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Effect of metadoxine on striatal dopamine levels in C57 Black mice

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Abstract—In the present study, we examined the effect of metadoxine on striatal levels of dopamine, 5-hydroxytryptamine (5-HT) and their metabolites in male C57 Black mice. Striatal content was assayed after systemic administration of metadoxine ranging from 1 $\mu g\ kg^{-1}$ to 500 mg kg^{-1}. Striatal dopamine increased 1 h after treatment with metadoxine (150 mg kg^{-1}), but the most notable effect was obtained 24 h after the drug administration. At this time a plateau was reached; the two major metabolites of dopamine showed the same trend. Seven days after metadoxine administration, striatal dopamine approached the control values. Over the same time intervals, striatal 5-HT increased to a lesser extent and 5-hydroxyindoleacetic acid did not differ significantly from controls. Striatal dopamine increased significantly at a dose of 250 $\mu g\ kg^{-1}$ up to a dose of 1 mg kg^{-1} metadoxine; no further increment was observed between 1 and 500 mg kg^{-1} metadoxine. Administration of each component at doses equimolar to 1 mg metadoxine showed that pyridoxine produced only a mild increase in striatal dopamine compared with controls. We suggest that the metadoxine-induced striatal dopamine increase is obtained by increasing synthesis of dopamine.

Previous studies have shown that metadoxine modifies several biochemical mechanisms involved in ethanol-induced liver diseases. The compound induces an increase in hepatic and cerebral ATP concentration and in the intracellular transport of amino acids (Felicioli et al 1980). Metadoxine is also able to interact with central neurotransmitters; it increases the release of GABA and acetylcholine from the frontoparietal cortex of freely moving guinea-pigs (Antonelli et al 1984b). This increase is associated with a pronounced anxiolytic action, as demonstrated by a conflict test. In clinical studies, performed in patients with acute alcohol intoxication, metadoxine improves the acute behavioural effects of alcohol (Moroni 1987). This effect has been attributed to the fact that metadoxine shortened the plasma half-life of ethanol, but it also could be due to interaction of metadoxine with cerebral neurotransmitters such as dopamine in specific brain areas. Indeed, one component of metadoxine, pyroglutamic acid, is able to antagonize the increase in dopamine efflux caused by glutamate in guinea-pig striatum (Antonelli et al 1984a). The other component, pyridoxine causes a mild increase in rat striatal dopamine (Pfeiffer & Ebadi 1972).

In the present study, we examined the striatal content of dopamine, 5-hydroxytryptamine (5-HT) and their metabolites after systemic administration of different doses of metadoxine in mice.

Materials and methods

Animals. Male C57 BL/6N mice (Charles River), 8 weeks old, 22–24 g, were housed in groups of six per cage in a climate-controlled room (22°C) with a 12 h light-dark cycle for at least four days before use. Food and water were freely available. Food consisted of standard optimal diet for mice (Piccioni, Italy). The commercial food pellets contained approximately 12 mg kg^{-1} pyridoxine (2 mg was naturally present in the various components of the pellet, while 10 mg was added to the pellets).

Experimental design. Mice were divided into six groups. The first received intraperitoneal injections of 0·2 mL 0·9% NaCl (saline); the others received single doses of metadoxine (150 mg kg^{-1}) (Lab. Baldacci, Pisa, Italy) dissolved in 0·2 mL saline. Animals were killed 1, 12, 24 h, 3 and 5 days after treatment.

Different doses of metadoxine, ranging from 1 $\mu g\ kg^{-1}$ to 500 mg kg^{-1} were used. Animals were divided into nine groups: controls, receiving 0·2 mL saline intraperitoneally, and the other 8 groups receiving 1, 10, 50, 250 $\mu g\ kg^{-1}$ or 1, 15, 150, 500 mg kg^{-1} metadoxine dissolved in 0·2 mL saline. Animals were killed 24 h after metadoxine administration.

In an experiment to investigate the effect of each component of metadoxine (pyridoxine and pyroglutamic acid), animals were divided into four groups: the first comprised control animals; the second and the third groups received 0·43 mg kg^{-1} pyroglutamic acid and 0·57 mg kg^{-1} pyridoxine, respectively; these doses are equivalent to 1 mg kg^{-1} metadoxine. The fourth group received metadoxine (1 mg kg^{-1}). All substances were dissolved in 0·2 mL saline. Animals were killed by cervical dislocation 24 h after treatment.

Assay procedure. Brains were removed and the striatum was dissected, using the external walls of the lateral ventricles as the internal limits, and the corpus callosum as the external boundary, as described by Glowinski & Iversen (1966) with minor modifications. Immediately after dissection, the striatum was frozen on dry-ice until assayed.

Striatum samples were homogenized using a microsonicator in 0·1 M ice-cold perchloric acid (0·6 mL) containing a known concentration (10 pg μL^{-1}) of dihydroxybenzylamine (DBA) as the internal standard. An aliquot of the homogenate (50 μL) was assayed for protein (Lowry et al 1951). After centrifugation at 8000 *g* for 10 min at 0°C, an aliquot of the clear supernatant (20 μL) was injected into a high performace liquid chromatograph. This consisted of a precolumn, a C18 reverse phase column (Beckmann, San Ramon, CA, USA) and an amperometric electrochemical detector M560 (Waters Associates, Milford, MA, USA). The mobile phase contained, in 1 L deionized distilled water, 1250 mg heptane sulphonic acid, 120 mg EDTA, 6 mL phosphoric acid (85%), 9 mL triethylamine and 18 mL acetonitrile. The mobile phase (filtered and degassed) was delivered at a flow rate of 1·5 mL min^{-1} and a pressure of 2000 psi.

The applied potential was set to 0·77 V. A standard curve was prepared using known amounts of dopamine, 5-HT and their metabolites, dissolved in 0·1 M perchloric acid containing a constant amount (10 pg μL^{-1}) of the internal standard (DBA), as used for tissue samples. The standard curve for each compound (monoamine or its metabolites) was determined using the regression analysis of ratios of the peak areas (compound area/DBA area) for various concentrations of each compound. The correlation index for the standard curves was greater than 0·996.

Results are expressed as the mean ± s.e. of values assayed in duplicate in animals ($n > 4$). Effects of metadoxine were statistically evaluated using the two-tailed Student's *t*-test ($P < 0.05$).

Results

As shown in Fig. 1, striatal dopamine (156 ± 4 ng mg^{-1} in controls) increased 1 h after treatment (178 ± 5 ng mg^{-1}), but the most notable effect was obtained 12 and 24 h after metadoxine

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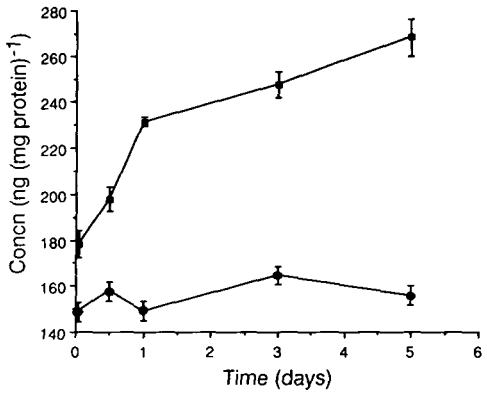

FIG. 1. Time course of striatal dopamine. Animals were killed 1, 12, 24 h, 3 and 5 days after a single dose of metadoxine (150 mg kg^{-1}, i.p., ■). Controls (●) were killed at the same times after a single injection of 200 μL saline intraperitoneally. Values are given as means ± s.e. for groups of six animals.

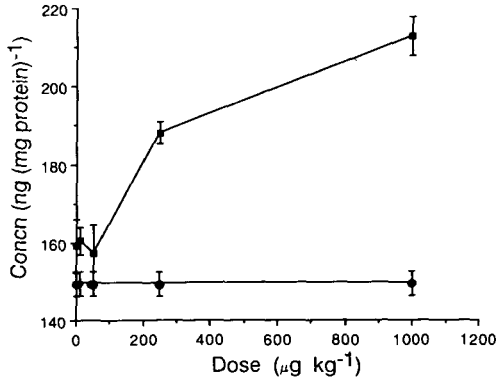

FIG. 2. Striatal dopamine after different doses of metadoxine (■). Animals were killed 24 h after treatment with different doses of metadoxine ranging from 1 μg kg^{-1} to 1 mg kg^{-1}. Controls (●) received a single injection of 200 μL saline. Values are given as means ± s.e. for groups of six animals.

administration (197 ± 5 and 231 ± 1 ng mg^{-1}, respectively). At this time as shown in Fig. 1, dopamine concentration reached a plateau, although levels continued to augment three and five days after treatment (247 ± 3 and 268 ± 8 ng mg^{-1}, respectively). The two major metabolites of dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), showed the same trend (Table 1).

Seven days after metadoxine administration, striatal dopamine levels approached the values of controls (data not shown).

Striatal 5-HT increased to a lesser extent: after 24 h, striatal 5-HT was not significantly different from controls (8·3 ± 1·2 and 6·0 ± 0·4 ng mg^{-1}, respectively). The increase in 5-hydroxyindolacetic acid (5HIAA) was less evident and the maximum levels, observed five days after treatment did not differ significantly from controls (7·7 ± 0·6 and 5·9 ± 0·6 ng mg^{-1}, respectively).

Using different doses of metadoxine (1 μg kg^{-1}–500 mg kg^{-1}), we evaluated the variations of striatal monoamine content 24 h after treatment. As shown in Fig. 2, striatal dopamine began to increase significantly at 250 μg kg^{-1} metadoxine (188 ± 2 ng mg^{-1} in comparison with 149 ± 3 ng mg^{-1} of controls) and the increase continued up to the dose of 1 mg kg^{-1} (212·74 ± 4·982 ng mg^{-1}). Dopamine metabolites (DOPAC and HVA) showed the same increment (data not shown).

In the range of doses between 1 and 500 mg kg^{-1}, no further increase in striatal dopamine levels was observed (data not shown). Striatal 5-HT after a dose of 1 mg kg^{-1} metadoxine increased slightly with respect to controls (7·5 ± 0·4 and 5·8 ± 0·3 ng mg^{-1}, respectively) while striatal 5HIAA after metadoxine (1 mg kg^{-1}) did not differ from controls (data not shown). Doses of metadoxine ranging from 1 to 500 mg kg^{-1} did not cause any further increment in striatal indolamine.

The effect of each component of metadoxine is shown in Fig. 3. The administration of 570 μg kg^{-1} pyridoxine (a dose equimolar to 1 mg metadoxine) produces a slight increase in striatal dopamine compared with controls (175 ± 3 and 148 ± 3 ng mg^{-1}, respectively). The administration of an equimolar dose of pyroglutamic acid (430 μg kg^{-1}) did not produce any change in striatal dopamine in comparison with 1 mg kg^{-1} metadoxine (222 ± 6 ng mg^{-1} compared with 148 ± 3 ng mg^{-1} for controls).

Table 1. Time course of striatal DOPAC and HVA levels after a single dose of metadoxine (150 mg kg^{-1}).

	DOPAC (ng (mg protein)$^{-1}$)	HVA (ng (mg protein)$^{-1}$)
Controls	8·4 ± 0·4	16·0 ± 0·6
1 h	11·5 ± 0·8*	20·8 ± 1·6
12 h	13·7 ± 1·6*	24·0 ± 1·3*
24 h	14·6 ± 0·6**	24·8 ± 1·9*
3 days	15·3 ± 1·2**	26·0 ± 1·6*
5 days	15·5 ± 0·6**	28·0 ± 1·7**

Results are the means ± s.e. of six mice per group. *P < 0·05; **P < 0·01 compared with controls.

Discussion

Metadoxine is the salt of pyridoxine and pyroglutamic acid, and our data reflect the combined actions of these two components.

Pyridoxine (one of the three forms of vitamin B_6) plays an important role in the central nervous system as a co-factor for decarboxylases. These enzymes are involved in the biosynthetic pathways for several neurotransmitters; for instance, dopamine, noradrenaline and 5-HT (Lovenberg et al 1962), tyramine and tryptamine (Meister 1965), histamine (Buffoni 1966), and GABA (Scriver & Whelan 1969) are synthesized or metabolized

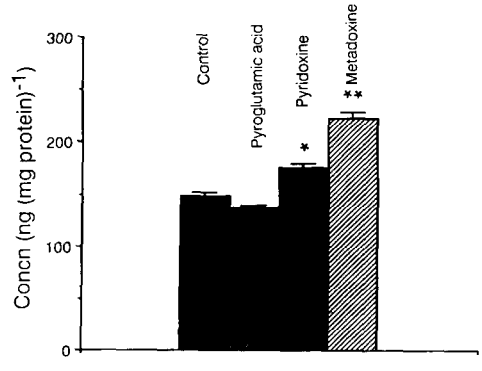

FIG. 3. Effect of each component of metadoxine on striatal dopamine. Animals were killed 24 h after single injections of saline (controls), pyridoxine, pyroglutamic acid or metadoxine. Pyridoxine (0·570 mg kg^{-1}) and pyroglutamic acid (0·430 mg kg^{-1}) were administered at doses equimolar to metadoxine (1 mg kg^{-1}). Values are given as means ± s.e. for groups of six animals. *P < 0·05; **P < 0·01 compared with controls.

by means of B_6-dependent enzymatic reactions. In recent years, vitamin B_6 has been recognized to be of great importance in the growth and development of neurons and synaptogenesis (Groziak & Kirksey 1989), particularly for the dopaminergic system (Guilarte et al 1987).

Pyridoxine alone, injected intraperitoneally into the rat at a dose of 100 mg kg^{-1} (Pfeiffer & Ebadi 1972) or used to supplement the diet of the rat at 10 times the optimal value (Guilarte 1989), has been reported to increase striatal dopamine by about 30 and 25%, respectively.

In our study, performed on the mouse, we replicated this small increase in striatal dopamine described with pyridoxine in the rat, but the most important finding was that pyridoxine with pyroglutamic acid increased striatal dopamine by 42% compared with control values at doses below 1 mg kg^{-1}. This effect was unexpected but it could be due to differences of animal species or treatment procedure.

The increase in striatal dopamine could be the effect of the enhancement of the activity of DOPA-decarboxylase, promoted by a surplus of its co-factor. This hypothesis has already been advanced for in-vitro experiments (Christenson et al 1970) and it has been postulated that this happens also in-vivo (Ebadi et al 1973). Alternatively, an increase in striatal dopamine might be the result of a reduced release of the neurotransmitter from dopaminergic terminals caused by an inhibitory action of GABA. Indeed, it has been reported that pyroglutamic acid releases GABA into the cerebral cortex (Antonelli et al 1984a). However, this is in contrast with the present findings where we found a similar increase in striatal dopamine and its extracellular metabolite HVA. From this point of view, the effect of metadoxine is different from that of γ-hydroxybutyric acid, or its analogue γ-butyrolactone, which increases brain dopamine (Gessa et al 1966) and decreases the levels of dopamine metabolites, as the consequence of both an inhibition of the firing rate of dopaminergic neurons (Roth et al 1980) and an increase in dopamine synthesis (through an activation of tyrosine hydroxylase inside these neurons (Morgenroth et al 1976)). In spite of several speculations, the mechanism of action of the metadoxine-induced dopamine increase remains unexplained. Electrophysiological studies, as well as the use of brain microdialysis, could partly clarify this action. As suggested above, it is likely that this effect is obtained by increasing newly synthesized dopamine by pyridoxine.

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