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Increase in Mouse Brain Regional Noradrenaline Turnover after L-Dopa Administration

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Abstract—Noradrenaline (NA) and its major metabolite, 3-methyl-4-hydroxyphenylethylene glycol (MHPG) were measured by HPLC with electrochemical detection in five mouse brain regions after L-dopa treatment. Noradrenaline concentration increased significantly, by 30–40%, in the three terminal regions of the locus coeruleus; cortex, hippocampus and cerebellum, but did not change in hypothalamus and brainstem. In mice whose central NA levels had been depleted by prior treatment with the neurotoxin, DSP-4, remaining NA neurons in these terminal regions were still able to respond with an increase in NA after L-dopa loading. The NA metabolite, MHPG, increased several-fold in all five regions in both saline and DSP-4-pretreated mice. Thus, L-dopa may be a useful precursor of NA in specific brain regions, particularly when NA is depleted.

Alterations of central noradrenaline (NA) systems, loss of noradrenergic cells (Forno 1966; Mann et al 1983) and deficits in transmitter level (Fahn et al 1971), occur in Parkinson's disease, in addition to the major deficits in nigrostriatal dopaminergic systems. L-dopa has been used successfully in the treatment of Parkinson's disease and studies in animals have helped to define its effects on striatal dopamine (DA) metabolism, time course, and interaction with other neurotransmitter systems. L-Dopa lies in the synthetic pathway to noradrenaline as well as to dopamine and several studies have evaluated its effectiveness as a precursor for this amine (Everett & Borcherding 1970; Maj et al 1971; Romero et al 1972; Keller et al 1974; Dolphin et al 1976; Edwards & Rizk 1981).

Although it seems that noradrenaline is involved in changes in locomotor activity occurring after dopa treatment, it has been difficult to show an alteration in NA level (Everett & Borcherding 1970; Maj et al 1971; Benkert et al 1973; Keller et al 1974; Dolphin et al 1976). Few of these studies have included a regional evaluation of NA metabolism or included animal models in which noradrenaline had been previously depleted to approximate the human disorder more closely. Current HPLC technologies easily allow the analysis of NA and its major metabolite, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), in small tissue samples. Thus, we have looked at the effectiveness of L-dopa in increasing NA turnover, as assessed by changes in level of NA and MHPG, in normal mice and in mice pretreated with the neurotoxin, DSP-4, which specifically lesions the noradrenergic terminals of the locus coeruleus (LC), while leaving dopaminergic systems intact (Ross 1976; Jonsson et al 1981).

Materials and Methods

Male, CD-1 mice, 30 g (Charles River Breeding Labs, St. Constant, Que.) were housed (4-5/cage) under standard conditions of lighting (lights on 0700 to 1900 h) with free access to food and water (Purina rat and mouse chow). Mice were injected intraperitoneally with 0·9% NaCl (saline) or DSP-4 (50 mg kg^{-1}, dissolved in saline) and two weeks later received either L-dopa (100 mg kg^{-1}, i.p.) or its diluent (0·001 M HCl). All injections were given in a volume of 10 mL kg^{-1}. One hour after injection, a time at which the peak effect on NA and its metabolites occurs (Romero et al 1972; Edwards & Rizk 1981), all mice were decapitated and brains rapidly removed and dissected on a chilled glass plate into five regions, (cortex, hippocampus, cerebellum, hypothalamus and brainstem) (Glowinski & Iversen 1966). Tissue was immediately frozen on dry ice and kept at −80°C until assay.

Tissue regions were weighed, placed in 0·5 mL of 0·1 M perchloric acid, dihydroxybenzylamine (DHBA) added as internal standard, sonicated and centrifuged (12 000 *g* for 15 min). The clear acid supernatant was adjusted to pH 8·6 with 1 mL of 1M Tris-HCl, pH 8·6, and NA, DA and dopa isolated on mini-alumina columns (Felice et al 1978). After being washed with 5 mM TRIS-HCl, pH 8·6, followed by distilled, deionized water, the amines were eluted from the alumina with 300 μL of 0·5 M acetic acid. The effluent from the alumina column, containing MHPG, was collected and MHPG extracted into ethyl accetate. After a wash with 0·5M potassium bicarbonate, the ethyl acetate fraction was evaporated to dryness in a Speed-Vac concentrator (Savant Instruments, Hicksville, NY) and the sample resuspended in 125 μL of 0·01 M HCl.

Fractions of the NA and MHPG isolates were placed on an LC system consisting of a Waters dual piston pump; Rheodyne Model 7125 Injector; Beckman-Altex Ultrasphere I.P. column (4·6 mm × 15 cm) (5 μm reverse phase particles); and detected electrochemically with a TL-5 glassy carbon electrode (BAS, West Lafayette, IN) set at a potential of +0·78 V versus an Ag/AgCl electrode. The mobile phase for the amines pumped at a flow rate of 1·5 mL min^{-1}, consisted of 0·05 M sodium phosphate, monobasic, pH 3·2, 0·1 mM EDTA, and 0·3 mM sodium octyl sulphate, with 6% methanol, and for MHPG, 0·05 M sodium acetate, pH 5, 0·1 mM EDTA with 5% methanol. Detection limits for the catecholamines (DOPA, NA and DA) were 50 pg with average recoveries of 60%. Recovery of MHPG calculated from known amounts of pure standard run through the entire procedure was 60% with a detection limit of 100 pg.

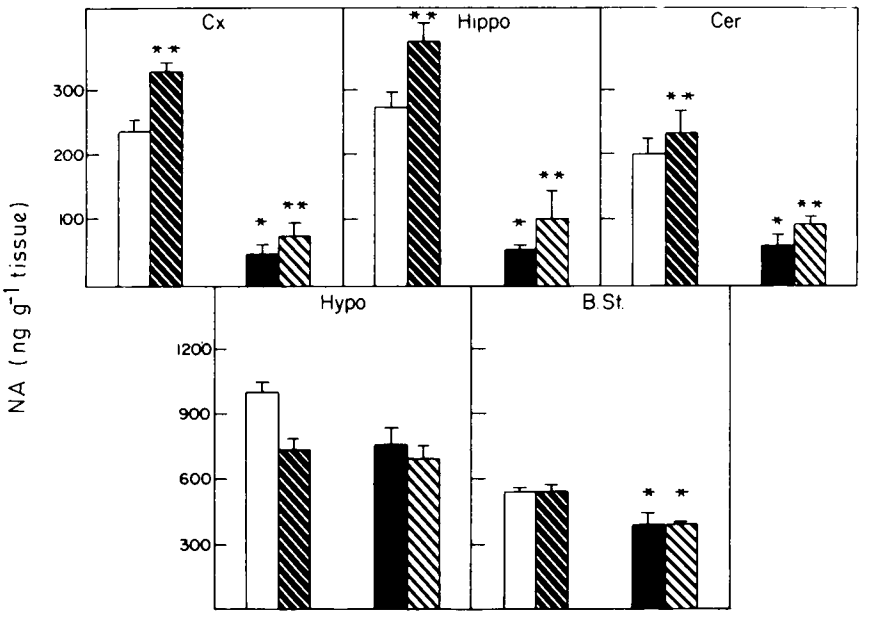

FIG. 1. Regional noradrenaline levels one hour after administration of L-dopa to saline or DSP-4 pretreated mice. One hour after administration of L-dopa (100 mg g^{-1} i.p.) or vehicle, mice were killed and brain regions analysed for NA by HPLC. Brain regions examined included cortex (Cx), hippocampus (Hippo) cerebellum (Cer), hypothalamus (Hypo) and brainstem (B.St.). Half of the mice had been previously treated with the NA neurotoxin, DSP-4, two weeks before. From left to right, saline-pretreated controls are represented by the white bars; saline + L-dopa by the black-striped bar; DSP-4 pretreated controls by the black bars and DSP-4 + L-dopa by the white-striped bars. Data were analysed by two way analysis of variance. $*P < 0.01$ significantly differs from saline-pretreated mice. $**P < 0.01$ significantly differs from its vehicle-injected control.

All reagents were of analytical grade and obtained from Fisher Laboratories (Mississauga, Ont.); HPLC standards and L-dopa from Sigma Chemical Company (St. Louis, MO). DSP-4 was generously provided by Astra Labs (Astra Läkemedel AB, Södertälje, Sweden).

Data are expressed as mean ± standard error of the mean and were analysed by two-way analysis of variance or Student's *t*-test where appropriate.

Results

In control mice, NA levels in cortex, hippocampus and cerebellum were significantly increased 1 h after administration of L-dopa to levels 39, 37 and 28% above their control values of 283 ± 16, 275 ± 22 and 202 ± 12 ng g^{-1}, respectively (Fig. 1). No change was observed in brainstem or hypothalamic NA levels.

Administration of the neurotoxin, DSP-4 causes a long-lasting depletion of noradrenaline, primarily in the terminals of the LC noradrenergic brainstem nucleus. In DSP-4 treated mice, cortical, hippocampal and cerebellar NA was severely diminished to 48 ± 15 ng g^{-1} (20% of control); 54 ± 8 ng g^{-1} (20% of control) and 60 ± 16 ng g^{-1} (30% of control). Reductions in the cell body region, i.e. brainstem, and in hypothalamus are significant, but far less extensive (30–40% reduction) (Fig. 1). After L-dopa loading in the NA-depleted mice, increases in noradrenaline were again confined to the three terminal regions. Although NA levels were still not returned to normal in these regions, the percentage increases exceeded those in the saline-pretreated controls (55% in cortex and cerebellum and 89% in hippocampus; Fig. 1).

Increases in MHPG after dopa administration, far exceeded those seen in the parent amine and were roughly 1·5 to 2-fold greater than normal in both saline and DSP-4 pretreated mice. In the case of the metabolite, no clearly discernible difference in the five brain regions was observed, with the exception of a significantly lower increase in hypothalamic MHPG under normal conditions (Fig. 2).

The concentration of L-dopa itself showed a regional variation in accumulation 1 h after its intraperitoneal administration. Levels of dopa in non-dopa treated mice were virtually zero; while in both saline and DSP-4-pretreated mice, dopa accumulated to a greater extent in cortex, hippocampus, and cerebellum, than in hypothalamus and brainstem (Table 1).

With the alumina extraction method and LC conditions used we were also able to measure the DA level in these five brain regions. Normally, the DA level was low, about 40 ng g^{-1} in cortex, hippocampus, and brainstem, except for hypothalamus, with normal levels of 274 ± 53 ng g^{-1}. After L-dopa injection, DA increased several-fold in all five regions rising to values that often exceeded the concentration of noradrenaline normally present (Fig. 3). There was no significant difference in the rise of DA in DSP-4-pretreated mice compared with controls. DSP-4 itself had no effect on regional DA levels.

Discussion

L-Dopa can serve as a precursor for central nervous system (CNS) noradrenaline as well as for dopamine. This can be observed as a rise in the NA level in the terminal regions (i.e. cortex, hippocampus and cerebellum) of the LC, a major noradrenergic nucleus.

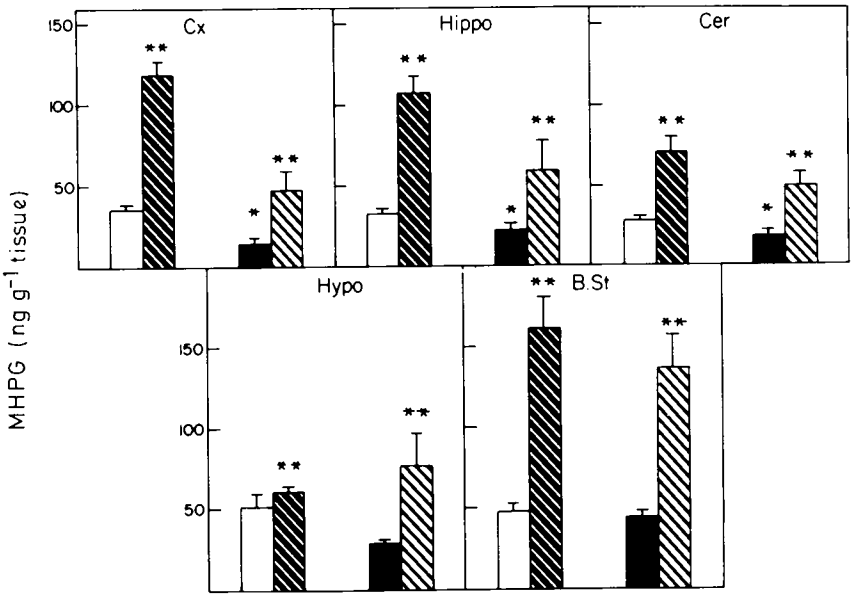

FIG. 2. Regional MHPG levels 1 h after administration of L-dopa to saline- or DSP-4-pretreated mice. One hour after administration of L-dopa (100 mg g^{-1} i.p.) or vehicle mice were killed and brain regions analysed for MHPG by HPLC. Coding for groups as in Fig. 1.

Table 1. Regional dopa accumulation after L-dopa administration. Male, CD-1 mice (groups of 5–6) were pretreated with saline or DSP-4 (50 mg kg^{-1} i.p.) and two weeks later received an injection of L-dopa (100 mg kg^{-1} i.p.). All mice were killed 1h after dopa injection and brain regions analysed for dopa. Values are mean ± standard error. Tissue levels of dopa in mice not receiving L-dopa injections were undetectable.

Brain region	Saline (ng g^{-1})	DSP-4* (ng g^{-1})
Cortex	390 ± 114	459 ± 76
Hippocampus	242 ± 37	359 ± 84
Cerebellum	469 ± 92	499 ± 94
Hypothalamus	150 ± 14	233 ± 109
Brainstem	86 ± 10	107 ± 46

* No significant differences between saline- and DSP-4-pretreated groups.

The effect of dopa on NA is complex, affecting its synthesis, release and metabolism, thus altering both the NA and MHPG level. Dopa administration may directly result in (i) an increase in synthesis of NA (with an increase in NA and MHPG level); (ii) displacement of NA from storage sites directly by dopa or indirectly by DA formed by dopa (with a decrease in NA and increase in MHPG level); and (iii) an alteration in NA methylation by consumption of methyl groups as dopa is metabolized to 3-*O*-Me-dopa (with an increase or no change in NA level and a decrease in MHPG) (Romero et al 1972; Edwards & Rizk 1981; Keller et al 1974). The importance or predominance of one or other of these mechanisms may vary in different brain regions and the

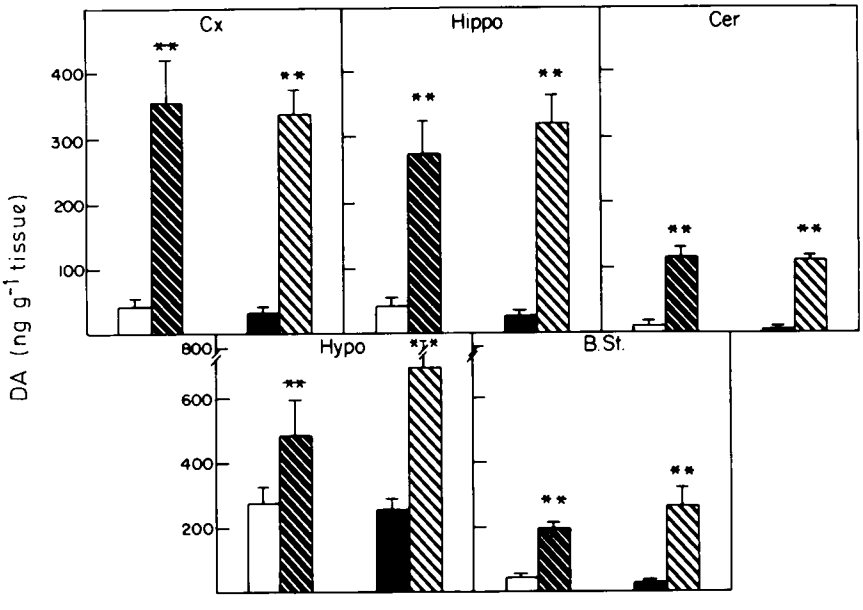

FIG. 3. Regional DA levels 1 h after administration of L-dopa to saline- or DSP-4-pretreated mice. One hour after administration of L-dopa (100 mg g^{-1} i.p.) or vehicle, mice were killed and brain regions analysed for DA by HPLC. Coding for groups as in Fig. 1.

tissue levels of NA and MHPG measured reflect the sum of all these events.

In our study, dopa accumulation appeared to be greater in NA terminal regions (cortex, hippocampus and cerebellum) where active NA carrier mechanisms may also recognize the amino acid precursor and concentrate it. In the terminal regions there appears to be a greater conversion of dopa to NA and net synthesis and hence, an increase in NA level is observed. In the cell body area (i.e. brainstem) release of stored NA seems to predominate, so that while MHPG values still rise, probably reflecting metabolism of the displaced NA, no increase in NA level occurs.

The effect of dopa on hypothalamic NA was minimal. If anything, there was a slight decrease in NA suggesting that the dopa taken up was converted primarily to DA with subsequent displacement of NA rather than increased synthesis. MHPG levels increased only slightly in control animals suggesting that at least in hypothalamus, *O*-methylation of NA to MHPG is not the main source of metabolism (Edwards & Rizk 1981) or that *O*-methylation to MHPG was limited by rapid methylation of L-dopa itself (Romero et al 1972). Levels of 3-*O*-Me-dopa were not determined in this study to evaluate the possibility of a regional variance in its production. Measurement of hypothalamic NA turnover using deuterated L-dopa also indicates there is a minimal effect on synthesis (Freed & Murphy 1978). These authors suggest that there is a further rate-limiting step in hypothalamic NA synthesis at the level of dopamine β-hydroxylase (DBH) or access to it. In the NA-depleted mouse, increases in hypothalamic DA are even greater and we do see an increase in MHPG level reflecting metabolism of displaced NA.

Thus not all NA terminals respond in the same way to a dopa load. This difference may be based on differences in regional uptake of the precursor (both into the nerve terminal and into storage vesicles); regional activity of synthetic (DBH) and/or degradative (*O*-methyl transferase) enzymes; or the origin of noradrenergic input. Measurement of noradrenergic parameters in whole brain represents an averaging of all regions, with the greatest contribution from brainstem and hypothalamus. This may explain why several previous studies looking at whole brain NA levels report no differences after L-dopa administration (Everett & Borcherding 1970; Maj et al 1971; Benkert et al 1973; Keller et al 1974; Dolphin et al 1976). Our studies indicate the need for careful, regional analysis of transmitter turnover since there is a non-uniform response of NA after L-dopa administration.

Following NA depletion in the cerebral and cerebellar cortices by DSP-4, remaining noradrenergic neurons are still able to respond to an L-dopa load by an increase in NA and MHPG level. In this study, NA levels are not restored to control values but the percent increases in NA in DSP-4-treated mice are greater than in control animals receiving dopa. In cortical and hippocampal regions, the net amount of NA formed exceeds that which would have been predicted on the basis of an 80% loss of NA and presumably, DBH activity (Ross 1976). An enhancement of NA synthesis has also been reported following depletion by reserpine or α-methyl-*p*-tyrosine (AMPT) administration (Maj et al 1971; Dolphin et al 1976). Elevation of NA after L-dopa was noted under these conditions, where no change had been seen under control conditions.

Dopamine level increased significantly in all five regions examined, roughly to the same extent in both control and DSP-4 pretreated mice. The net rise of dopamine in these regions (200–300 ng g^{-1} above control levels) is less than that we have seen in striatum (about 1000 ng g^{-1} above control levels, from 8·5 to 9·56 $\mu g\ g^{-1}\ g^{-1}$), but DA is now appearing in regions where its levels are normally low (cortex and hippocampus) or undetectable (cerebellum). Thus, many of the effects of L-dopa may be mediated via actions on noradrenergic systems or on extra-striatal dopaminergic neurons.

Deficits in the LC are usually a constant feature of Alzheimer's disease (Mann et al 1984) and Parkinson's disease (Forno 1966; Mann et al 1983) particularly in demented, parkinsonian patients (Gaspar & Gray 1984). The DSP-4-treated rodent may prove useful in modelling noradrenergic losses seen in these degenerative disorders. L-Dopa may increase NA as well as DA turnover in parkinsonian patients and may also be a useful adjunct to therapy in some cases of Alzheimer's disease.

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