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Dopamine turnover and glutathione oxidation: Implications for Parkinson disease

(hydrogen peroxide/monoamine oxidase/nigrostriatal tract)

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ABSTRACT **Parkinson disease is characterized by a major loss (≈80% or more) of dopaminergic nigrostriatal neurons and by an increased turnover of neurotransmitter by surviving neurons of the nigrostriatal tract. In theory, increased turnover of dopamine should be associated with an oxidative stress derived from increased production of hydrogen peroxide. The peroxide is formed during the oxidative deamination of dopamine by monoamine oxidase. In experiments with mice, increased presynaptic turnover of dopamine was evoked by injection of reserpine, which interferes with the storage of dopamine in synaptic vesicles. Loss of dopamine and formation of deaminated metabolites were accompanied by a significant rise (87.8%) in the level of oxidized glutathione in brain. This change was observed in the striatum, which is richly innervated by dopamine terminals, but not in the frontal cortex, which receives a much sparser innervation by catecholamine nerve terminals. The rise in oxidized glutathione was seen even though dopamine terminals constitute only 1% or less of the mass of the striatum. Clorgyline, an inhibitor of monoamine oxidase type A, blocked the formation of oxidized glutathione. These observations confirm that a selective increase in neurotransmitter turnover within nigrostriatal nerve terminals can evoke a change in cellular redox status. We suggest that an oxidative stress may play a role in the natural history of Parkinson disease.**

The factors responsible for loss or dysfunction of nigrostriatal dopamine neurons in Parkinson disease are not understood. It is well established by neurochemical assay at autopsy (1) and in animals with partial lesions of the nigrostriatal tract (2, 3) that surviving dopamine neurons are characterized by increased turnover of neurotransmitter, an apparent compensatory response to the loss of neurons. Because the turnover of dopamine by monoamine oxidase [MAO; monoamine:oxygen oxidoreductase (deaminating), EC 1.4.3.4] is associated with the formation of a cellular oxidant, namely, hydrogen peroxide, increased neuronal activity could, in theory, be associated with an oxidative stress. In other systems, increased generation of H_2O_2 results in either tissue damage or overt cellular destruction. We report here that increased presynaptic metabolism of neurotransmitter alters the redox state of dopamine nerve terminals in the striatum.

The H_2O_2 that is generated by MAO (a mitochondrial enzyme) is scavenged by glutathione (GSH) peroxidase (reduced-glutathione:hydrogen-peroxide oxidoreductase, EC 1.11.1.9) (4, 5), leading to the formation of glutathione disulfide (GSSG).

$$\text{dopamine} + O_2 + H_2O \xrightarrow{\text{MAO}} H_2O_2 + NH_3 + \text{3,4-dihydroxyphenylacetaldehyde} \quad [1]$$

$$H_2O_2 + 2\ \text{GSH} \xrightarrow{\text{GSH peroxidase}} \text{GSSG} + 2\ H_2O \quad [2]$$

$$\text{GSSG} + \text{NADPH} + H^+ \xrightarrow{\text{GSSG reductase}} 2\ \text{GSH} + \text{NADP}^+ \quad [3]$$

Normally, GSSG is efficiently reduced by glutathione reductase (reduced NADP:oxidized-glutathione oxidoreductase, EC 1.6.4.2) and, as a consequence, levels of GSSG in various tissues, including brain (6), constitute only 1% or less of the total glutathione (GSH + GSSG). Levels of GSSG can rise, however, during exposure of tissues to added peroxides or peroxide-generating cell toxins (7–9). The ratio of oxidized to reduced glutathione reflects, in part, the redox state of the tissue.

To evoke increased presynaptic turnover of dopamine, we used the drug reserpine. Reserpine prevents the storage of dopamine in neuronal transmitter vesicles by interfering with the transport of dopamine from the cytosol into the vesicles (10). As a result, the metabolism of dopamine by mitochondrial MAO is accelerated. The net effect is the disappearance of dopamine and the simultaneous formation of acidic metabolites (11, 12). This action mimics increased neuronal activity, where increased release and reuptake of transmitter leads to increased presynaptic oxidation of dopamine. We assessed the effect of reserpine on the redox status of dopaminergic nerve terminals in the striatum by measuring changes in the levels of GSSG.

MATERIALS AND METHODS

Reserpine (Serpasil; CIBA Pharmaceutical) was dissolved in 20 μl of glacial acetic acid and diluted into 0.3 mM dextrose in distilled water. Clorgyline (May & Baker, Dagenham, England) was prepared in isotonic saline.

Male Swiss–Webster mice (25–30 g; Ace Breeders, Boyertown, PA) received intraperitoneal injections of reserpine (10 mg/kg); control mice received the injection vehicle alone. Where indicated, mice received the MAO inhibitor, clorgyline (2.5 mg/kg, i.p.) 18 hr prior to injection of reserpine. Two hours after reserpine, the mice were rapidly decapitated, the striatum and frontal cortex were dissected over ice, and tissues were homogenized in 10 vol of cold 0.4 M perchloric acid containing 0.1 mM diethylenetriaminepentaacetic acid (a metal chelator).

Spectrophotometric assays for GSSG were carried out with a modification (6) of the enzymatic recycling procedure of Teitze (13), which is based on the activity of GSSG reductase. GSH was first removed (7) by reaction with *N*-ethylmaleimide, followed by removal of the *N*-ethylmaleimide by chromatography over Sep-Pak C_{18} cartridges (Millipore/Waters). After the addition of 0.4 mM 5,5′-dithiobis(2-nitrobenzoic acid), 0.17 mM NADPH, and GSSG

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Abbreviations: MAO, monoamine oxidase; GSSG, oxidized glutathione; GSH, reduced glutathione.

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Table 1. Effect of reserpine on GSSG levels in striata of mice, with and without pretreatment with clorgyline

Exp.	Striatal GSSG, μM			Rise in GSSG, μM	
	Control (*A*)	Reserpine (*B*)	Reserpine/clorgyline (*C*)	Reserpine (*B* − *A*)	Reserpine/clorgyline (*C* − *A*)
1	5.1 ± 0.4	10.4 ± 0.2	4.9 ± 0.3*	5.3	−0.2
2	6.1 ± 0.6	12.4 ± 0.1	6.7 ± 0.0*	6.3	0.6
3	6.4 ± 0.3	11.2 ± 0.3	7.5 ± 0.0*	4.8	1.1
4	12.8 ± 0.2	17.1 ± 0.0	13.5 ± 0.3*	4.3	0.7
Mean	7.6 ± 1.8	12.8 ± 1.5	8.2 ± 1.9*	5.2 ± 0.4	0.6 ± 0.3

Reserpine was administered at 10 mg/kg for 2 hr. The dose of clorgyline (2.5 mg/kg, 18 hr) used produces a selective inhibition of MAO type A in brain (15, 16). Data are the mean ± SEM with three or four mice per group.
*$P < 0.001$ compared to reserpine alone (values in column *B*). Individual experiments were compared by a two-tailed Student *t* test and the pooled means by a paired *t* test.

reductase at 16 μg/ml, the rate of color formation (rate of formation of 5-thio-2-nitrobenzoate) was monitored between 1 and 4 min at 412 nm and ambient temperature. A Stasar III flow-through spectrophotometer (Gilford) was used. Rates were corrected for the blank rate in the absence of added tissue extract and compared to a standard curve obtained with known amounts of GSSG, similarly corrected for the blank.

Dopamine and its acidic metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, in the acidified homogenates of striatum were measured by HPLC with electrochemical detection (14).

RESULTS

In eight independent experiments with three or four mice per group, the levels of GSSG were significantly elevated 2 hr after the administration of reserpine: GSSG in the striatum rose by 4.3–6.3 μM (mean 5.1 μM, $P < 0.001$ in each experiment). The increment in GSSG was 87.8 ± 10.7% (mean ± SEM) of the basal levels seen in corresponding control groups. Therefore, mobilization of dopamine by reserpine is associated with a substantial rise in striatal levels of GSSG.

Over this time period, the mean level of dopamine in the striatum fell from 50.9 μM to 1.4 μM ($n = 6$), while the steady-state levels of the acidic metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, doubled (from 4.8 μM to 8.8 μM and from 5.9 to 12.3 μM, respectively). These results confirm the expected increase in MAO activity. The mean rise in GSSG (5.1 μM) is readily accommodated by the H_2O_2 that would be generated during the observed loss of dopamine (49.5 μM). It should be noted that the change in dopamine concentration in the striatum does not take into account the metabolism of newly synthesized dopamine during exposure to reserpine.

Table 1 shows the results of experiments in which reserpine was administrated with clorgyline, a selective inhibitor (15, 16) of MAO type A. In each of four experiments, clorgyline suppressed the rise in GSSG ($P < 0.001$); the mean suppression was 88%. The results are consistent with an inhibition of MAO within dopamine nerve terminals that, in rodents, contain mainly or exclusively MAO type A (17, 18). The extensive suppression by clorgyline indicates a relative lack of participation by MAO type B, such as that present in glia (19). Clorgyline alone did not affect GSSG (controls, 5.6 ± 0.6 μM, and clorgyline-treated, 5.8 ± 0.1 μM; $n = 3$ per group).

The striatum is relatively enriched with dopamine nerve terminals, whereas the frontal cortex receives a sparser innervation by catecholamine (dopamine and norepinephrine) terminals (20, 21). A marked differential was seen between the responses of the striatum and cortex (Table 2). Whereas the striatum exhibited a significant rise in GSSG after reserpine, the frontal cortex did not. The mean change in GSSG in three experiments was +5.0 ± 0.3 μM in the striatum and −0.3 ± 0.6 μM in the cortex. Therefore, the response parallels the degree of innervation by catecholamine terminals.

DISCUSSION

The current technology, as applied to brain, requires that a very small mass of monoamine nerve terminals, embedded within a much greater mass of brain tissue, be viewed against the background of the tissue as a whole. Because GSH is ubiquitously distributed, the loss in GSH would be difficult to detect. However, the basal level of GSSG is quite low and, therefore, an elevation in GSSG within a small tissue compartment can be observed as a significant rise in the average tissue level of GSSG. It is probable that the rise in GSSG seen in the striatum after injection of reserpine is localized mainly to dopamine nerve terminals. In that event, the results signify very much higher concentrations of GSSG within dopamine terminals. It is apparent that the rate of production of H_2O_2 evoked by reserpine is sufficient to override cellular mechanisms for reducing GSSG.

Reserpine promotes MAO activity in other monoamine-secreting neurons. It is reasonable to expect that significant elevations in GSSG also take place in mesolimbic and mesocortical dopamine terminals, as well as in projections of central norepinephrine and serotonin neurons. The striatum,

Table 2. Comparison of the effects of reserpine on GSSG levels in mouse striatum and frontal cortex

Exp.	Tissue GSSG, μM					
	Striatum			Frontal cortex		
	Control	Reserpine	Increase in GSSG	Control	Reserpine	Increase in GSSG
1	5.2 ± 0.5	10.7 ± 0.3*	5.5	5.4 ± 0.6	4.1 ± 0.2	−1.3
2	8.5 ± 0.3	13.4 ± 0.2*	4.9	8.3 ± 0.4	9.0 ± 0.2	0.7
3	3.6 ± 0.3	8.2 ± 0.3*	4.6	3.6 ± 0.3	3.4 ± 0.2	−0.2
Mean	5.8 ± 1.4	10.8 ± 1.5*	5.0 ± 0.3	5.8 ± 1.4	5.5 ± 1.8	−0.3 ± 0.6

Reserpine was administered at 10 mg/kg for 2 hr. Data are the mean ± SEM with three or four mice per group.
*$P < 0.001$ compared to controls. Individual experiments were compared by a two-tailed Student *t* test and the pooled means by a paired *t* test.

with its relatively dense innervation by monoamine neurons, provided the means to study GSSG in a selected neuronal type. The frontal cortex, with its sparser innervation, served as an essential negative control (Table 2). Improvements in technology will be required to facilitate the study of monoamine neurons in brain regions that are less densely innervated than the striatum.

The observation that reserpine elevates striatal GSSG *in vivo*, reported here, is supported by *in vitro* observations with isolated nerve terminals. When striatal synaptosomes are incubated with reserpine and L-dopa (0.04–1.0 mM), the levels of GSSG rise significantly and the rise is blocked by clorgyline (22, 23). The addition of L-dopa, an immediate precursor of dopamine, is required *in vitro* because endogenous dopamine is lost during the preparation of synaptosomes. *In vivo*, however, the high intracellular pool of dopamine, which has been estimated at 50 mM within striatal nerve terminals (24), along with continued biosynthesis of fresh dopamine, provides adequate substrate for neuronal MAO.

A therapeutic trial of "antioxidants" to slow the progression of Parkinson disease (25) is predicated, in part, on the theory that an oxidant stress derived from the turnover of neurotransmitter by MAO contributes to the loss of dopamine neurons (26). The current data show that accelerated presynaptic metabolism of dopamine can lead to a detectable alteration in the redox status of dopamine terminals. To the best of our knowledge, a direct demonstration of an oxidant stress under these circumstances has not been achieved previously.

In other biological systems, enhanced production of H_2O_2 is associated with cell damage and destruction. Examples are phagocytosis (27) and cell or organ-specific redox-cycling toxins, such as paraquat, doxorubicin, and alloxan. Within the nervous system, 6-hydroxydopamine and 6-aminodopamine are examples of redox-cycling toxins that selectively destroy catecholamine neurons (28). However, in each of these examples, the production of H_2O_2 is undoubtedly more vigorous than that evoked within dopamine nerve terminals by reserpine and, moreover, strong evidence exists for contributory roles by other species, which include hypochlorous acid, reactive quinones, superoxide, and the hydroxyl radical. Increased production of H_2O_2 evoked by reserpine does not, by itself, evoke a rapid neuronal destruction, such as that seen with 6-hydroxydopamine. Nonetheless, a milder oxidative stress that can persist for years in nigrostriatal neurons in Parkinson disease (namely, evidence at autopsy for increased production of deaminated metabolites) would be expected to evoke biologic sequelae.

We suggest that accelerated turnover of dopamine in Parkinson disease can affect the vitality of dopamine neurons and contribute to variations or abnormalities in dopaminergic neurotransmission, particularly during long-range therapy with L-dopa. At the far end of a spectrum of possible sequelae, a persistent oxidative stress, coupled to significant local loss of GSH, may promote a faster than normal rate of neuronal senescence. This possibility remains to be explored. Alternatively, elevated GSSG can affect the properties of sulfhydryl-dependent enzymes and structural proteins by the formation of mixed disulfides, and it can also serve as a "third messenger" to alter enzymatic systems or promote a variety of cellular changes (29–31).

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