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The Neurotoxicity of Glutamate, Dopamine, Iron and Reactive Oxygen Species: Functional Interrelationships in Health and Disease: A Review – Discussion

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The fact that glutamate, dopamine, iron and reactive oxygen species are potentially individually highly neurotoxic molecules is well known. The purpose of this review is to examine the less well known complex ways in which their normal biological, as well as their neurotoxic activity, are interconnected in relation to fundamental neuronal functions. These functions include synaptic plasticity (formation and removal of synapses), endocytosis-based recycling of receptors for neurotransmitters and neuromodulators, the role of the redox balance between reactive oxygen species and antioxidants in synaptic function, and the possible role of iron–catecholamine complexes in antioxidant protection and intraneuronal iron transport. These systems are closely involved in several diseases of the nervous system including Parkinson's disease, schizophrenia and Alzheimer's disease. In all these oxidative stress and a failure of antioxidant defenses are involved. In the former two the neurotoxicity of catecholaminergic o-quinones is important. In the latter excessive oxidation of neuronal membranes and excessive endocytosis and receptor recycling may be an important factor.

Keywords: Dopamine, Endocytosis, Glutamate, Iron, o-quinones, Parkinson's disease, ROS, Schizophrenia

INTRODUCTION

One of the most remarkable facts about the brain is how it manages to use highly toxic molecules in its normal function because of its built-in defenses against their neurotoxicity. However, this situation contains the seeds for the development of several brain diseases when this balance between neurotoxicity and neuroprotection is disturbed. The potentially toxic molecules covered in this review include glutamate, dopamine, iron, and reactive oxygen species (ROS) of various kinds that interact in complex ways. I will review first

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the normal functions mediated by these interrelationships and then cover the diseases that are relevant. These include Parkinson's disease, schizophrenia and Alzheimer's disease.

THE GLUTAMATE SYNAPSE: BASICS

Recently much progress has been made in integrating data from a number of disciplines into a coherent account of the events at the glutamate synapse that mediate normal synaptic plasticity (i.e. the growth and removal of existing synapses and the construction of new synapses) that underlie many aspects of neural computation and learning (Smythies, 1997). Figure 1 shows a simplified account of the glutamate synapse. There are three main types of glutamate receptor most of which are found on dendritic spines. The AMPA receptor controls a fast channel permeable mainly to Na^+ ions. Action of this receptor depolarizes the post-synaptic membrane. The NMDA receptor controls a slow ionic channel that conducts mainly Ca^{2+} ions. This channel is normally blocked by a Mg^{2+} ion and cannot function unless the Mg^{2+} ion is removed by (partial) depolarization of the membrane. Thus the NMDA receptor acts as a Hebbian co-incidence detector and allows entry of Ca^{2+} ions only when the membrane is already partly depolarized. The third type of glutamate receptor is the group of metabotropic receptors (mGlur), all of which do not control any channel but are linked to G-proteins that trigger post-synaptic cascades in which guanosine nucleotides and protein phosphorylation play key roles. This protein phosphorylation stimulates *inter alia* actin polymerization and growth of the cytoskeleton and so spine growth.

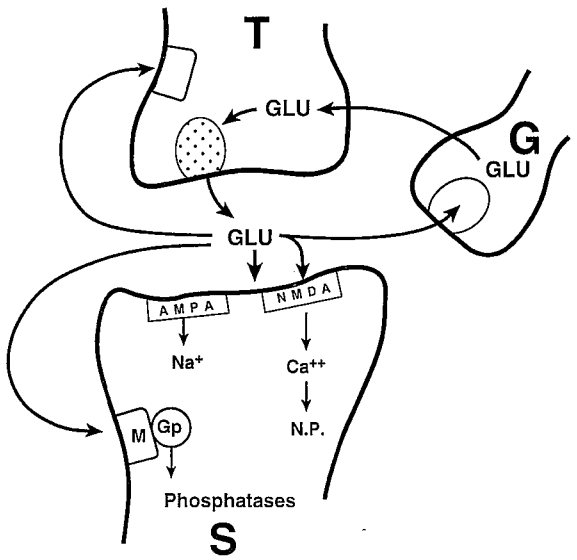

FIGURE 1 Simplified diagram of the glutamate synapse: AMPA, NMDA types of glutamate receptor; G glial cell; GLU glutamate; Gp G protein; M metabotropic glutamate receptor; N.P. nucleases, proteases, lipases; S spine; T axon terminal.

The Ca^{2+} inflow triggered by the NMDA receptor activation has a complex cascade (i) activation of neurodestructive proteases, nucleases and lipases; (ii) activation of phospholipase A2 (PLA2) which mobilizes arachidonic acid (AA) from the cell membrane. The AA in turn activates the enzyme prostaglandin H synthase (cyclooxygenase) (PGHS). This forms the rate-limiting step in prostaglandin synthesis by converting the AA into prostaglandin H, and, in the process, generating large quantities of neurotoxic ROS (ROS – such as the superoxide anion, hydrogen peroxide and the hydroxyl free radical); and (iii) activation of nitric oxide synthase (NOS) which produces nitric oxide from arginine and also generates ROS as a by-product (Fig. 2).

I have presented the hypothesis elsewhere (Smythies, 1997) that a key role in synaptic plasticity is played by the redox balance at the glutamate synapse, both inside the synaptic cleft and inside the spine and adjacent dendrite, between neurotoxic oxidants (i.e. ROS and reactive nitrogen species (RNS) such as the nitric oxide radical NO and peroxynitrite ONOO) and neuroprotective antioxidants. The most toxic ROS is the extremely reactive hydroxyl radical which will attack the nearest biological molecule, be it protein, nucleic acid or lipid. Hydrogen peroxide is the least reactive and can freely diffuse through the cytoplasm and across membranes. However,

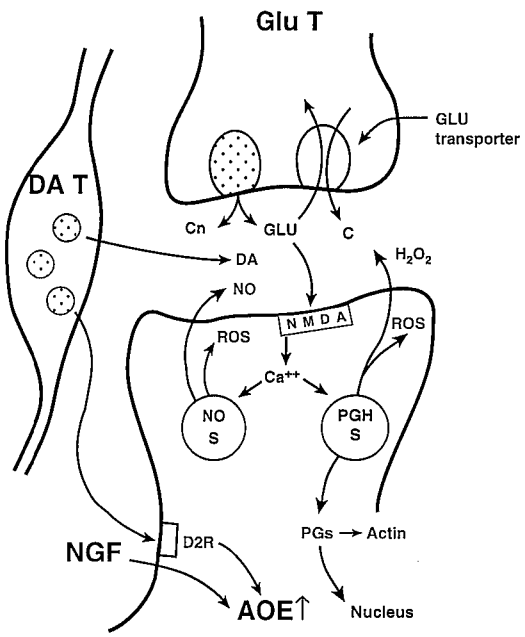

FIGURE 2 Diagram of the redox situation at the glutamate synapse: AOE antioxidant enzyme; C ascorbate (vitamin C); Cn carnosine; D2R dopamine D2 receptor; DA dopamine; Glu glutamate; NGF nerve growth factor; NO nitric oxide; NOS nitric oxide synthase; PGHS prostaglandin H synthase; T axon terminal.

on contact with free iron, superoxide or the nitric acid radical, it is converted by Fenton reactions to the hydroxyl radical. The superoxide anion comes somewhere in between. Nitric acid has two redox forms – the weakly neuroprotective nitrosium ion NO^+ and the strongly oxidant neurotoxic nitric oxide radical NO. Nitric oxide itself is freely diffusible and so, like hydrogen peroxide, it can act as a volume messenger in neural tissue and as a retromessenger to the glutamate synapse.

The high rate of production of ROS and RNS by the post-synaptic cascade associated with the NMDA receptor demands the presence of adequate antioxidant cover to avoid destruction of the synapse itself. Fortunately the glutamate synapse possesses a number of antioxidant protective systems:

(1) The glutamate transporter that terminates the action of glutamate at the synapse, obtains its energy to do so from an Na^+/K^+ ATPase, but it also exchanges ascorbate (vitamin C) for glutamate (Rebec and Pierce, 1994). The mechanism for this appears to be competition for a common storage site in the pre-synaptic terminal (Grünewald, 1993). Ascorbate is one of the principal (mainly extracellular) antioxidants in the brain. Thus, at the same time as the pro-oxidants hydrogen peroxide and nitric oxide produced by the enzymes of the post-synaptic cascade are diffusing back into the glutamate synaptic cleft, the antioxidant ascorbate is also being released into the synaptic cleft by this exchange mechanism. Ascorbate actions at the receptor level are, however, complex. It also inhibits dopamine uptake (Berman and Hastings, 1997) and it can block NMDA, adrenergic, 5-HT and DA receptors (Cammack *et al.*, 1991). Ascorbate inhibits NMDAr evoked currents possibly by altering the charge on the NMRAr protein (Gozlan and Ben-Ari, 1995). It inhibits Na^+/K^+ ATPase and DA sensitive adenylate cyclase (Milby *et al.*, 1981). Pierce *et al.* (1995) found the effect of ascorbate on glutamate systems to be dose dependent. At low doses pre-synaptic effects predominate leading to promotion of glutamate effects, whereas at high doses inhibitory effects on the NMDAr molecule predominate. Ascorbate also raises the level of mRNAs for catecholamine-synthesizing enzymes in neurons (Seitz *et al.*, 1998). Levels of ascorbate in brain are well in excess of that required for collagen synthesis and hydroxylation mechanisms.

(2) The antioxidant dipeptide carnosine co-localizes with glutamate in the synaptic vesicle and is released together with glutamate into the synaptic cleft (Sassoe-Pugnetto *et al.*, 1993). Carnosine scavenges hydroxyl radicals and aldehydes, dismutes superoxide anions, and protects neurons against MDA- and beta-amyloid-induced neurotoxicity (Hopkiss *et al.*, 1997; Hipkiss, 1998).

(3) The NMDAr protein has a redox site, oxidation of which (2-SH to -SS-) down-regulates the receptor. This is neuroprotective as it shuts off the main supply of ROS in the post-NMDAr cascade.

(4) Some 40% of glutamatergic synapses have a non-synaptic dopaminergic bouton-en-passage closely attached to one side. Dopamine is a volume transmitter and can diffuse through the neuropil to reach its own receptors on the presynaptic axon terminal and on the dendritic spine. Dopamine reuptake sites are located at a distance from release sites indicating volume transmission (Sesack *et al.*, 1998). However, dopamine can also diffuse into the adjacent glutamate synapse. The neurotoxicity of dopamine is mediated by its quinone derivatives acting on NMDArs not on dopamine receptors (Shachar *et al.*, 1995; Cadet and Kahler, 1994; Lieb *et al.*, 1995; Michel and Hefti, 1990; Ohmori *et al.*, 1996).

Learning depends to a great extent on positive reinforcement and dopamine plays a prominent role in signaling 'reinforcement received' particularly to the prefrontal cortex (Schultz, 1997; Taber and Fibiger, 1997). The redox hypothesis of synaptic plasticity (Smythies, 1997) suggests that the redox balance between neuroprotective antioxidants and neurotoxic oxidants is maintained by the level of dopamine, which has three possible mechanisms for its antioxidant function. Thus, the receipt of positive reinforcement will increase dopamine release which will tend to promote those synapses active at the time. Likewise a decrease in dopamine release will tend to lead to the deletion of synapses active at that time.

There is much evidence from *in vitro* studies that dopamine (and other catecholamines) can function as antioxidants (Liu and Mori, 1995). These authors propose that "The monoamine metabolism provides an antioxidant effect in the brain against oxidant and free-radical induced damage, because we consider the monoamines and their metabolites, in addition to their well recognized role as neurotransmitters, to be a group of endogenous antioxidants in the brain." In other *in vitro* systems dopamine has been found to inhibit the oxidation of polyunsaturated fatty acids by free radicals (Sam and Verbeke, 1995), to inhibit the oxidation of linoleic acid with a potency equal to vitamin E, and to show, in addition, potent scavenging effects on superoxide anions and hydroxyl radicals (Yen and Hsieh, 1997). The oxidation of beta-phycoerythrin is completely prevented by dopamine (Kang *et al.*, 1998).

The three putative biological *in vivo* mechanisms of dopamine antioxidant action are as follows.

(1) Dopamine exerts a direct antioxidant function by redox cycling between the molecule of dopamine and the molecule of its primary auto-oxidation product dopamine o-quinone. When a molecule of dopamine reduces an ROS, it is itself converted to dopamine o-quinone. This reaction is reversible and the o-quinone is converted back to dopamine by an ambient antioxidant such as ascorbate or glutathione. Dopamine o-quinone is also metabolized by 5-cysteinylization or 5-glutathionylization to products which themselves are antioxidants. However, if the supply of cysteine or of glutathione fail, then dopamine o-quinone can be converted irreversibly by ring closure to dopaminochrome (Carstam *et al.*, 1991; Cheng *et al.*, 1996; Odh *et al.*, 1994) to which I will return below.

(2) The activation of dopamine D2 receptors induces the synthesis of an antioxidant enzyme (probably superoxide dismutase) (Sawada *et al.*, 1998) inside the neuron.

(3) The third mechanism reflects a paradigm change that has recently occurred in cell biology relating to the mechanism by which receptors for neurotransmitters and neuromodulators function. It used to be thought that when a receptor located in the membrane bound a molecule of the transmitter/modulator it underwent a conformational change. This in some cases opened an ionic channel and in other cases activated a second molecule (such as a G-protein) which started a post-synaptic cascade. The receptor itself was supposed to eject that molecule of transmitter/modulator and then wait in the membrane for the next, when the whole process would be repeated. It was recognized that the receptor molecule was eventually replaced, possibly because of accumulated oxidative damage.

It is now known that, whereas receptors that control ionic channels may behave like this, the G-protein and other similar receptors not linked to ionic channels do not (Koenig and Edwardson, 1997; Mukherjee *et al.*, 1997). Instead, when one of these binds a molecule of the transmitter/modulator (e.g. a neuropeptide, catecholamine or acetylcholine), the receptor–ligand complex is rapidly endocytosed inside a clathrin-lined pit which converts to a vesicle inside the postsynaptic neuron. This is rapidly transported (~10 min) to the tubulovesicular endosome system (Fig. 3). Here, the vesicle membrane fuses with the membrane of the early endosome and delivers the receptor–ligand complex into the lumen of the endosome where the acidic environment leads to the dissociation of the ligand from the receptor protein. The complex is then transported to the late endosome. The receptor protein leaves the endosome and is subjected to a triage process. Some molecules (presumably ones damaged by oxidation or other factors) are transmitted to the lysosome for degradation, the rest are recycled back to the cell membrane. Endosome membrane is also recycled back to the surface. The whole cycle in some cases takes around 30 min (e.g. for NGF receptors Zapf-Colby and Olefsky, 1998). This membrane recycling can be rapid and massive. For example, Bretscher and Aguado-Velasco (1998) report that the large synaptic terminals of gold fish retinal bipolar cells 'take up their surfaces' once every 23 s. Rouze and Schwartz (1998) found that these cells turn over their entire membranes once every two minutes.

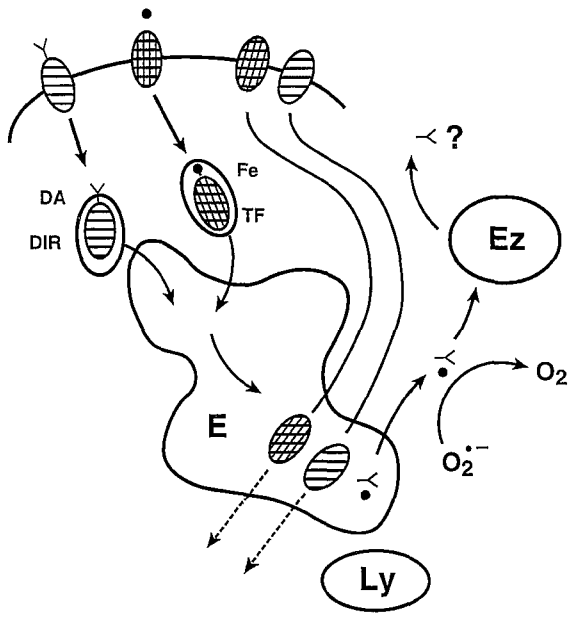

FIGURE 3 Postulated role of iron and dopamine at the endocytotic site: DA dopamine; D1R dopamine D1 receptor; E endosome; Ez iron-using enzyme; Ly lysosome; TF transferrin.

If the ligand is a polypeptide this is transmitted to the cell nucleus where it plays an essential role in gene expression (Jans and Hassan, 1998). As Koenig and Edwardson (1997) say in the case of polypeptide transmitters "... the purpose of endocytosis is to capture the ligand for consequent use by the cell." The fate of other ligands is less clear. For our present purposes it is of particular interest that dopamine G-protein related receptors are also rapidly and robustly endocytosed following transmitter binding (Dumartin *et al.*, 1998). The D1 receptor is endocytosed by clathrin-lined vesicles that also transport the iron transporter transferrin (Vickery *et al.*, 1998). The D2 receptor is endocytosed by non-clathrin lined vesicles that do not also transport transferrin (Vickery *et al.*, 1998). So the question arises what could be the function, if any, of dopamine inside the postsynaptic neuron? Koenig and Edwardson (1997) say that it is unlikely that low affinity agonists (like muscarine or dopamine) would be internalized in sufficient quantity to "cause significant receptor activation in endosomes". However, the relationship between internalization and the intrinsic activity of the ligand is non-linear and so very weak partial agonists can produce significant receptor internalization (Szekeres *et al.*, 1998). Moreover, the role of intracellular dopamine may not be receptor activation but something quite different. One possible mechanism is suggested by a recent paper by Zhao *et al.* (1998) who

reported that catechol–iron complexes in general form very potent antioxidants because of a complex redox cycle involving ferrous–ferric transformations and semiquinone formation. This mechanism effectively transforms 5 molecules of superoxide into 2 molecules of oxygen and 3 of hydrogen peroxide. Dopamine is very effective in this system (O'Brien, 1998). Since in the post-dopamine D1 receptor endosome system, transferrin and the D1 receptor–dopamine complex co-localize in the same endosome, this would enable chelatable iron and dopamine to be in close contact. The path of iron from the late endosome to its target – the iron-containing enzymes being synthesized in the neuron (that include enzymes like tyrosine and tryptophan hydroxylase and certain mitochondrial enzymes) is not clear. There have been suggestions that a small molecule acts as the carrier (Bradbury, 1997; Jacobs, 1977). However, Vyoral and Petrák (1998) using gel electrophoresis could not find any evidence for these. They suggested that the endosome is physically in contact with all the structures that use iron (mitochondria, ribosomes, etc.) and that the iron is transported from one to the others by means of an extensive system of channels and tubes. On the other hand Breuer *et al.* (1997) found that the catalytic potential of iron was highest while in transit between the endosomes and cytosolic ligands. Breuer *et al.* (1995) also found that iron is released from endosomes and enters a cytoplasmic pool at a concentration of 0.3–0.5 mM. The mean transit time through the chelatable pool is 1–2 h. Moreover Moos and Morgan (1998) have recently presented experimental evidence for the existence of low-molecular weight transporters for iron in the brain and cerebrospinal fluid. They suggest that citrate or ascorbate might act in this way. Perhaps, to explain Vyoral and Petrák's results, the postulated iron–dopamine complex is carried attached to some protein. In either event the function of dopamine inside the post-synaptic neuron could be the same as I have suggested for it in the synapse – antioxidant protection of the spine and dendrite. An added advantage would be the safe transport of iron to its cytoplasmic destinations. Free iron is far too toxic to be loose in the cytoplasm. The dopamine complex-mediated iron transport could be transported in the cytoplasm or within extensions of the endoplasmic tubules to the sites of iron usage proposed by Vyoral and Petrák, or both. In the second case superoxide anions could enter the endosomes from their main sites of production i.e. the mitochondria, to which the endoplasmic tubes would give direct access. In the first case the superoxide anions would encounter the dopamine–iron complexes in the cytoplasm. Qian *et al.* (1997) have reviewed the whole question of how iron is transported from inside the endosome to its cytosolic targets. Their conclusion is that little is known about this subject, that there may be multiple carriers (e.g. p97, integrin, H^+-ATPase and the Tf receptor). There may also be different transporters in different cells, and that the evidence favors some type of carrier protein rather than an iron-conducting channel. So, if the dopamine–iron complex forms in the endosome, it may be transmitted to the cytosol by such a carrier mechanism. Alternatively, there may be separate transport mechanisms for iron and dopamine out of the endosome and the complex forms only in the cytosol. Qian *et al.* (1997) state that iron is maintained in a chelatable pool in the cytoplasm after leaving the endosome. Catecholamine–iron complexes function as iron siderophores for the bacterium *Listeria monocytogenes* by which it acquires iron from the environment and transports it into the cell (Coulanges *et al.*, 1997) by means of a ferric reductase in the membrane. Perhaps the endosome membrane also has a similar ferric reductase system. Mitochondria, descended from bacteria, may do the same.

This system may explain the remarkable finding reported in 1985 by Blake *et al.* that a combination of the standard iron-chelator desferrioxamine (100 mg) plus prochlorperazine (25 mg) produces a profound and prolonged coma in rats and humans, whereas these drugs given singly had no such effect. These researchers explained

this effect as follows. Desferrioxamine is a hydrophilic iron chelator and prochlorpromazine is a lipophilic iron chelator. They showed that this drug combination acts synergistically in transferring iron across a layer of chloroform between two water compartments. They therefore suggested that this combination produces a rapid flux of iron (and copper) out of neurons and this flux produces a disturbance of serotoninergic and noradrenergic function that leads to coma. However, it may be the loss of intraneuronal iron rather than the transmembrane flux that is important. Coma is usually associated with disturbances of the glutamate/GABA systems rather than with disturbances of serotonin and noradrenergic systems, which are more related to mood disturbances, cognitive effects and ordinary sleep. For example, most general anesthetics act by potentiating GABA systems. Ketamine acts at anesthetic doses by inhibiting glutamate NMDArs. Thus the coma induced by the combination of desferrioxamine and prochlorperazine may be due to blockade of the NMDAr due to low levels of iron in the post-synaptic neuron, produced by the combined chelation effect described by Blake *et al.* (1985), that interferes with the postulated essential antioxidant effect of the dopamine–iron complexes. Also the side chain of prochlorpromazine somewhat resembles a polyamine such as spermidine. There is a polyamine modulatory site on the NMDAr. So, in addition, a possible antagonist effect of prochlorperazine at the polyamine site on the NMDAr may be involved.

Since desferrioxamine is hydrophilic it may be asked how it could cross the cell membrane in the manner required by this hypothesis. The answer may be supplied by Ollinger and Brunk (1995) who report that desferrioxamine is taken up in the case of hepatocytes by endocytosis and is then transported to the acidic endosome. Furthermore, it inhibits the peroxidation and lysis of lysosomal membranes by chelating intralysomal iron.

In addition to dopamine, endocytosis of receptor/neuromodulator complexes has been reported for adrenergic receptors (Cao *et al.*, 1998; Ferguson *et al.*, 1998; Hirasawa, 1998; Von Zastrow and Kobilka, 1994), muscarinic cholinergic receptors (Sorensen *et al.*, 1998), somatostatin receptors (Beaudet *et al.*, 1998; Boudin *et al.*, 1998), galinin receptors (Fuxe *et al.*, 1998), neurotensin receptors (Vandenbulcke *et al.*, 1998), various types of opoid receptors (Coscia *et al.*, 1998; Jordan *et al.*, 1998), NGF and its receptor TrkA (Grimes *et al.*, 1996; Zapf-Colby and Olefsky, 1998), mGlurs (Liu *et al.*, 1998) and EGFR (Skarpen *et al.*, 1998). In some cases the internalized ligand has been shown to be physiologically active (Grimes *et al.*, 1996; Skarpen *et al.*, 1998). Endocytosis is modulated by GAP-43 and rabaptin-5 (Neve *et al.*, 1998). It may also be significant that the AMPA gluR2 receptor interacts with NEM-sensitive fusion protein which is involved in vesicle formation, fusion and transport (Jahn, 1998).

The redox theory, of course, describes the possible mode of action of only one section of a most complex process involving many other systems relating to synaptic plasticity. For example, there is good evidence that changes in synaptic efficacy that take place in a short time span (minutes, hours) are the substrate for working memory and that this is in turn related to reversible changes in the ionic conductance of NMDA and AMPA/kainate receptors brought on by their phosphorylation in a consensus site on one of the intracellular loops. This process is carried out by protein kinases. Hevroni *et al.* (1998) have shown that stimulation of glutamate receptors by kainic acid leads to an increase in mRNA levels for no less than 66 identified genes relating to synaptic plasticity.

So, in summary, it is suggested that dopamine promotes synaptic growth by three different antioxidant mechanisms (1) by redox cycling between dopamine and dopamine o-quinone (2) by its action on D2 receptors in promoting the synthesis of antioxidant enzymes and (3) by redox cycling of dopamine–iron complexes acting as potent intraneuronal scavengers of superoxide anions.

NEUROTOXICOLOGICAL ASPECTS

The mechanisms proposed for the antioxidant effects of dopamine carry inherent risks. Under certain circumstances, such as low ambient antioxidant cover and low levels of cysteine and glutathione, further dopamine oxidation can occur which results in the production of highly neurotoxic free radical dopamine o-semiquinones. These can form covalent links to sulfhydryl groups on proteins. Dopamine neurotoxicity is mediated via the production of DA quinones. The end metabolite of this pathway is neuromelanin (Fig. 4). Another protective enzyme against DAQ toxicity is catechol-O-methyltransferase (COMT). This O-methylates dopamine hydroquinone to an inactive product and so helps to prevent formation of the toxic o-semiquinone. As COMT is one of the enzymes inactivated by dopamine o-semiquinone, the possibility of a vicious circle exists. The diseases in which this system may be involved include Parkinson's disease, schizophrenia and Alzheimer's disease.

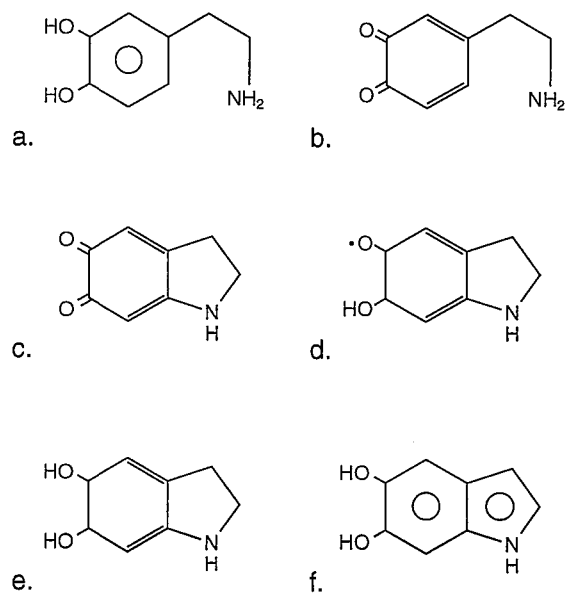

FIGURE 4 (a) dopamine; (b) dopamine o-quinone; (c) dopaminochrome; (d) dopamine o-semiquinone; (e) dopamine o-hydroquinone; (f) 5,6-dihydroxyindole.

Parkinson's Disease

This disease is due to the destruction of the neuromelanin-containing cells particularly in the substantia nigra pars compacta (SNpc) but also in the ventral tegmental area (both dopaminergic), and the locus coeruleus and A1–A3 group in the medulla (both norepinephrinergic). Neuromelanin is composed mainly of a complex polymer of 5,6-dihydroxyindole, and possibly 5-cysteinyldopamine, on a glycoprotein matrix. It represents the final end product of the dopamine and norepinephrine oxidation pathways. Interestingly, the C1–C3 adrenergic groups in the medulla contain neuromelanin and so there is now direct evidence that adrenochrome itself occurs in the brain (Gai *et al.*, 1993), since the presence of neuromelanin in a neuron means that the parent catecholamine o-quinone must be present, or have been present, too. Normally neuromelanin is neuroprotective as it is a potent antioxidant and chelates large quantities of potentially toxic heavy metals. However, in excess it becomes neurotoxic, largely by physical disruption of cellular function by massive amounts of the dense polymer. The SNpc in Parkinson's disease shows signs of severe oxidative stress and evidence of excess production of dopamine o-quinones. There is excessive oxidation of proteins in the cortex and substantia nigra (Castellani *et al.*, 1996; Jenner and Olanow, 1998), excess products of lipid oxidation in the SNpc (Nakamura *et al.*, 1997), mitochondrial defects (Itoh *et al.*, 1996; Schapira *et al.*, 1992) probably due to dopamine quinone toxicity (Zhang *et al.*, 1998), and low glutathione levels (Merad-Boudia *et al.*, 1998; Nakamura *et al.*, 1997; Pearce *et al.*, 1997), excess dopamine o-quinone synthesis (Mattammal *et al.*, 1995), and excess levels of 5-cysteinyldopamine in the CSF – which also indicates increased dopamine quinone synthesis (Cheng *et al.*, 1996). Shen and Dryhurst (1996) also found low GSH levels in the SNpc but normal levels of its oxidized product GSSG, which suggested that the GSH is depleted by forming 5-glutathionyldopamine rather than by scavenging

ROS. This finding is unique to the SNpc in Parkinson's disease and is not seen in other neurodegenerative disorders. Toffa *et al.* (1997) found that reduced levels of GSH by itself did not lead to neural damage but sensitized the cell to attack by toxins. Iron levels in the SNpc are raised some threefold (Ben-Shacher *et al.*, 1992). However, this iron is located in the neuromelanin granules (Jenner and Olanow, 1998) and may be secondary to the neuronal degeneration. Chiueh and Rauhala (1998) have pointed out that iron is involved in tyrosine hydroxylase function and that SNpc neurons have high levels of transferrin and so can take up large amounts of iron and synthesize more dopamine than can other dopamine neurons. Increased iron is found in the basal ganglia in other neurodegenerative diseases (e.g. multiple system atrophy and Huntingdon's disease). Levels of serum transferrin receptor concentrations are strongly correlated with mortality rates in Parkinson's disease but not in controls (Marder *et al.*, 1998). The numbers of cells that contain NF-kB (a redox-modulated transcription factor) are raised some 70-fold in Parkinson's disease (Merad-Boudia *et al.*, 1998). Hemoxygenase levels are increased in Lewy bodies (Schipper *et al.*, 1998) which leads to release of free iron. Lastly Muthane *et al.* (1998) have found that poor Indians, who eat a largely vegetarian diet, show only one-tenth the incidence of Parkinson's disease of Europeans and also have significantly less (40%) neuromelanin in their brains. Parsees in Bombay, who eat meat, had European levels of incidence. However, the poor Indians also eat a great deal of curry spices and these are potent inhibitors of iron uptake from the gut, which may be significant.

Thus, in summary, one can suggest that the oxidative pathway of dopamine and norepinephrine metabolism may play an important role in the etiology of Parkinson's disease.

Schizophrenia

In 1954 Hoffer *et al.* reported that adrenochrome is a psychotomimetic agent. It produces subtle disorders of perception (disturbances of color and shape vision), thought disorder, altered social responses and paranoia of the type often seen in schizophrenia – but no vivid visual hallucinations of the LSD type. This was confirmed by three other groups of workers (Grof, 1963; Schwartz *et al.*, 1956a; Taubman and Jantz, 1957). No such tests unfortunately have been carried out on its close relatives, noradrenochrome or dopaminochrome. Post-mortem studies have shown that schizophrenics have raised levels of 5-cysteinyldopamine in the striatum (Carlsson *et al.*, 1994) suggesting increased production of dopamine o-quinone. Adrenochrome also produces EEG and behavioral changes in animals (Schwartz *et al.*, 1956b). At the biochemical level adrenochrome inhibits COMT (White and Wu, 1975), promotes the synthesis of prostaglandins in brain tissue *in vitro* (Wolfe *et al.*, 1976), promotes the secretion of nerve growth factor by L-M cells (Murakami *et al.*, 1993), inhibits hexokinase and succinic dehydrogenase (Grof, 1963), and acts as a powerful stimulant of guanylcyclase activity in cell free systems (Liang and Sacktor, 1978). But it is not clear which, if any, of these have physiological significance.

We saw earlier that defenses against catecholamine o-semiquinone formation include an effective antioxidant system, a normal transmethylation system and adequate levels of cysteine in the neuron. Schizophrenics show defects in all three areas.

(1) Signs of increased oxidative stress have been found in schizophrenics: increased lipid peroxides (McCreadie *et al.*, 1995) and TBARS (Mahadik *et al.*, 1998) in serum, raised serum malonyldialdehyde and breath pentane (Reddy and Yao, 1996), decreased serum antioxidants albumin and bilirubin (Yao *et al.*, 1998) and raised superoxide production by white blood cells (Melamed *et al.*, 1998). Antioxidant defenses have been reported to be compromised by several groups although there is lack of agreement as to details (Abdalla *et al.*, 1986; Cuénod *et al.*, 1997; Mahadik and Mukherjee, 1996; Reddy *et al.*, 1991). Buckman *et al.* (1987, 1990) have reported that

there is a highly significant negative correlation between the activity of the antioxidant enzyme glutathione peroxidase in red blood cells and the degree of cortical atrophy in schizophrenia.

(2) The level of activity of two enzymes in the one carbon cycle involved in transmethylation reactions – methionine adenine transferase and serine hydroxymethyl transferase – have been found to be underactive in schizophrenia in red cells and in the brain but also in depression (see Smythies *et al.*, 1997 for a review) and overactive in mania.

(3) One of the most consistent findings in schizophrenia is that methionine given to many chronic schizophrenics produces an acute exacerbation of their illness (Antun *et al.*, 1971). One explanation for this is that methionine competes with cysteine for uptake into neurons and so leads to a deficiency of intraneuronal glutathione. This would compromise the general antioxidant protection of the cell and also leads to increased catecholamine o-semiquinone production by failure of protective catecholamine 5-glutathionylization.

(4) There have also been some very preliminary reports of abnormal neuromelanin in schizophrenia (see Smythies, 1996 for details).

It might be argued that adrenochrome mimics only the positive symptoms of schizophrenia. However, unlike mescaline and LSD, we found it to induce some negative symptoms such as severe social withdrawal and paranoia. Furthermore, it is difficult to compare the effects of a single dose of a drug with a long drawn-out process like chronic schizophrenia. Certainly the aminochrome hypothesis is meant only to cover one part of a very complex system. Other factors, such as various neurodevelopmental effects – in particular migration of certain neurons during the second trimester – are likely to be important in certain cases.

Alzheimer's Disease

There is abundant evidence that Alzheimer's disease is associated by marked oxidative stress in the cortex and elsewhere produced by beta-amyloid with oxidative damage to proteins, lipids and nucleic acids. The point I wish to stress here is that, in Alzheimer's disease, the endosomes in neurons are much larger than normal with increased levels of proteases (Cataldo *et al.*, 1997). This supports the hypothesis that the endosomes act as a triage system and separate oxidatively damaged proteins derived from the cell membrane (which are transmitted to liposomes for dismantlement and recycling of the normal portions and excretion of the damaged portions) from undamaged proteins (that are recycled back to the cell membrane). This suggests that the repair of oxidatively damaged membrane proteins is done in the endosome system rather than *in situ* in the membrane.

Glutamate neurotoxicity is also of course extremely important for other diseases of the central nervous system, such as epilepsy and stroke, but these lie outside the scope of this review.

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