative metabolism,[29,30] damage mitochondrial DNA (mDNA).[31] Such mDNA damage increases exponentially with age.[32] The defective proteins coded for by the damaged mDNA are incorporated into mitochondria, and electron transport chain components preceding the damaged protein become reduced, leading to greater free radical (superoxide) production,[33–35] oxidative processes, and depletion of cellular antioxidants such as glutathione,[36] and exacerbation of mitochondrial damage, in a vicious cycle, which may be responsible, in part, for neurodegenerative diseases (Fig. 4). This hypothesis is supported by reports of high degrees of oxidative damage as well as damaged mitochondria in tissues from patients with neurodegenerative disease.[33,37–41] The presence of nonprotein bound $Fe^{3+}$ in the cerebrospinal fluid[42] increases the likelihood for the formation of highly reactive hydroxyl radicals

through the Haber-Weiss reaction, in which $Fe^{3+}$ receives an electron from superoxide, becoming the $Fe^{2+}$ ion, which can then react with hydrogen peroxide to produce hydroxide ion and hydroxyl radical through the Fenton reaction. $Fe^{3+}$ ions are freely accessible in the cerebrospinal fluid and in many regions of the brain.[43]

Excitotoxic mechanisms, neurotransmitter metabolism, and mitochondrial dysfunction can occur together and reinforce each other; for example, dopamine metabolism in the substantia nigra can add to oxidative stress in mitochondria (Fig. 4), while excitotoxic mechanisms and mitochondrial dysfunction may be linked in that increased intracellular calcium, which is part of the excitotoxic process, can lead to free radical formation in mitochondria and exacerbate free-radical induced damage to these organelles[44] (Fig. 1).

### *Involvement of metal ions and metalloproteins in brain aging and disease*

The brain contains a high iron content, especially in the globus pallidus and substantia nigra,[45,46] yet the transferrin content of cerebrospinal fluid indicates that it is near iron saturation.[47] It has been hypothesized that the brain accumulates such high iron contents early in life because iron is essential for proper binding of some neurotransmitters to their receptors, possibly assisting in learning and memory.[48] However, a consequence is that iron overload in certain regions of brain is easily achieved. For example, in Parkinson's disease, substantia nigra total iron content is increased 77% compared to other brain regions.[49] In Alzheimer's disease, neurofibrillary tangle is increased in neurons, and increased iron concentrations are associated with neurons containing neurofibrillary tangle.[50] Such increased iron levels are hypothesized to increase free radical mediated alterations in Alzheimer's disease.[51] The high iron content in brain coupled with the high degree of unsaturation of brain lipids leads to extremely rapid peroxidation in brain lipids;[52] iron increases brain peroxidation both in vitro[10] and in vivo.[9,53]

### *Cellular sources of oxidants*

During their respiratory burst, macrophages produce superoxide and nitric oxide, which combine very rapidly (rate constant $6.7 \times 10^9$ $M^{-1}s^{-1}$) to form the cytotoxic molecule, peroxynitrite. Microglia, the resident macrophages of brain, spinal cord, and retina, comprise approximately 20% of the total glial cell population in the central nervous system and are known to produce a strong respiratory burst activity.[54] Although the free radicals produced by microglia may be protective to the brain by destroying infectious organisms, brain degenerative diseases are associated with dramatic proliferation of the microglial cells, which may lead to excessive radical production.[55,56] In this way neurodegenerative diseases may be similar to inflammatory diseases.

### *Neurotoxins*

Many exogenous neurotoxins may act via formation of reactive oxygen species, and the existence of cellular responses common to many neurotoxins has led to the hypothesis that free radical formation may be the "final common pathway" of neurotoxic action of many agents.[57] Agents that have been shown to induce oxidative stress in brain include methyl mercury,[58] toluene,[59] 6-hydroxydopamine,[60] nonlethal levels of cyanide,[61] and manganese.[62] New information on manganese, however, indicates that at physiological concentrations it is a potent antioxidant that protects dopamine neurons against iron-induced neurotoxicity in vivo.[63] Of particular interest is the compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which induces symptoms similar to those of Parkinson's disease in nonhuman primates. The neurotoxic effect of MPTP is probably mediated by its metabolite, 1-methyl-4-phenylpyridinium (MPP+), which is thought to block mitochondrial electron transport through complex I, leading to ATP depletion;[64] in addition, however, MPTP administration has also been shown to induce oxygen radical formation.[65–69] Superoxide reacts with FeS clusters of mitochondrial electron transport complexes, releasing iron, which further exacerbates damage propagation. It has been suggested that both effects may be linked due to increased oxygen radical formation in mitochondria in which electron transport is blocked.[57]

### *Microvasculature changes*

Because brain and neural tissue are dependent on an uninterrupted supply of oxygen, they are particularly vulnerable to changes in the vasculature that occur with aging or in disease conditions such as diabetes. There is some evidence for oxidative damage with age in the microvasculature of the brain. Mooradian and Smith reported increases in concentrations of conjugated dienes in lipids of the cerebral vasculature with age.[70] Antioxidant enzymes in brain microvasculature undergo changes in activity with age as well; superoxide dismutase activity increases, while glutathione peroxidase and catalase activities decrease or remain unchanged.[71] In addition, brain and peripheral nerve vasculature is subject to atherosclerotic changes, hy-

pothesized to be associated with oxidative events, in aging and diabetes.

*Peptide toxicity*

In Alzheimer's disease, amyloid β-protein accumulates in plaques. It has recently been proposed[72] that free radicals are involved in the aggregation of amyloid β-protein,[73] and that amyloid β-protein can itself form peptidyl free radicals.[74] It is hypothesized that the free radical form of amyloid β-protein is involved in the crosslinking of soluble amyloid β-protein to form amyloid aggregates, and that the free radicals can also be propagated to cell membranes and proteins.[72]

*The antioxidant defense system in the brain*

Brain contains little catalase activity.[75] Of the other major antioxidant enzymes, superoxide dismutase is localized mainly in neurons[76] while glutathione peroxidase is localized mainly in astrocytes.[77] Astrocytes are also the main location of glutathione.[78] These enzyme deficits and localizations suggest that neuronal tissue may be especially vulnerable to $H_2O_2$ production, with subsequent formation of hydroxyl radical.

## CAN LIPOATE ACT AS A THERAPEUTIC ANTIOXIDANT IN BRAIN?

*Criteria for an antioxidant to be effective in brain*

Antioxidants that accumulate in brain and neuronal tissue are potential candidates for prevention or treatment of disorders involving oxidative damage during disease progression. In particular, thiol antioxidants may be good candidates for use in brain disorders; glutathione is depleted in conditions such as cerebral ischemia-reperfusion[79] and Parkinson's disease,[80,81] and glutathione depletion can lead to neurological damage.[82]

However, thiol antioxidants that are effective in vitro or in other tissues may not accumulate in brain. For example, the thiol antioxidant glutathione would seem an ideal candidate for treating many brain disorders. But it is not absorbed in usable form from the diet. *N*-Acetylcysteine, which provides cysteine for glutathione synthesis, and which is readily absorbed and transported,[83] is an alternative, but side effects include nausea, vomiting, and diarrhea.[84] Another alternative, glutathione esters, are absorbed but may not enhance glutathione levels in brain tissue. For example, in a study by Anderson et al.,[85] mice were injected intraperitoneally with glutathione monoethyl ester. In the kidney, liver, and heart, tissue levels of GSH rose, but in brain there was no increase. Hence, in brain disorders, increasing thiol levels via administration of glutathione monoesters may not be feasible, unlike in other tissues.

*Properties of α-lipoate as a metabolic antioxidant*

α-Lipoate may be considered a "metabolic antioxidant" in two ways. First, α-lipoate has long been known for its role in oxidative metabolism;[86–88] as lipoamide, it is an essential cofactor in mitochondrial α-keto acid dehydrogenase complexes. Hence, it is essential for normal oxidative metabolism, and that was long thought to be its only role. However, exogenously supplied α-lipoate is rapidly taken up by cells and reduced to dihydrolipoate (Fig. 5). The reducing power for this comes from both NADH and NADPH,[89] and α-lipoate may modulate NADH/NAD+ and NADPH/NADP+ ratios, thus affecting numerous aspects of cell metabolism. α-Lipoate is, thus, intimately connected to cell metabolism and redox state.

α-Lipoate is especially promising as an antioxidant acting against mitochondrial dysfunction, due to its intimate role in metabolism. A major site of reduction of α-lipoate to dihydrolipoate is by the mitochondrial α-keto acid dehydrogenase complexes (Fig. 6). Hence, dihydrolipoate would be present at the site of oxidant generation. Another way in which mitochondria may be important in neurodegeneration is through alterations in their effects on calcium homeostasis. In this regard, the recent observation of Sen and Roy[90] with indo-1 loaded T-cells in a flow-cytometric system that α-lipoate partially protects against oxidant-induced perturbation of intracellular calcium homeostasis may be relevant to its beneficial effects in neurodegenerative disorders noted by Greenamyre.[91] Consistent with this

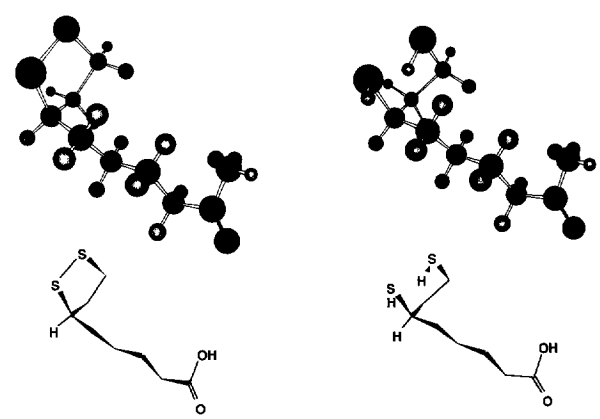


Fig. 5. Structures of α-lipoate and dihydrolipoate.

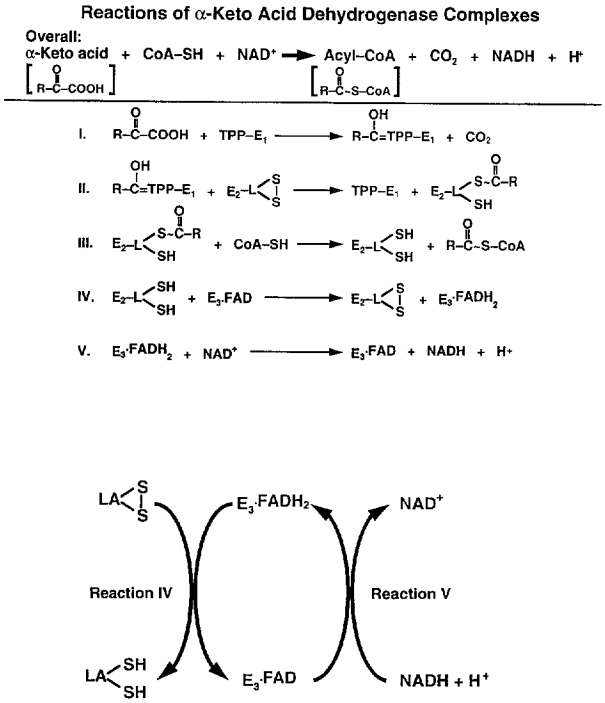


Fig. 6. Major physiological reaction of free lipoate with α-keto acid dehydrogenase complexes in mitochondria. E3 = dihydrolipoamide dehydrogenase. In the presence of free lipoate, reactions IV and V are reversed from their normal direction, and free lipoate is the substrate rather than the lipoyl lysine moeity of the E2 enzyme.

is the report of Christoph Richter (M. Schweitzer and C. Richter, submitted) that α-lipoate also inhibits mitochondrial calcium transport, suggesting that α-lipoate has specific effects on intracellular calcium homeostasis.

In contrast to glutathione, α-lipoate is readily absorbed from the diet, transported, taken up by cells, and reduced to dihydrolipoate in various tissues, including brain.[79,92] The dihydrolipoate thus formed is also exported from cells and can provide antioxidant protection to the extracellular compartment and to nearby cells. Numerous studies demonstrate that both α-lipoate and dihydrolipoate are antioxidants (for a review, see Packer et al., 1995)[93] α-Lipoate scavenges hydroxyl radicals, hypochlorous acid, nitric oxide (NO), peroxynitrite,[94] hydrogen peroxide and singlet oxygen. It also chelates iron, copper, and other transition metals. Dihydrolipoate is an even more potent antioxidant; in addition to those species acted upon by α-lipoate, it scavenges superoxide radical and peroxyl radicals actively formed in many metabolic processes and during the course of lipid peroxidation (Table 2). It appears to have very low toxicity, and is used in the treatment of patients with diabetic neuropathy in Germany.[93] Data of Chiueh et al. suggest that dihydrolipoate can suppress iron-stimulated hydroxyl radical formation, lipid peroxidation, and nigral injury (K. P. Mohanakumar and C. C. Chiueh, unpublished data).

Maintenance of intracellular glutathione levels is essential to protecting neurons from excitotoxic insult,[95] and α-lipoate administration increases intracellular glutathione levels by 30–70%. This has been shown both in cell culture[96,97] and in vivo.[96] Such elevations in GSH cannot be explained by reduction of GSSG, because GSSG is normally present at less than 1% the concentration of GSH.[98]

Of particular interest is the fact that α-lipoate scavenges NO (H. Kobuchi and L. Marcocci, personal communication). Nitric oxide is a neurotransmitter,[99] a potent antioxidant,[9,10] and a suspected cytotoxic agent;[100] of the tissues so far examined, the brain contains the highest NO synthase (NOS) activity.[101] Specific proteins appear to tether NOS in neuronal membranes to target NO production to specific areas; disruption of such specific targeting may contribute to damage in pathologic conditions.[102] NO has been proposed to be involved in both excitotoxic[100,103] and protective effects[104] in NMDA receptors. In addition, NO protects against iron-induced lipid peroxidation and brain injury in vivo.[9,10] The modulation of NO levels in brain and neural tissue by α-lipoate may have effects in such diverse conditions as ischemia-reperfusion, excitotoxic damage, neurodegeneration, learning and cognition, and aging,[100] which should provide fruitful research opportunities, as little is now known about the effects of lipoate on NO-mediated processes in neural tissue.

Another effect of α-lipoate and dihydrolipoate that may have significant consequences in brain as well as other tissues is their effect on signal transduction, as exemplified by inhibitory effects on NF-κB and AP-1 (a dimer of c-*fos* and c-*jun* proteins) activation.[105] NF-κB[106] is a transcription factor that increases the expression of genes involved in viral activation such as human immunodeficiency virus type 1 and those involved in adhesion and inflammatory responses,[107] and is regulated through redox mechanisms.[108,109] Incubation of

Table 2. Reactive Oxygen Species Scavenged by α-Lipoate and Dihydrolipoate

| | Scavenged by | |
|---|---|---|
| Oxidant | α-Lipoate | Dihydrolipoate |
| Hydroxyl radical | + | + |
| Singlet oxygen | + | – |
| Hypochlorite | + | + |
| Nitric oxide radical | + | + |
| Peroxynitrite | + | + |
| Superoxide radical | – | + |
| Peroxyl radical | – | + |
| Hydrogen peroxide | + | + |

human Jurkat T-cells in medium containing 4 mM α-lipoic acid completely inhibits NF-κB activation induced by tumor necrosis factor α or phorbol myristate 13-acetate.[110] Recently, the DNA binding activity of NF-κB was found to be enhanced by DHLA and inhibited by α-lipoic acid.[111] Inhibition of NF-κB DNA binding induced by a nonreducing environment or by exposure to a thiol-oxidizing agent, diamide, was reversed by DHLA. In Wurzburg cells, a clone of Jurkat T-cells in which NF-κB activity can be increased by exposure to $H_2O_2$, the activation of NF-κB induced by 0.25 mM $H_2O_2$ was completely blocked by 1 mM α-lipoate pretreatment (C. K. Sen, personal communication). Sen et al. have found that in Jurkat T-cells loaded with low levels of intracellular lipoate (13 picomol/$10^6$ cells) and dihyrdrolipoate (6.5 picomol/$10^6$ cells) $H_2O_2$-induced disturbance in intracellular calcium homeostasis is markedly suppressed.[90] Pretreatment of T-cells with 1 mM α-lipoate also downregulated expression of adhesion molecules, e.g., ICAM 1.[90] The effects of both α-lipoic acid and DHLA on the expression of the growth-regulating gene c-*fos* have also been investigated.[112] Jurkat T cells preincubated with either α-lipoic acid or DHLA were exposed to TPA, an activator of c-*fos* expression. Cells incubated in DHLA exhibited less c-*fos* mRNA expression, compared to controls, whereas those preincubated with α-lipoic acid exhibited greater expression compared to controls. The interplay of these compounds and their effects on NF-κB and AP-1 in a physiological cellular environment is still difficult to predict.

Exogenously supplied α-lipoate accumulates in brain and peripheral nerves. We have found that rats fed α-lipoate exhibited a 50% reduction in accumulation of lipid peroxidation products from induced lipid peroxidation in three different regions of brain, indicating that α-lipoate has been transported into brain tissue and is protective.[113] Others have found by direct measurement that α-lipoate, administered intravenously to rats in a single dose of 25 mg/kg, appeared in free form in the brain, reaching a level of 2.14 nmol/g wet tissue at 1 h after administration and 0.5 nmol/g wet tissue at 24 h.[79] In an extensive study using [7,8-$^{14}$C] α-lipoic acid,[114] Borbe and Peter showed that α-lipoic acid reaches peak levels in cortex, retina, optic nerve, sciatic nerve, femoral nerve, and spinal cord of rats, within one-half hour of administration of an oral dose of 10 mg/kg; by 24 h levels declined to about 5% of those at one-half hour. Over 21 d of daily oral administration (10 mg/kg/d) levels rose progressively and remained relatively stable in the same regions of CNS and peripheral nerves. This study conclusively demonstrates that α-lipoate is taken up by all areas of the CNS and peripheral nerves. α-Lipoate contains a chiral carbon and exists as two enantiomers, the natural R-enantiomer and the S-enantiomer. Although little work has been done regarding the relative absorption and bioavailability of each, a recent study indicates that the R-enantiomer is more readily absorbed and reaches higher plasma levels than the S-enantiomer.[115]

The combination of uptake, accumulation in brain, interrelationship with metabolism, antioxidant properties, and reducing effects on other antioxidants appears to confer on α-lipoate properties of an ideal therapeutic thiol to treat or prevent brain and nervous system disorders involving free radical processes.

## CEREBRAL ISCHEMIA-REPERFUSION INJURY

### *Oxidative stress in ischemia-reperfusion*

Thus far, one of the most studied effects of α-lipoate is in cerebral ischemia-reperfusion. In addition to reactive oxygen species produced during normal metabolism, another major mechanism by which brain may suffer free radical-induced injury is through ischemia-reperfusion (e.g., during stroke, head trauma, or cardiac arrest). During ischemia-reperfusion, reactive oxygen species form.[116–118] One major consequence of this is the depletion of glutathione.[119] Antioxidants have been shown to reduce the resulting neuronal damage.[120–122] The antioxidant properties of α-lipoate, as well as its ability to increase cellular levels of glutathione, indicate that it would be a potent therapeutic agent in this condition. Several models have been used to demonstrate that α-lipoate has protective effects against ischemia-reperfusion injury.

### *Effects of α-lipoate in ischemia-reperfusion injury*

In a cell culture model of hypoxia, cultured neurons (primary neuronal cultures derived form 7-d-old chick embryo telencephalon), were exposed to cyanide, followed by washout.[123] In control hypoxic cultures, protein and ATP concentrations decreased due to neuronal death, but in hypoxic cultures pretreated with dihydrolipoate, protein concentration and ATP concentration were maintained at normal levels, indicating protection from hypoxic damage.[123] α-Lipoate was ineffective in this system if added 1 h before hypoxia, but was protective if added 24 h before; both the R- and the S-enantiomer were equally effective.[124] These results indicate that α-lipoate is probably being reduced to dihydrolipoate in these cells, and that pathways of reduction for both the R- and the S-enantiomers operate in neuronal cells. In our studies we have found that the S-enantiomer is the preferred substrate of glutathione reductase, present in the cytosol and mitochondrial ma-

trix, while the R-enantiomer (the natural form present in the E2 enzyme of α-keto acid dehydrogenase complexes) is preferred by dihydrolipoamide dehydrogenase (the E3 enzyme), in mitochondria (Fig. 7).[89] Thus, it seems likely that both NADH and NADPH pathways are responsible for reduction of α-lipoate in neuronal cells to its more potent antioxidant form, dihydrolipoate.

Neuroprotective effects have also been found in animal models. Prehn et al., in a study with middle cerebral artery occlusion in mice, demonstrated that in this model of focal ischemia, treatment with dihydrolipoate, but not α-lipoate, reduced the size of the infarct.[123,125] Backhaus et al., using the same model, also found a protective effect of dihydrolipoate but not α-lipoate.[126] In an investigation of the mouse model of focal cerebral ischemia, Wolz and Krieglstein found that α-lipoate administered subcutaneously was protective, but α-lipoate administered either intraperitoneally or intracisternally was not (P. Woltz and J. Krieglstein, submitted). The authors speculate that, at least in the case of intraperitoneal administration, α-lipoate was metabolized in the liver so that brain levels remained low and no protective effect was seen. The R- and S-enantiomers of α-lipoate were also tested in this study; both were found to be effective if administered 2 h before induction of ischemia, but the S-enantiomer was more effective than the R-enantiomer if administered only 1 h before ischemia. These results confirm studies with cells in tissue culture such as human lymphocytes and keratinocytes indicating that both glutathione reductase (NAPDH) and dihydrolipoamide dehydrogenase (NADH) pathways of α-lipoate reduction operate in vivo, although it is possible that reduction is occurring at some other location than brain.

Cao and Phillis also observed a protective effect of α-lipoate against ischemia-reperfusion injury in the Mongolian gerbil model, which offers the advantage of more complete ischemia.[127] Gerbils treated with α-lipoate for 7 d prior to ischemia-reperfusion exhibited less change in locomotor activity and less damage to the CA1 hypocampal pyramidal cell layer, compared to saline-treated controls.

The work of Panigrahi et al.[79] illustrates the necessity of the choice of an appropriate antioxidant for treatment of brain disorders. In rats subjected to reperfusion following ischemia induced by bilateral carotid artery occlusion, pretreatment with GSH isopropyl ester had no protective effect, consistent with the studies of Anderson et al.,[85] in which GSH levels in brain were not increased by administration of glutathione esters. In contrast, pretreatment with α-lipoate (25 mg/kg given intravenously immediately before ischemia) reduced mortality from 78 to 26% in the 24 h following reperfusion. This represents the most remarkable effect of a therapeutic substance in protection against cerebral ischemia-reperfusion so far known. Lipoate pretreatment also almost completely abolished ischemia/reperfusion-induced losses of glutathione in the cortex, striatum, and hippocampus. α-Lipoate pretreatment dramatically decreased lipid peroxidation in these brain areas also; animals pretreated with α-lipoate experienced a 60% average rise in thiobarbituric acid reactive substances (TBARS) while untreated animals experienced a 225% average rise in TBARS. Hence, in this study α-lipoate treatment was shown to be effective in preventing antioxidant loss, attenuating oxidative damage, and dramatically increasing survival after cerebral ischemia-reperfusion.

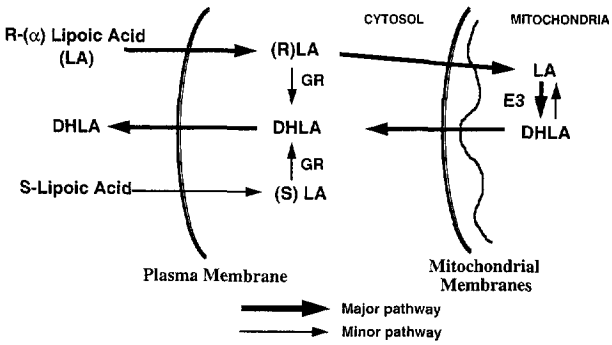


Fig. 7. Enzymes of cell metabolism that reduce lipoate. GR = glutathione reductase. E3 = the mitochondrial pyruvate dehydrogenase E3 enzyme, dihydrolipoamide dehydrogenase.

The exact mechanisms of cerebral ischemia-reperfusion injury have not been elucidated, but free radical mechanisms are generally thought to play a role. Free radical formation may be initiated by release of free iron ion. Because dihydrolipoate chelates iron, it may operate at this step to reduce ischemia-reperfusion damage. Also, as stated above, both dihydrolipoate and α-lipoate are potent scavengers of reactive oxygen free radical species, such as superoxide, hydroxyl, nitric oxide radicals, and hydrogen peroxide, and hence, they may operate here to reduce ischemic injury. In addition, dihydrolipoate also regenerates other antioxidants. Current evidence indicates that dihydrolipoate can reduce glutathione disulfide,[128] dehydroascorbate and the semidehydroascorbyl radical,[129,130] or ubiquinone,[131–133] all of which can contribute to vitamin E regeneration from its radical oxidized form, as well as to reduce thioredoxin[134,135] (Fig. 8). Indeed, dihydrolipoate exhibits thiol reductase activity.

Any or all of these redox actions of dihydrolipoate may contribute to α-lipoate's ability to decrease damage from ischemia-reperfusion. To further elucidate the mechanisms of α-lipoate's action in ischemia-reperfusion in various brain regions, the regional distribution

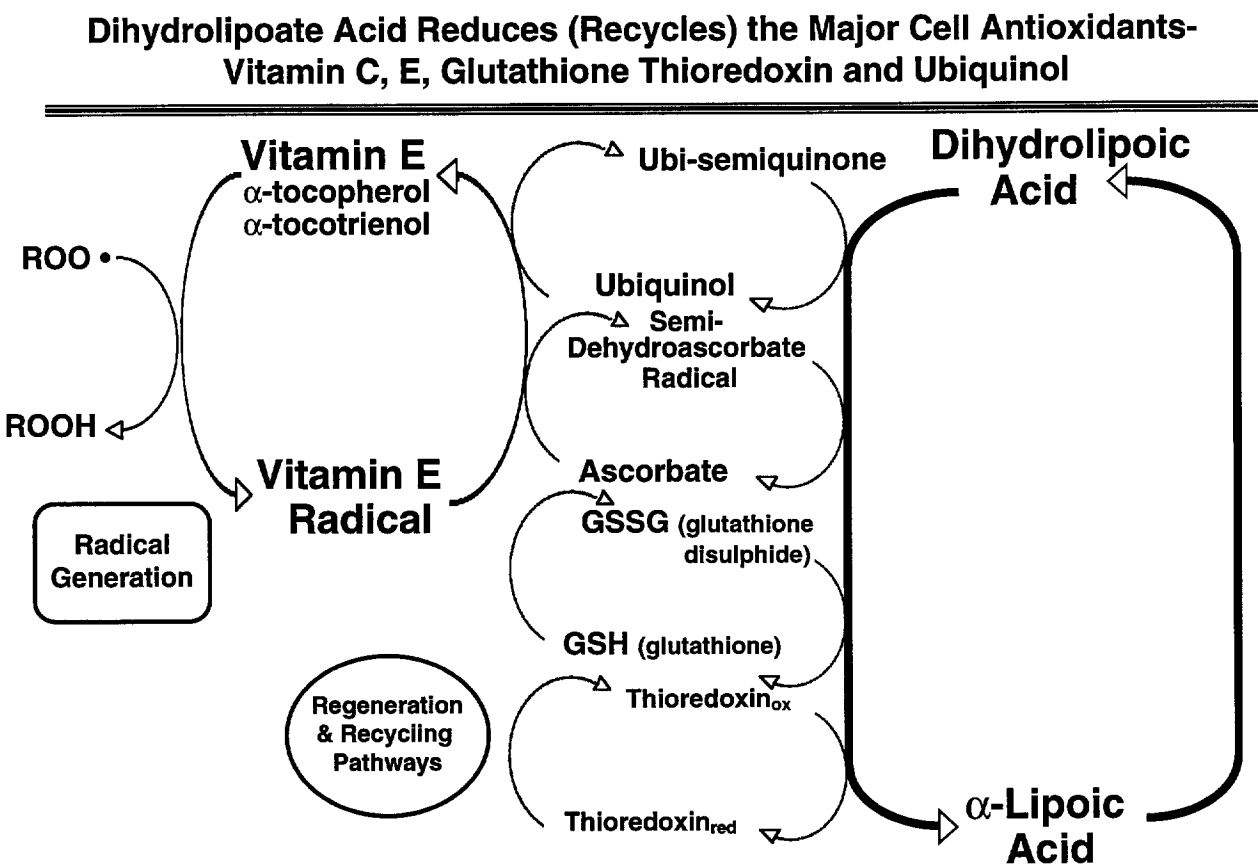


Fig. 8. Pathways by which dihydrolipoate recycles vitamin E and other antioxidants.

of α-lipoate would be of interest to investigate, using new imaging techniques, EPR tomography and biochemical studies of lipoate content in brain regions.

## NEURODEGENERATIVE CONDITIONS

Chronic and age-related neurodegenerative conditions, such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease have been hypothesized to have an oxidative component. Several oxidative mechanisms may be involved in each disease, but the relative importance of each mechanism probably differs in each.

### *Aging and memory loss: excitotoxic mechanisms*

In aging, both oxidative stress and excitatory imbalance may be part of the cause of loss of function such as memory.[136] Stoll et al. have demonstrated that α-lipoate has shown potential in treating aging-related behavioral declines.[123,137–138] Aged mice (20–23 months) receiving oral α-lipoate (100 mg/kg body weight for 15 d) exhibited improved performance in an open-field memory test, and 24 h after the first test α-lipoate-treated animals performed better than young animals.[137,138] There was no effect of treatment with α-lipoate on memory in young animals. The α-lipoate–treated animals exhibited decreased age-related *N*-methyl-D-aspartate (NMDA) receptor declines compared to controls, but showed no improvement in muscarinic, benzodiazepine, or $\alpha_2$-adrenergic receptor deficits. The authors concluded that α-lipoate's free radical scavenging ability may improve NMDA receptor density, leading to improved memory. In cortical neurons dihydrolipoate enhanced NMDA receptor-mediated whole cell responses by reducing the redox site on the receptor[139] and such an effect may partially explain the memory improvement in the aged mice.

Protective effects of α-lipoate against excitotoxins have been demonstrated both in vitro and in vivo. Müller and Krieglstein found that 1 μM α-lipoate added to cultured rat hippocampal neurons 2 h after plating and after every medium replacement protected against cell damage induced by 1 mM glutamate; 18–20 h after glutamate treatment, 91% of nonlipoate treated cells were damaged (assessed by trypan blue dye exclusion) whereas only 8% of lipoate-treated cells lost viability.[124] Greenamyre et al.[91] observed that the

intraperitoneal administration of α-lipoate or dihydrolipoate (10 mg/kg body weight for 10 d) decreased rat striatum lesions induced by excitotoxins, which affect NMDA receptors and which may lead to calcium influx as well as generation of NO and other free radicals.[44] In animals that received NMDA, striatal lesion size was reduced 49% by α-lipoate treatment and 41% by dihydrolipoate treatment. In animals receiving malonic acid, α-lipoate reduced lesion size by 45% and dihydrolipoate by 68%. These authors suggest several possible explanations: dihydrolipoate alters the activity of α-ketoglutarate dehydrogenase complex and thus might enhance mitochondrial function;[140] lipoate can increase ATP levels;[141] or the effects of lipoate and dihydrolipoate may be mediated through their own antioxidant effects or their regenerative effects on other antioxidants. Among the explanations offered, the latter appears most likely.

*Parkinson's disease: neurotransmitter metabolism and metal ions*

Parkinson's disease is a neurodegenerative disease in which an oxidative stress hypothesis for disease pathogenesis is well supported. It is characterized by the loss of dopaminergic neurons in the nigrostriatal system. The increased $Fe^{3+}$ content, especially in the substantia nigra[49] and the production of $H_2O_2$ during dopamine metabolism in dopaminergic neurons[142] point to the possibility that these neurons are damaged by highly reactive hydroxyl radicals, perhaps arising from the Haber-Weiss or Fenton reactions. It has also been proposed that copper ion release, in the presence of L-DOPA and its metabolites, may be an important mechanism of neurotoxicity, because L-DOPA and dopamine cause extensive oxidative DNA damage in the presence of $H_2O_2$ and traces of copper ions[143] (B. Halliwell, J. P. E. Spencer, and O. I. Aruoma, personal communication). Mitochondrial respiratory chain dysfunctions in different tissues from Parkinson's patients have also been identified.[39,144,145] As mentioned previously, MPP+, the metabolite of the neurotoxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP), inhibits the mitochondrial respiratory chain complex I, increases hydroxyl radical generation,[65,66] induces neuronal loss in the substantia nigra, and causes symptoms similar to Parkinson's disease[146,147] in monkeys and other animal models. The oxidative stress hypothesis is further supported by the increased production of the superoxide anion radical in the mitochondria and increased superoxide dismutase activity in the substantia nigra from Parkinson's patients.[33] Total iron is increased and ferritin is reduced in the substantia nigra pars compacta in patients with Parkinson's disease.[148] Therefore, $Fe^{3+}$, $H_2O_2$, and $O_2$ are all likely present and available for the Haber-Weiss or Fenton reaction, leading to oxygen damage by the hydroxyl radical. Increased levels of malondialdehyde[37] and of hydroperoxides, and decreased levels of glutathione,[81,149] the degree of which is correlated with the severity of the Parkinsonism,[81] have been found in the substantia nigra of Parkinsonian patients compared to normal controls,[38] thus supporting the hypothesis of oxidative stress and neuronal death in Parkinson's disease. The decreases in glutathione occur early in disease pathology, suggesting that it is a primary event.[150] It has been hypothesized that treatment with L-DOPA, a common treatment for Parkinson's disease, may accelerate neuronal degeneration by increasing free radical levels[151,152] and depleting glutathione;[143,153] this is prevented by dihydrolipoate (B. Halliwell, personal communication). However, the major metabolite of L-DOPA in the brain is not dopamine but rather 3-methyldopa, which has a higher redox potential and may not cause oxidative stress.

In experimental models of Parkinson's disease it has been established that inhibition of the enzyme responsible for the oxidative metabolism of dopamine, monoamine oxidase B (MAO-B), can diminish oxidative stress to dopaminergic neurons and prevent neuronal degeneration.[154] A large scale, double-blind, placebo-controlled multicenter clinical trial of selegiline (deprenyl), which inhibits MAO-B, showed that it can prolong the time until patients require L-DOPA treatment by 10 months.[155] This study suggests beneficial effects in the use of other agents that reduce oxidative stress in the treatment of Parkinson's disease.

There are a number of sites in the chain of reactions of oxidative stress where α-lipoate or dihydrolipoate can be effective (Fig. 9), especially in the scavenging

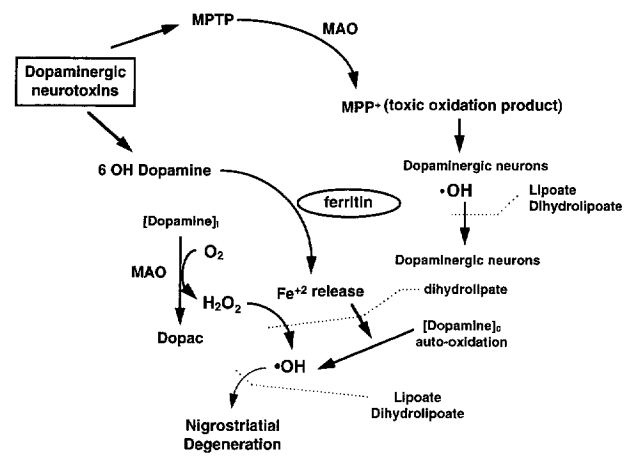


Fig. 9. Oxidative stress and Parkinson's disease showing possible sites of effects of α-lipoate.

of superoxide and hydroxyl radicals. These scavenging activities could interrupt the chain of degenerative reactions at key points.

As mentioned previously, Parkinson's disease is associated with reduced levels of glutathione,[81,149] and the ability of α-lipoate administration to increase cellular glutathione levels may prove helpful in preventing the antioxidant depletion in the disease. In addition, S-nitroso glutathione, a NO donor, is a potent antioxidant in vivo,[9] which in turn, protects nigral neurons against oxidation stress/injury caused by hydroxyl radical or ferrous ion.

Götz et al. showed that, in mice given MPTP and diethylthiocarbamate (which potentiates the effects of MPTP), pretreatment with α-lipoate did not restore dopamine in striatum but was able to maintain the normal ratio of reduced to oxidized ubiquinone.[110] In cultured cells from the mesencephalon of C57BL/6 mice, treatment with lipoic acid resulted in partial restoration of both $^{3}$H-dopamine uptake and dopamine content after exposure of the cells to MPP+, but did not improve cell survival; only the naturally occurring R-enantiomer was effective.[156] In addition, α-lipoate treatment has been shown to increase $^{14}$C-deoxyglucose uptake in substantia nigra,[157] zona compacta, and zona reticulata (T. A. Seaton, P. Jenner, D. Marsden, in preparation). Because altered glucose utilization has been shown to occur in the MPTP primate model of Parkinson's disease,[158] the α-lipoate-stimulated alteration of glucose utilization combined with normalization of reduced and oxidized ubiquinone ratios suggests that α-lipoic acid may potentially correct metabolic abnormalities in Parkinson's disease.

In a rat model of Parkinson's disease 6-hydroxydopamine is used to induce free radical-mediated nigrostriatal lesions through metal-catalyzed free radical formation and semiquinone formation;[159,160] it has been shown that 6-hydroxydopamine is capable of generating 20 times the amount of hydroxyl radical than that which can be generated by dopamine.[161] Pretreatment of animals for 5 d with α-lipoic acid partially prevented the neurotoxic effect of 6-hydroxydopamine treatment, as shown by partial protection of dopamine, homovanillic acid, and dihydroxyphenylacetic acid (Table 3); the protection offered was similar to that seen with vitamin E and the iron chelator desferral (M. B. H. Youdim, personal communication). However, in another study in which the destructive effects of 6-hydroxydopamine were potentiated by prior depletion of glutathione by buthionine sulfoximine (BSO) treatment, treatment with lipoate did not reverse either the glutathione depletion or 6-hydroxydopamine-induced neurotoxicity.[162] Similar results have been seen in BSO-treated lymphocytes, in which treatment with α-lipoate did not restore depleted glutathione, but in untreated cells α-lipoate increased intracellular glutathione (C. K. Sen, D. Han, and S. Roy, personal communication); hence, this system does not seem to be a good model of the physiological condition. In vivo, similar to the lazaroid U-78517F, K. P. Mohanakumar and C. C. Chiueh report (unpublished observations) that intranigral coadministration of dihydrolipoate with ferrous citrate prevents oxidative nigral injury elicited by •OH or ferrous citrate.

Table 3. Striatal Homovanillic Acid and Dihydroxyphenylacetic Acid in 6-Hydroxydopamine-Treated Rats, With and Without α-Lipoic Acid Treatment[a]

| | (ng/mg Tissue) | |
|---|---|---|
| | Homovanillic Acid | Dihydroxyphenylacetic Acid |
| Control | 0.822 | 0.679 |
| 6-OHDA | 0.240 | 0.210 |
| 6-OHDA + lipoate 50 mg•kg$^{-1}$•5 d$^{-1}$ | 0.190 | 0.195 |
| 6-OHDA + lipoate 100 mg•kg$^{-1}$•5 d$^{-1}$ | 0.314 | 0.281 |
| 6-OHDA + lipoate 200 mg•kg$^{-1}$•5 d$^{-1}$ | 0.486 | 0.429 |

[a] Data from M. B. H. Youdim (personal communication).

### *Alzheimer's disease: peptide toxicity*

A variety of oxidative effects have been implicated in Alzheimer's disease. Mitochondrial abnormalities have been found in Alzheimer's disease,[163,164] and lipid peroxidation has been observed in the frontal lobe of Alzheimer's patients.[165] Age-related changes in the cerebral microvasculature[166] and peptide free radicals[72] have also been suggested as being involved in Alzheimer's disease; aggregated beta amyloid peptide fragments can stimulate formation of free radicals such as hydroxyl radical.[167] The evidence for involvement of free radicals in neuronal cell death is at present indirect,[168] but a role for α-lipoic acid in prevention or treatment of this disorder is suggested by the variety of ways in which free radicals have been proposed to be involved. Research is hampered by the lack of a good animal model.

### *Diabetic neuropathy and diabetes-related complications: microvasculature effects*

*Oxidative stress in diabetic neuropathy.* Microvascular effects are especially prominent in diabetes complications, including diabetic neuropathy. Experimental models of diabetes exhibit reduced endoneural blood flow and oxygen tension, resulting in neuropathy. This

process of endoneural hypoxia is associated with an increase of oxidative stress and an impairment of nerve conduction velocity,[169] which can be prevented by antioxidants.[170,171] In a study of streptozotocin-induced diabetes in rats, it was found that, in sciatic nerve, superoxide dismutase showed decreased activity by 25% and increased conjugated dienes, which was apparent as early as one month after induction of diabetes.[169]

In addition to its antioxidant properties, $\alpha$-lipoate has been reported to induce sprouting of neurites in culture,[172,173] increase glucose uptake,[174] and chelate transition metals,[175] which are thought to be involved in diabetic complications including neuropathy.[176] Hence, it is a logical choice for treatment of diabetic neuropathy.

*Experimental studies.* In a large study using the streptozotocin-diabetic rat model, Nagamatsu et al. investigated the effect of $\alpha$-lipoate administration on diabetic neuropathy in diabetic rats and in diabetic vitamin E-deficient rats.[177] Diabetes increased nerve vascular resistance by about 100% and decreased nerve blood flow by 50%. $\alpha$-Lipoate administration in diabetic animals (20, 50, and 100 mg/kg; IP, 5×/week) reversed these trends in a dose-dependent manner. Sciatic nerve GSH was reduced in diabetic rats to about 65% control values, and was also completely restored to normal values by $\alpha$-lipoate. $\alpha$-Lipoate did not restore conduction velocity in sciatic and caudal nerves. But nerve conduction velocity in digital nerves was restored to normal, even at the lowest dose of $\alpha$-lipoate (20 mg/kg IP 5×/week), after 3 months of administration, but not after 1 month. These results are consistent with a free radical component in diabetic neuropathy, which is eliminated by $\alpha$-lipoate, with concomitant elimination of most physiological indices of neuropathy.

In four human clinical studies, two of which were placebo controlled double blind, treatment of diabetic patients with $\alpha$-lipoate resulted in clinical improvement.[178–181] For example, in one study, before treatment six patients had moderate pain and four had severe pain, but after 21 d of intravenous administration of $\alpha$-lipoate (200 mg daily), five patients had no pain, four had moderate pain, and only one had severe pain.[178] More recently, a multicenter randomized double-blind placebo-controlled study[181] with 328 Type 2 diabetic patients showed that clinical measures of diabetic polyneuropathy (numbness, pain, paresthesia, and burning sensation) improved significantly after 3 weeks of intravenous administration of $\alpha$-lipoate five times per week at doses of 1200 or 600 mg The improvement had not declined after 3 weeks (when the study was terminated) and further improvements with continued therapy seem likely. Although in these studies neurophysiological measures, such as vibration sense and nerve conduction velocity, did not change with lipoate administration, none of the studies lasted more than 15 weeks. A period of several years is required to slow progress of diabetic neuropathy due to normalization of blood glucose levels, so it reasonable that no measurable neurophysiological changes occurred over less than 4 months of $\alpha$-lipoate treatment. Consistent with this explanation is the fact that in the steptozotocin-diabetes study of Nagamatsu et al.[177] no improvement in digital nerve conduction velocity was seen after 1 month of $\alpha$-lipoate supplementation in diabetic rats, but conduction velocity was restored to normal by 3 months of supplementation.

*Other complications of diabetes related to brain.* Diabetes results in accelerated development of atherosclerosis, and atherosclerotic lesions in brain blood vessels can exacerbate such conditions as stroke. High blood glucose leads to the appearance of advanced glycosylation end-products (AGE), which can interact with their cellular AGE receptors to generate oxidative stress and activate the transcription factor NF-$\kappa$B.[182] This causes a variety of responses in endothelial cells that accelerate progression of atherosclerosis.[182] $\alpha$-Lipoate and other antioxidants inhibit NF-$\kappa$B activation,[105,110,183–185] and $\alpha$-lipoate prevents AGE-mediated activation of NF-$\kappa$B,[186] and hence, may be helpful in delaying the onset of diabetes-induced atherosclerosis and, in turn, reduce the incidence of brain disorders related to atherosclerotic lesions.

Another possible beneficial effect offered by $\alpha$-lipoic acid is its ability to increase glucose uptake. This has been shown in vitro,[187] in vivo in rats,[188] and humans,[189] and in rat brain.[157] Enhanced glucose uptake by cells provides fuel for both the pentose phosphate shunt and oxidative phosphorylation, enhancing cellular levels of NADPH and NADH. These, in turn, will affect the redox status of glutathione and lipoic acid, which may help the cell maintain a state of redox balance following oxidative challenges, thus potentially slowing development of neurodegenerative diseases such as Parkinson's disease.

*Other neurodegenerative diseases (HIV dementia)*

Studies are currently planned to investigate the effects of $\alpha$-lipoate in other neurodegenerative diseases. One study involving 32 patients will examine the effects of $\alpha$-lipoate in HIV dementia (K. Kieburtz, personal communication)

## INBORN ERRORS OF METABOLISM

Another therapeutic role for $\alpha$-lipoate may be related to its metabolic effects besides its antioxidant

properties. Children affected by inborn errors of pyruvate metabolism display nervous system abnormalities, including ataxia and severe mental retardation. These central nervous system effects appear to be caused by inadequate synthesis of neurotransmitters.[190] There are two general types of errors of pyruvate metabolism: deficiency of the pyruvate dehydrogenase complex, and deficiency of pyruvate carboxylase. Case reports concerning the effects of α-lipoate on these deficiencies are intriguing but not yet conclusive.

*Pyruvate dehydrogenase deficiency*

Though no definitive studies have been done, case reports suggest that α-lipoate treatment may be beneficial in slowing brain and neuronal degeneration in some of these disorders, and allowing psychomotor progress. In a case of dihydrolipoamide dehydrogenase (a component of the pyruvate dehydrogenase complex) deficiency, α-lipoate supplementation at a dose of 25–50 mg/kg body weight produced dramatic improvement in lactic and pyruvic acidemia. Therapy was started at 15 months of age, and this patient continued to improve for 2 years, with slow but consistent gains in neuromuscular development.[191] The mechanism for the improvement is not known, because α-lipoate is covalently bound to the E2 enzyme of the dehydrogenase complexes and not to dihydrolipoamide dehydrogenase (E3 component). Lactate homeostasis also responded to pharmacological supplements of lipoate in a 2-d-old subject with pyruvate dehydrogenase deficiency.[192]

*Pyruvate carboxylase deficiency*

Similar beneficial effects of α-lipoate supplementation have been observed in cases of pyruvate carboxylase deficiency. A 2-year-old patient suffering from pyruvate carboxylase deficiency and subsequent mental and motor retardation was given 100 mg/day α-lipoate orally, along with thiamine, and experienced cessation of eye-rolling, sighing respiration, and periodic vomiting, and markedly decreased blood lactate levels.[193] In one child in which pyruvate carboxylase deficiency was found, intellectual and emotional improvement was noted after α-lipoate administration, though little improvement in motor development was seen.[194] It is again not clear by what mechanism α-lipoate may be exerting beneficial effects, because α-lipoate is not a component of pyruvate carboxylase.

In another case, the biochemical mechanism was also not determined, but a defect in the oxidation of α-keto acids was suspected due to the presence of increased concentrations of α-ketoglutarate in the blood. Two siblings had been affected and died before the age of 4 years. A third sibling was also affected but was administered α-lipoate, first by oral route and then intramuscularly. In this patient signs of psychomotor abnormalities, especially eye-rolling and sighing respiration, ceased, and there was a remarkable improvement in mood; the individual lived to over 6 years of age.[195]

The mechanism of α-lipoate's mode of action in these cases is unknown, and may involve either its metabolic role or its antioxidant effects, or both. Although α-lipoate treatment has not been successful in all cases of pyruvate decarboxylase deficiency or pyruvate dehydrogenase deficiency,[196–198] its effectiveness in some cases, together with its relative lack of toxic effects, make it attractive for the future treatment of these disorders in which the brain and the central nervous system are severely affected.

*Other metabolic defects*

In one other case study, a patient suffering from chronic progressive opthalmoplegia and mitochondrial cytopathies in both brain and skeletal muscle was given oral α-lipoate for 7 months.[199] Treatment with lipoate caused brain phosphocreatine levels to increase 86%, back to normal levels, decreased intracellular ADP, and increased phosphorylation potential, which is a measure of the available energy to the cell.[200]

## OTHER POTENTIAL THERAPEUTIC USES OF α-LIPOATE IN BRAIN AND NEURAL DISORDERS

*Cadmium poisoning*

The brain is a major target of acute cadmium poisoning, which causes metabolic alterations in rat brain.[201] Such alterations may lead to oxidative damage, which enhances peroxidation of membrane lipids.[202] Even small doses of cadmium can harm brain tissues, causing large production of free radicals.[203] In rats injected with cadmium chloride, lipoate injection (IP) 30 min later at a dose of 30 mg/kg body weight completely abolished cadmium-induced lipid peroxidation in brain, and restored ATPase activity, catalase activity, and glutathione levels back to normal.[204] The effect on glutathione was particularly dramatic; brain glutathione levels decreased 63% in rats treated with cadmium alone, whereas in rats treated with cadmium and α-lipoate, there was a modest 4% increase in brain glutathione levels.

*n-hexane inhalation*

Hexane inhalation causes severe neuropathy. In rats exposed to inhalation of *n*-hexane (constant exposure

to 700 ppm), α-lipoate treatment delayed the onset of severe neuropathy by 3 weeks: control rats displayed severe motor neuropathy by 6 weeks, while in rats which also received α-lipoate, severe neuropathy did not appear until the ninth week.[205]

### *Wilson's disease*

Wilson's disease is a genetic disorder of copper metabolism in which neurologic and psychiatric abnormalities are often present in association with copper accumulation in the liver . Current treatment involves use of a chelating agent such as penicillamine; however, penicillamine may cause worsening of the neurological symptoms.[206] α-Lipoate is a copper chelator[175,207] and has shown promise in case studies for the treatment of this disorder, increasing copper excretion in the urine, normalizing liver function, and reducing the severity of symptoms.[208–210]

## CONCLUSION

α-Lipoate, a natural metabolic antioxidant substance, has been shown in numerous types of neurological disorders to potentially be effective in both prevention and treatment, and it is clinically safe.[93,211] It has a range of effects that appear to make it an ideal therapeutic antioxidant for treatment of brain and neurodegenerative disorders in which free radical processes are involved, not least of which is its ability to cross the blood–brain barrier and accumulate and be reduced in brain tissue. Diabetic polyneuropathy is regarded as a chronic alteration of peripheral nerves involving oxidative stress. The effects of α-lipoate treatment in diabetic neuropathy are dramatic, and future clinical trials may reveal its usefulness in other neurodegenerative conditions and aging-related diseases, possibly even in aging of the brain itself.

Several intriguing areas remain to be explored concerning the effects of α-lipoic acid on brain physiology and pathophysiology. α-Lipoate has effects on gene regulation, as exemplified by its modulation of NF-κB and AP-1 activation. This may be a factor in disorders involving inflammation, or in other neurodegenerative processes in which these factors are activated.[105] Studies still need to be conducted to determine the regional distribution and reduction of α-lipoate in various areas of the brain, as well as to determine the effective dose. What is the most effective route of administration, oral or injection? The stereospecificity of reduction and the effects of the two enantiomers has been little studied, but the work of Jenner et al. on glucose uptake in the brain[157] makes clear that this may be an important factor in refining therapy for brain disorders using α-lipoate. Studies thus far have focused on the antioxidant effects of α-lipoate, but its metabolic effects may be equally important. These questions open many avenues for future exploration of the potential therapeutic effects of α-lipoate.

Especially intriguing is the role of altered thiol homeostasis in brain disorders, demonstrated in such conditions as ischemia reperfusion,[212] exposure to haloperidol,[213,214] and exposure to t-butylhydroperoxide.[215] This may prove to be a central mechanism in oxidative stress-induced damage in brain. Administration of lipoate has been shown to have remarkable effects on tissue thiol status, increasing intracellular GSH levels,[96,97] probably by reducing extracellular cystine to cysteine, which bypasses the cystine transporter (D. Han *et al.*, submitted). Thus, α-lipoate may prove to be especially potent for the treatment of brain disorders related to reactive oxygen species.

*Acknowledgements* — This paper is dedicated to the memory of Dr. Simon Wolff, University College London, who passed away unexpectedly on November 25, 1995. Dr. Wolff was a pioneer in the elucidation of glycation mechanisms in relation to oxidation stress and diabetes. He was a gifted scholar and an extraordinary person who will be long remembered.

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## ABBREVIATIONS

AGE—advanced glycosylation end-products
AP-1—activation protein 1
BSO—butathione sulfoximine
CNS—central nervous system
DHLA—dihydrolipoic acid
L-DOPA—L-3,4-dihydroxy-phenylalanine
MAO-B—monoamine oxidase B
MPP+—1-methyl-4-phenylpyridinium
MPTP—1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NMDA—*N*-methyl-D-aspartate
NF-$\kappa$B—nuclear factor kappa B
NO—nitric oxide
NOS—nitric oxide synthase
TBARS—thiobarbituric acid reactive substances