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EFFECTS OF NIGROSTRIATAL PATHWAY STIMULATION AND TROPOLONE ON PLASMA CONCENTRATION OF 3,4-DIHYDROXYPHENYLACETIC ACID

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The concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in regions of rat brain have been shown to vary according to the functional state of dopaminergic neurons. Experimental conditions which lead to increased rates of impulse flow through the nigro-neostriatal pathway result in increased concentrations of DOPAC and HVA in the striatum, and conditions which inhibit impulse flow lead to decrements in DOPAC and HVA [1-4].

Previous studies in this laboratory have shown that, during electrical stimulation of the nigrostriatal pathway at 15 Hz, DOPAC levels in the striatum are increased by 200 per cent within 20 min. Further electrical stimulation does not cause a progressive accumulation of DOPAC [5], suggesting that this metabolite is rapidly cleared from brain. This view is further supported by the finding that DOPAC in the striatum declines to undetectable levels 30 min after inhibition of monoamine oxidase [5,6]. These findings suggested that DOPAC produced upon activation of central dopaminergic neurons may enter the circulation and that this route may constitute a major mechanism of removal of this metabolite from brain. If this were the case, measurements of plasma concentrations of DOPAC could provide an index of activity of dopaminergic neurons in the brain.

To investigate this possibility, we measured DOPAC in the plasma of rats (Sprague-Dawley males, 200-250 g) after unilateral stimulation of the nigrostriatal pathway for 60 min at 15 Hz [5]. In a series of experiments tropolone was used to block the conversion of DOPAC to HVA. Blood was collected by cardiac puncture and centrifuged at 10,000 g for 20 min. Fifty ng of 3,4-dihydroxyphenyl-d_3 acetic-d_2 acid (Merck, Sharp & Dohme, Canada) was added to 1 ml samples of plasma acidified with 1 ml of 0.5 N HCl, or to 1 ml of 10,000 g supernatants of striatal tissue homogenized in 0.1 M formic acid. DOPAC was extracted into 5 ml ethyl acetate which was then evaporated to dryness under N_2. The pentafluoropropionyl derivative of DOPAC was formed by reaction with pentafluoropropionic

acid anhydride and pentafluoropropanol. Quantitation was performed by the technique of selected ion monitoring using a Finnigan model 3200 quadrupole electron impact mass spectrometer. Ions at m/e 387 and 392, originating from endogenous and deuterated DOPAC, respectively, were monitored. Experimental values were determined by extrapolation from appropriate standard curves. The identity of the endogenous compound was confirmed by comparing the ratios of two ions (m/e 387 and 415) derived from endogenous DOPAC with the ratio of those same two ions derived from authentic DOPAC.

The results presented in Table 1 indicate that a measurable and significant ($P < 0.05$) increase in plasma DOPAC concentration occurred after electrical stimulation of the nigrostriatal pathway on one side of the brain. A large increase in the concentration of DOPAC was also observed in the striatum ipsilateral to stimulation (Table 2).

Table 1. Effects of nigrostriatal pathway stimulation and tropolone treatment on plasma DOPAC concentration*

Treatment	N	DOPAC (ng/ml plasma)
Controls	7	2.0 ± 0.06
1-hr Stimulation	7	2.5 ± 0.16[+]
Tropolone (50 mg/kg)	8	4.8 ± 0.44[‡]
Tropolone + 1-hr stimulation	6	6.3 ± 0.53[§]

*All animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) 60 min prior to sacrifice. Tropolone (50 mg/kg, i.p.) was injected 15 min before chloral hydrate. Results are expressed as mean ± S.E.M.

[+]$P < 0.05$ from controls. [‡]$P < 0.001$ from controls. [§]$P < 0.05$ from tropolone-treated controls.

Table 2. Effects of nigrostriatal stimulation and tropolone treatment on DOPAC concentration in the striatum*

Treatment	Tissue	DOPAC (μg/g striatum)
1-hr Stimulation (N = 7)	Striatum ipsilateral to stimulation	3.05 ± 0.10[+]
	Striatum contralateral to stimulation	0.91 ± 0.06
1-hr Stimulation + tropolone (N = 6)	Striatum ipsilateral to stimulation	2.03 ± 0.11[‡]
	Striatum contralateral to stimulation	1.06 ± 0.12

*Animals were treated as described in Table 1. Results are expressed as mean ± S.E.M.

[+]$P < 0.01$ from striatum contralateral to stimulation. [‡]$P < 0.05$ from striatum contralateral to stimulation.

The difference in magnitude between the stimulation-induced increase of DOPAC in the striatum and that in the plasma is probably due to the dilution of the metabolite in a relatively large volume of blood, and its rapid conversion to other compounds by conjugation or O-methylation by liver catechol-O-methyltransferase (COMT).

The administration of tropolone (50 mg/kg, i.p.), a COMT inhibitor, 15 min prior to stimulation induced significant alterations in the pattern of DOPAC accumulation both in the brain and in plasma.

The concentration of DOPAC in the plasma of tropolone-treated animals was increased to 2[illegible]0 per cent of control levels (Table 1), suggesting that O-methylation is a major route of metabolism of free DOPAC in the plasma. Stimulation of the left nigrostriatal pathway for 60 min led to a further increase in plasma DOPAC which was significantly higher than that induced by tropolone alone.

Administration of tropolone did not cause a significant increase in the accumulation of DOPAC in the striatum (Table 2). This finding suggests that DOPAC can be removed from the brain by means other than O-methylation, such as conjugation [7] or by entering the circulation. The latter view is supported by the observations that a significant increase in the accumulation of DOPAC occurs in the plasma after stimulation of the nigrostriatal pathway or after inhibition of COMT (Table 1).

The results presented in this communication suggest that changes in the concentration of DOPAC in the plasma reflect changes in the functional activity of dopaminergic neurons in the brain. These findings raise the possibility that some aspects of central nervous system function can be studied by monitoring neurotransmitter metabolites in the plasma. We are currently investigating the effects of pharmacologic treatments which increase impulse flow in central dopamine neurons on the plasma concentrations of dopamine metabolites.

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