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DOPAMINE UPTAKE IN STRIATAL SYNAPTOSOMES EXPOSED TO PEROXIDATION "*IN VITRO*"

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Abstract—The uptake of [^{3}H]dopamine by synaptosomes from rat brain striatum was biphasic, the rapid phase requiring less than 1 min for completion and the slower phase occurring over a few minutes. This uptake of labeled dopamine was enhanced by prior exposure of the synaptosomes to Fe^{2+}, which gives rise to peroxidation of the synaptosomal lipids. Both lipid peroxidation and enhancement of dopamine uptake were unaffected by the presence of inhibitors of monoamine oxidase but were blocked by inclusion of *o*-phenanthroline in the preincubation medium. It is concluded that lipid peroxidation may be involved in certain neuropsychiatric disorders such as seizures evoked by exposure to high oxygen pressures.

Evaluation of neurotransmitter metabolism, including its uptake, release, synthesis and degradation in the brain "*in vivo*" is a difficult experimental problem. However synaptosomes isolated from the rat brain, a preparation of isolated presynaptic nerve endings, cause a net, sodium-dependent, veratridine-sensitive accumulation of dopamine [1]. Current thinking as to the mechanisms of biogenic amine accumulation has been reviewed recently [2–4]. There is general agreement that an energy-dependent, carrier-mediated transport is involved in the uptake of neurotransmitters at the plasma membrane level [4]. Considerable evidence has been accumulated that changes in membrane structure may result in a modified function of this carrier. In particular this may cause disturbance of dopamine uptake. Such membrane damage may be responsible for some neuropsychiatric disorders such as seizures evoked by exposure to high oxygen pressure (OHP) and the disturbances following ischemic episodes. It is generally agreed that subcellular fractions of the ischemic brain had increased rates of malondialdehyde (MDA) formation [5–8]. Lipid peroxidation modifies the lipid content of membranes [5, 9, 10], and in the present paper we have utilized Fe^{2+}-induced lipid peroxidation for "*in vitro*" modification of isolated synaptosomes. These synaptosomes are shown to have increased rates of dopamine uptake, consistent with the dependence of transport activity on the membrane lipid structure.

MATERIALS AND METHODS

Male Wistar rates (180–220 g) were used throughout the study. Synaptosomes were isolated from the striatum of rats essentially as described by Booth and Clark [11]. The final pellets were suspended in a modified Kreb's–Henseleit saline (140 mM NaCl, 5 mM KCl, 5 mM $NaHCO_3$, 1.3 mM $MgSO_4$, 1 mM Tris–phosphate, 2.5 mM $CaCl_2$, 10 mM Tris–HCl, pH 7.3) at protein concentrations of about 7 mg/ml. Total protein was measured by the method of Lowry *et al.* [12] using bovine serum albumin as the standard.

Peroxidation condition. Synaptosomes were diluted in the appropriate media to a protein concentration of approximately 0.2 mg/ml and preincubated as demanded by the experimental protocol with constant shaking at 30°.

After treatment the synaptosomes were collected by centrifugation, washed once and resuspended in the appropriate buffer. MDA was determined according to Rehncrona *et al.* [13].

Dopamine uptake. Both control and pretreated synaptosomes were suspended in the appropriate media to the required protein concentration, usually 1–2 mg/ml, and uptake measurements started by addition of the radioactive neurotransmitters [^{3}H]dopamine (sp. act. 34.2 Ci/mmole) (New England Nuclear). Samples (200 μl) were withdrawn at the time intervals indicated and rapidly centrifuged (Beckman microfuge) through a layer of silicone oil (sp. gr. 1.03) (General Electric, Waterford, NY). Radioactivity in the supernatants and in the pellets was counted in a Beckman LS-9000 liquid scintillation counter using an appropriate scintillant (Beckman Ready-Solv). Kinetic data were evaluated by transforming the hyperbolic uptake curves into their linear reciprocal form. The data were fitted to straight lines by linear regression analysis. The means and the S.E.M. were calculated and the levels of significance between the treated and the control groups were determined using the Student's *t*-test.

RESULTS

The time course of [^{3}H]dopamine uptake by synaptosomes is shown in Fig. 1. The uptake can be resolved into a rapid and a slower phase. The rate of dopamine transport was markedly increased when the membranes were submitted to non-enzymatic peroxidation. As the amount of Fe^{2+} was elevated

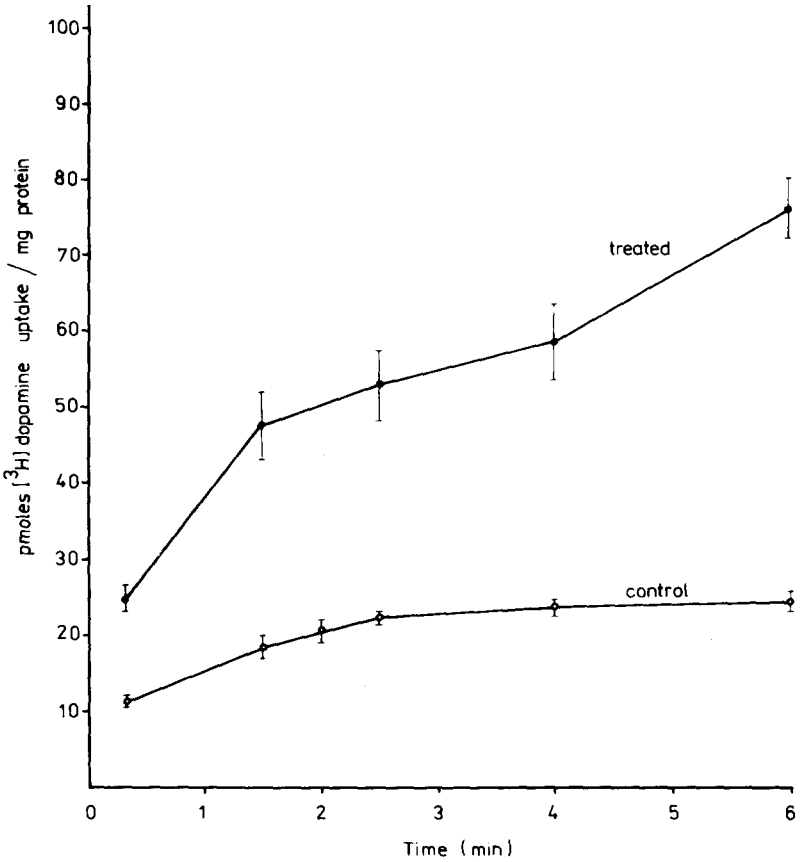

Fig. 1. Time course of [^{3}H]dopamine uptake. Results are the means ± S.E. of seven and three experiments respectively for control and treatment values. Each experiment was performed in duplicate. Synaptosomes were preincubated at approximately 0.2 mg protein/ml for 2 min with 120 μM Fe^{2+} and 200 μM ascorbic acid at 30°. After treatment the synaptosomes were collected by centrifugation, washed once and resuspended in Kreb's–Henseleit medium. Uptake of dopamine is determined immediately as described in Materials and Methods.

in the preincubation medium, the active uptake of dopamine increased. This increase was slight until a concentration of 60–90 μM was reached, then increased abruptly and above 120 μM neither the rapid nor the slower phase increased further (Fig. 2).

Figure 3 shows that pretreatment of synaptosomes with 60 μM Fe^{2+} and 200 μM ascorbic acid gave a maximal enhancement of dopamine transport with only 30 sec of preincubation and then slowly declined. After a 5-min preincubation the rapid-phase uptake after 20 sec of accumulation of dopamine was still 41% higher than the control value while the uptake of dopamine in the slower phase (U_{max}) had returned to the control value. It has been reported that Fe^{2+} stimulates non-enzymatic peroxidation of endogenous lipid of the brain cortex if ascorbic acid is present [14]. This reaction destroys polyunsaturated fatty acid moieties and can be monitored by measurement of MDA. A typical experiment showing the time-dependent production of MDA by striatum synaptosomes is presented in Fig. 4. It can be seen that MDA formation increased by the earliest measured time after addition of Fe^{2+} and ascorbic acid (1.88 ± 0.15 and 3.67 ± 0.09 nmoles/mg protein for control and treated synaptosomes respectively). By the end of the 5-min period of incubation with Fe^{2+} the level of MDA reached a value of 22.7 ± 1.6 nmoles/mg protein.

The rate of MDA formation increased as a biphasic function of Fe^{2+} concentration. There was a rapid increase in MDA formation for Fe^{2+} concentrations of 0–30 μM whereas at concentrations of 30–120 μM Fe^{2+} the rate of formation continued to increase but at a slower rate and as a nearly linear function of iron concentration.

In order to investigate the effect of different conditions of preincubation on the rate of neurotransmitter uptake, as well as on MDA production, synaptosomes were preincubated for 2 min at 30° in the presence of various compounds and then the dopamine uptake was measured. Tables 1 and 2 summarize the results with their statistical evaluation. Addition of ascorbic acid to the preincubation medium was without effect on both the uptake of dopamine and MDA formation. The simultaneous application of ascorbic acid (200 μM) and Fe^{2+} (60 μM) increased the uptake of [^{3}H]dopamine by about 40% as compared to the control value. It can be seen that after preincubation for 2 min under these conditions the concentration of MDA in synaptosomes increased almost 5.7 times. Addition of *o*-phenanthroline to the preincubation medium abolished the effect of Fe^{2+} on the uptake of neurotrans-

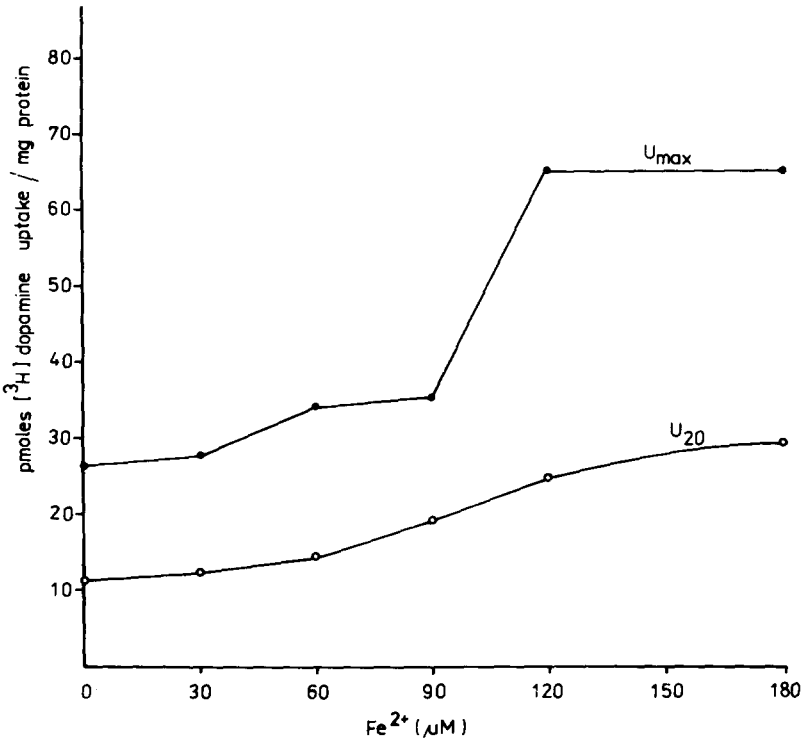

Fig. 2. Dose-dependent effect of pretreatment with Fe^{2+} upon [^{3}H]dopamine uptake. Synaptosomes were preincubated with different concentration of Fe^{2+} for 2 min as described in Fig. 1. Uptake at 20 sec (U_{20}) represents the rapid phase of accumulation of dopamine. U_{max} is the total amount of dopamine accumulated in the synaptosomes in the rapid and slow phases. The values were changed from the hyperbolic curves in Fig. 1 into their reciprocal straight-line forms. All of the data for pretreated synaptosomes were significantly different from the controls as determined by the Student's *t*-test ($P < 0.05$).

mitter and MDA formation. On the other hand, *o*-phenanthroline added only to the incubation medium was without effect on dopamine accumulation by either control or Fe^{2+}-treated synaptosomes. It should also be mentioned that the rates of uptake as well as MDA production were unaffected by the presence of an inhibitor of monoamine oxidase (10 μM pargyline).

DISCUSSION

We have previously reported that the kinetic constants for uptake of neurotransmitters are characteristic of the high-affinity system with rapid and slower phases [1]. It can be seen that after addition of 0.39 μM [^{3}H]dopamine its level in the striatal synaptosomes increased during the initial 150 sec and then remained nearly constant (Fig. 1). Active uptake has been reported to be sensitive to modification of the outer surface of bilayer [14] especially by the superoxide anion and hydroxyl radical.

It had been postulated by Weiss [15] that in the presence of a complexing agent the autoxidation of Fe^{2+} produces O_2^-. This view was supported by Nofre *et al.* [16] and Fridovich's group [17] who demonstrated the hydroxylating properties of Fe^{2+}–O_2 phosphate reagent. It is generally considered that lipid peroxidation occurs when peroxide, generated by O_2^- and superoxide dismutase, interacts with Fe^{2+} to form a hydroxyl radical. The hydroxyl radical then initiates a chain reaction involving alkyl radicals which interact with oxygen [18]. Lipid peroxidation results in the destruction of polyunsaturated fatty acid moieties in the membrane phospholipids [19]. According to Mead *et al.* [20] the major products of this peroxidation are epoxides of fatty acids of other olefins such as cholesterol. A similar radical mechanism is believed to be responsible for the oxidation of thiol groups [21].

The superoxide anion is both an oxidant and a reductant and hence can modify a variety of biologically important molecules. Thus, substances such as catecholamines are oxidized while cytochrome *c* and other heme derivatives are reduced [22, 23].

We feel that the preincubation system which we have employed duplicates or at least closely resembles the main non-enzymatic pathway of peroxidation in the brain [9]. Our studies indicate that lipoperoxidations modify the accumulation of dopamine by striatal synaptosomes. Moreover, since dopamine uptake per se was not *o*-phenanthroline-sensitive (Table 1) interaction of Fe^{2+} with the carrier in any fashion is not likely. The addition of pargyline to intact synaptosomes resulted in the irreversible inhibition of monoamine oxidase without any effect on the measured dopamine uptake.

We can not exclude the possibility that freshly accumulated dopamine is metabolized by other enzymatic or non-enzymatic systems which enhanced uptake of the ^{3}H label but our current knowledge

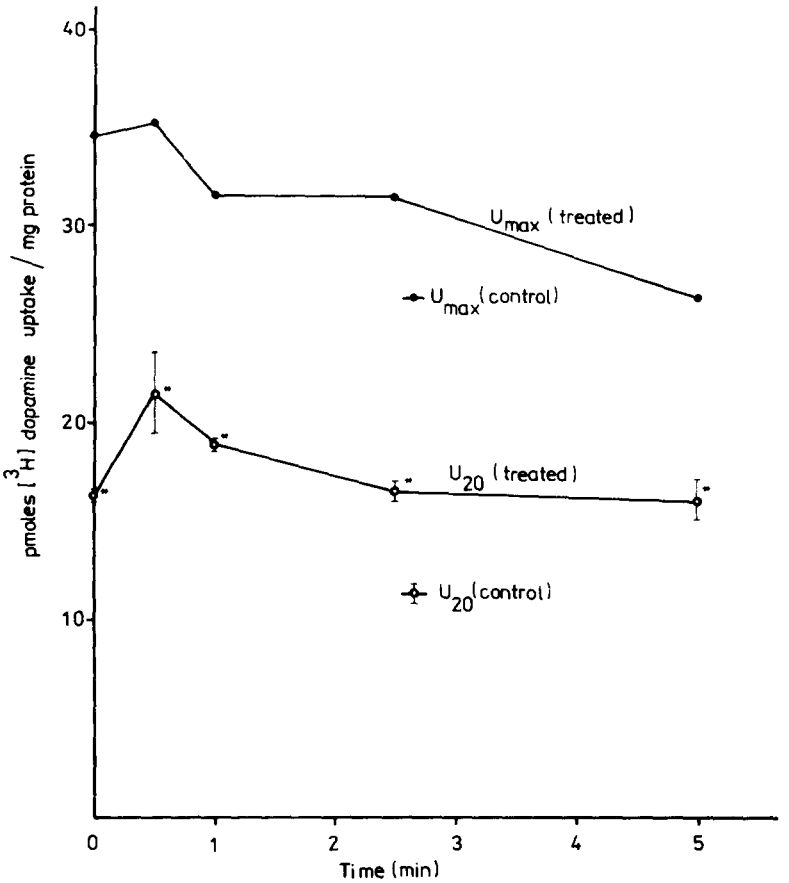

Fig. 3. Effect of time of incubation of synaptosomes with Fe^{2+} on the activation of [^{3}H]dopamine uptake. Synaptosomes were preincubated for indicated period with 60 μM Fe^{2+} and 200 μM ascorbic acid. The results are the means ± S.E. of four experiments performed in duplicate. U_{20} and U_{max} were calculated as described in Materials and Methods. Control values were 11.3 ± 0.4 and 26.3 pmoles/mg protein for U_{20} and U_{max} respectively. The data points were all different from the control values ($P < 0.01$).

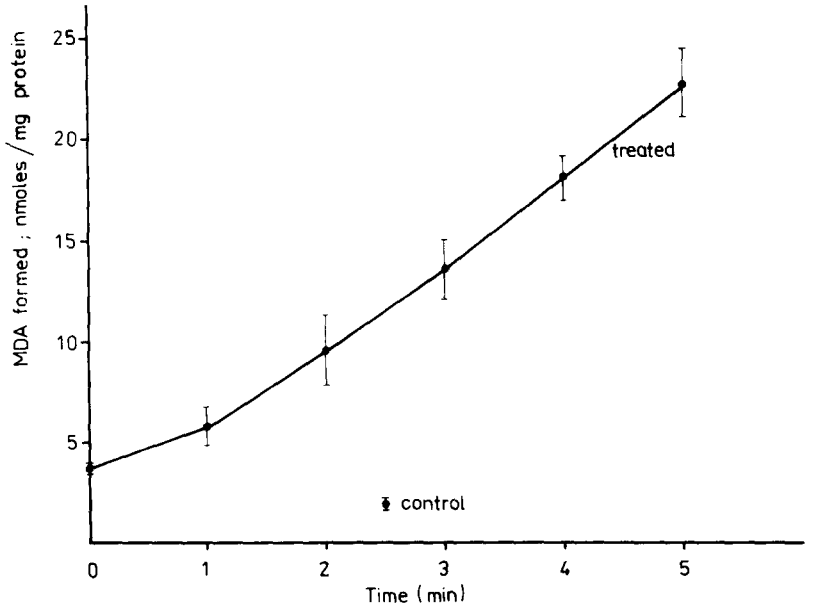

Fig. 4. Time-dependent production of malondialdehyde (MDA) by synaptosomes. The results are the means ± S.E. of four experiments. Control value was 1.88 ± 0.15 nmoles/mg protein and was independent of the time of incubation over this period (5 min). The experimental conditions were similar to those described in Materials and Methods.

Table 1. Effect of different conditions of preincubation on [^{3}H]dopamine uptake

Pretreatment condition	Uptake condition	Dopamine (% dopamine)
None	None	100
None	10 μM pargyline	97.1 ± 5.9 (3)
None	200 μM *o*-phenanthroline	92.8 ± 3.2 (2)
200 μM ascorbic acid	None	96.4 ± 5.5 (3)
60 μM Fe^{2+}	None	130.9 ± 4.5 (3)*
200 μM ascorbic acid, 60 μM Fe^{2+}	None	140.3 ± 7.0 (4)*
200 μM ascorbic acid, 60 μM Fe^{2+}, 200 μM *o*-phenanthroline	None	92.7 ± 1.5 (2)
200 μM ascorbic acid, 60 μM Fe^{2+}, 10 μM pargyline	None	138.7 ± 5.9 (3)*
200 μM ascorbic acid, 60 μM Fe^{2+}	200 μM *o*-phenanthroline	135.7 ± 2.1 (2)*

The results (means ± S.E.) are given as percentages of the control value.
Number of experiments, each performed in duplicate, is given in parentheses.
The time of pretreatment was 2 min and incubation with dopamine was also for 2 min at 30°.
* Different from the control value at a level of $P < 0.01$.

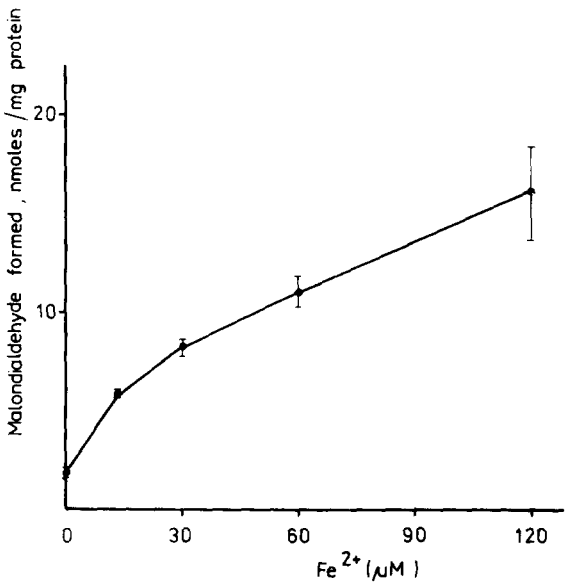

Fig. 5. Dependence of the production of malondialdehyde on the concentration of added Fe^{2+}. Synaptosomes were incubated for 2 min with different concentration of Fe^{2+} as described in Fig. 2. The results are the means ± S.E. for four experiments.

Table 2. Effects of changes in the composition of incubation medium on the malondialdehyde formation in synaptosomes

Incubation condition	Malondialdehyde (% of control)
None	100
200 μM ascorbic acid	113.8 ± 6.2
60 μM Fe^{2+}	568.8 ± 28.7*
10 μM pargyline	105.0 ± 6.5
200 μM *o*-phenanthroline	75.9 ± 5.2
200 μM ascorbic acid, 60 μM Fe^{2+}	585.6 ± 9.1*
200 μM ascorbic acid, 60 μM Fe^{2+}, 10 μM pargyline	546.3 ± 65.6*
200 μM ascorbic acid, 60 μM Fe^{2+}, 200 μM *o*-phenanthroline	104.5 ± 11.9

The results are the means ± S.E. of three experiments with the data expressed as percentages of the control value.
The time of incubation was 2 min at 30°.
* Different from control value at a level of $P < 0.001$.

of dopamine metabolism makes this unlikely. The observed relationship between peroxidation and dopamine uptake is consistent with the view that lipid peroxidation increases the activity of the dopamine translocase system [24].

The increase in the rate of dopamine accumulation probably reflects the conformational changes in the carrier and/or its environment in the membrane.

An increase in dopamine uptake may be involved in certain neuropsychiatric disorders, such as drug-induced Parkinsonism and seizures evoked by exposure to OHP. An increased net uptake of neurotransmitters has been reported to occur in synaptosomes isolated from animals given "*in vivo*" treatment with lithium [25]. This mechanism may be responsible for the neurological side effects of lithium carbonate administration [26–28].

The mechanism of seizures evoked by OHP is not fully elucidated. Complex biochemical changes in the CNS including inactivation of cellular enzymes, and increased formation of free radicals and lipid peroxides are known to occur during OHP exposure [10, 29].

No data are available so far on the effect of OHP upon dopamine uptake. However, we can postulate that OHP changes the biomembranes in a similar manner to that observed in this study for striatal synaptosomes.

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