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3,4-DIHYDROXYPHENYLACETIC ACID (DOPAC) AND THE RAT MESOLIMBIC DOPAMINERGIC PATHWAY: DRUG EFFECTS AND EVIDENCE FOR SOMATODENDRITIC MECHANISMS

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1 Drug effects on dopamine catabolism of the mesolimbic dopaminergic pathway have been investigated using a sensitive radioenzymatic assay for 3,4-dihydroxyphenylacetic acid (DOPAC).

2 Turnover of DOPAC was less rapid in the ventral tegmentum (containing somata and dendrites) than in the nucleus accumbens (containing nerve terminals): 9 and 115 ng g^{-1} min^{-1} for ventral tegmentum and nucleus accumbens respectively.

3 Reserpine (5 mg/kg, 1 h) elevated DOPAC concentration to a greater extent in ventral tegmentum than in nucleus accumbens.

4 Neuroleptic drugs elevated DOPAC levels in ventral tegmentum and nucleus accumbens. Thioridazine, sulpiride and clozapine, thought to act preferentially on the mesolimbic system, caused a similar elevation in both brain regions.

5 γ-Butyrolactone (750 mg/kg) caused a significant decrease in the DOPAC concentration in ventral tegmentum after 0.5 and 1 h, while DOPAC levels in nucleus accumbens were not significantly altered at these time intervals.

6 Similarities exist between the dopamine catabolism in somatodendritic and nerve terminal regions of mesolimbic dopaminergic neurones in the response to neuroleptic drugs, but differences in catabolism are evident following certain pharmacological treatments such as reserpine and γ-butyrolactone.

7 Dopamine release occurs in the somatodendritic region of mesolimbic dopaminergic neurones and release sites may be dendritic as has been found for nigrostriatal dopaminergic neurones.

Introduction

Central dopaminergic neurotransmission has been studied until recently almost exclusively in nerve terminal regions. However, histochemical evidence of intensely fluorescent varicosities in the substantia nigra, apparently arising from dendrites of dopaminergic cell bodies (Björklund & Lindvall, 1975), has led many workers to examine the functional role of dendritic dopamine stores (Korf, Zieleman & Westerink, 1976; Geffen, Jessell, Cuello & Iversen, 1976; Wilson, Groves & Fifkova, 1977). This work has suggested that dopaminergic neurones of the nigrostriatal pathway may release their transmitter at both dendritic and nerve terminal sites (Björklund & Lindvall, 1975; Geffen *et al.*, 1976).

By contrast dopaminergic neurones of the mesolimbic pathway have been poorly studied. They have their somata and dendrites in the area ventral tegmentum (the A10 group of Dahlström & Fuxe, 1964) and project axons to form terminals in a number of forebrain regions, including the nucleus accumbens septi (Lindvall & Björklund, 1974; Carter & Fibiger, 1977). Mesolimbic dopaminergic neurones appear to be involved in motor function (Pijnenburg, Honig & Van Rossum, 1976; Kelly & Moore, 1976) and psychotic disturbances (Stevens, 1973). Furthermore, neuroleptic drugs are considered to act on these neurones by blocking dopamine receptors (Matthysse, 1973; Bartholini, 1976; Colpaert, Van Bever & Leysen, 1976). Since neuroleptic drugs can elicit changes in dopamine synthesis in the substantia nigra (Westerink & Korf, 1976b), we endeavoured to determine whether dopamine catabolism in the somatodendritic region of the mesolimbic dopaminergic pathway could be altered by drug treatments.

A combination of microdissection and a sensitive radioenzymatic assay for 3,4-dihydroxyphenylacetic acid (DOPAC), an important acid metabolite (Roffler-Tarlov, Sharman & Tergerdine, 1971; Westerink & Korf, 1976a), was employed to examine dopamine catabolism in the somatodendritic regions of the mesolimbic pathway, and to compare these results with parallel studies in nucleus accumbens. A prelimi-

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nary account of these results has been published (Gundlach & Beart, 1978).

Methods

Dissection

Male Sprague-Dawley rats (150 to 200 g) were stunned by a blow to the neck, decapitated and their brains removed and chilled in ice.

All dissections were performed on an ice-chilled glass plate. A coronal section of mesencephalon was obtained by making one cut just rostral to the pons and another approximately 1 mm rostral to the caudal end of the mammillary body (König & Klippel, 1963; McGeer, Parkinson & McGeer, 1976). The coronal section was placed with rostral surface facing up and tissue enriched in the area ventral tegmentum and containing the interpeduncular nucleus (henceforth referred to as ventral tegmentum) was dissected free with a fine pair of scissors. No adjoining substantia nigra or posterior hypothalamus was included. Mean weight of samples was 5.7 ± 0.1 mg (n = 167). Nucleus accumbens was removed as described by Horn, Cuello & Miller (1974): mean weight 19.6 ± 0.2 mg (n = 167). Corpus striatum was dissected according to Glowinski & Iversen (1966): mean weight 61.7 ± 2.9 mg (n = 20). Substantia nigra was dissected from the same mesencephalic section as ventral tegmentum: mean weight 9.5 ± 0.6 mg (n = 14).

DOPAC assay

Dissected brain areas were homogenized with a glass-teflon microhomogenizer in ice-cold 0.1 M aqueous HCl containing 0.1% w/v disodium edetate (EDTA): the volume was adjusted according to the expected DOPAC concentration. Homogenates were stored at −20°C overnight. Samples were centrifuged at 10,000 g for 5 min at 4°C. Portions of the clear supernatant were used for the assay of DOPAC. Cerebellar homogenates served as tissue blanks and recoveries were checked by homogenizing cerebellum in a solution containing added DOPAC and carrying this through the assay procedure. Recovery was 75 ± 6% (n = 18) and levels have not been corrected. DOPAC was assayed by a modification of a radioenzymatic assay employing catechol-*O*-methyltransferase (COMT) and [^{3}H]-S-adenosyl methionine (SAM) (Argiolas, Fadda, Stefanini & Gessa, 1977), in which DOPAC is estimated after conversion to [^{3}H]-homovanillic acid (HVA).

Homogenate supernatants (50 µl) were incubated with 70 µl of freshly prepared incubation mix, which contained partially purified COMT (265 µg protein) (Da Prada & Zürcher, 1976), 0.86 M Tris(hydroxymethyl)aminomethane, 12.7 mM $MgCl_2$ and [^{3}H]-SAM (2 µCi), for 20 min at 37°C. The reaction was stopped by addition of 0.9 ml ice-cold 0.4 M aqueous perchloric acid containing 2 µg HVA as carrier. Potassium formate was added to give pH 2 to 3, and each sample was vortexed and allowed to stand in ice for 5 min. Samples were centrifuged at low speed for 2 to 3 min and the supernatant applied to a Sephadex G-10 column (2 cm long × 1 cm diameter; Westerink & Korf, 1975). Each pellet was washed with 1.0 ml of 10 mM formic acid by resuspension of the pellet and centrifugation. These supernatants were also applied to the columns, which were washed with 2 ml of H_2O and the [^{3}H]-HVA then eluted with 2 ml of 10 mM sodium potassium phosphate pH 8.5 into tubes containing 2 g of solid NaCl. The collected buffer was acidified with 0.1 ml of 1 M HCl and the [^{3}H]-HVA extracted into 4 ml of ethyl acetate. The tubes were centrifuged briefly at low speed and the aqueous layer frozen before removal of the organic phase. The ethyl acetate extract was decanted, washed with 2.5 ml of 10 mM HCl and after centrifugation 1 ml of ethyl acetate was transferred to a scintillation vial. The radioactivity was counted after adding 10 ml of 0.4% 2,5-diphenyloxazole, 0.01% 1,4-di(2-(5-phenyloxazolyl)-benzene in toluene by liquid scintillation spectrometry. Blanks and standards in the range 100 to 2000 pg were run concurrently with all samples.

Since other catechol acids (e.g. 3,4-dihydroxymandelic acid) could be converted to [^{3}H]-methoxy-derivatives, and extracted into ethyl acetate, the specificity of the procedure was checked. The elution profile from Sephadex of the radio-labelled reaction product was identical to that of authentic [^{3}H]-HVA. Chromatography of ethyl acetate extracts on Whatman No. 3 chromatography paper confirmed that essentially all of the radioactivity in samples from ventral tegmentum, corpus striatum and nucleus accumbens ran with an R_F identical to that of authentic HVA and custom synthesized [^{3}H]-HVA.

Drugs and reagents

Drugs were injected intraperitoneally in a volume of 2 ml/kg and controls received the appropriate vehicle. Neuroleptic drugs were dissolved in 10% tartaric acid, the pH adjusted (6 to 7.5) and the volume made up with saline. Apomorphine and reserpine were dissolved in 20% ascorbic acid. Probenecid was prepared in a 0.35 M KH_2PO_4 solution and adjusted to pH 7.5 with 2 M NaOH. γ-Butyrolactone was injected as the pure substance. All other drugs were dissolved in 0.9% w/v NaCl solution (saline).

S-adenosyl-L-[methyl-^{3}H]methionine (9.3 to 12.0 Ci/mmol) and [^{3}H]-HVA were obtained from The Radiochemical Centre, Amersham. The following

drugs were used: 3,4-dihydroxyphenylacetic acid, homovanillic acid, pargyline hydrochloride (Sigma); thioridazine, clozapine (Sandoz Ltd.); haloperidol (Searle); pimozide (Ethnor Pty. Ltd.); chlorpromazine (May & Baker); sulpiride (Delagrange); tropolone (Aldrich Chemical Co.); probenecid (Merck, Sharp & Dohme); reserpine, (+)-amphetamine sulphate (Koch-Light); γ-butyrolactone (Eastman Kodak Co.); and apomorphine hydrochloride (Macfarlan Smith). Sephadex G-10 was obtained from Pharmacia Fine Chemical and plastic disposable columns from Whale Scientific Inc. All other reagents were of the highest grade possible.

Unless otherwise stated, results are presented as mean ± s.e. mean. Statistical analysis was by Student's *t* test.

Results

Concentration and turnover of DOPAC

The radioenzymatic assay for DOPAC was found to be extremely effective for measuring DOPAC concentrations in small brain regions. By defining the sensitivity of the assay as the amount of DOPAC which, after conversion to [^{3}H]-HVA, contained twice the radioactivity present in the reagent blank, it was possible to determine 115 ± 2 pg (n = 34) of DOPAC in 50 to 100 μg wet wt. of tissue. The DOPAC concentration in the ventral tegmentum of control rats was 426 ± 24 ng/g wet wt. (n = 42), and that in nucleus accumbens was 1,587 ± 66 ng/g wet wt. (n = 45). Since concentrations in untreated and vehicle-injected controls were found to be almost identical, the above concentrations represent the pooled values from these two groups. Control levels in analogous regions of the nigrostriatal pathway were substantia nigra, 741 ± 57 ng/g wet wt. (n = 10), and corpus striatum, 1,389 ± 39 ng/g wet wt. (n = 10).

DOPAC turnover was studied by inhibiting its formation with the monoamine oxidase inhibitor, pargyline, administered intraperitoneally. Under control conditions the rate of disappearance of DOPAC after inhibition of monoamine oxidase with pargyline is a method of determining dopamine turnover (Westerink & Korf, 1976a). Although there was a rapid decline of DOPAC levels in both brain regions, the acid metabolite was easily measured at all time points studied. The rate of decline of DOPAC was found to be exponential in both regions and the $T_{\frac{1}{2}}$, rates of elimination and initial rates of loss are given in Table 1. Turnover was found to be less rapid in ventral tegmentum than in nucleus accumbens with $T_{\frac{1}{2}}$ being 11 min and 4 min in ventral tegmentum and nucleus accumbens respectively.

Effect of psychotropic drugs

Reserpine (5 mg/kg, 1 h pretreatment) elevated DOPAC concentrations in both ventral tegmentum and nucleus accumbens (Table 2). Thus reserpine, which releases dopamine from storage vesicles in nerve terminals where it is deaminated to DOPAC, acted similarly in both brain regions. However, DOPAC concentrations were elevated to a greater extent in ventral tegmentum ($P < 0.02$). Both the directly and indirectly acting dopamine agonists, apomorphine and amphetamine, produced no effect on DOPAC levels in both somatodendritic and nerve terminal regions. Probenecid produced a small significant increase in DOPAC level in the ventral tegmentum ($P < 0.05$), but had no effect in nucleus accumbens. The COMT inhibitor tropolone, caused little change in DOPAC concentrations in both regions (Murphy, Robinson & Sharman, 1969; Table 2).

Effect of neuroleptic drugs

Neuroleptic drugs produced significant increases in DOPAC concentrations in both ventral tegmentum

Table 1 Rate of elimination of DOPAC in ventral tegmentum and nucleus accumbens following treatment with pargyline

	Ventral tegmentum	*Nucleus accumbens*
Steady state concentration (ng g^{-1})	382 ± 28	1650 ± 100
$T_{\frac{1}{2}}$ (min)	11.5 ± 1.2	4.2 ± 1.1
Fractional rate of elimination (min^{-1})	0.025 ± 0.001	0.069 ± 0.002
Calculated initial rate of loss (ng g^{-1} min^{-1})	9.4 ± 0.9	114.5 ± 8.5

Groups of at least 6 rats were killed at 0, 5, 10 and 15 min after treatment with pargyline HCl (100 mg/kg i.p.). Data were analysed by the least squares method. The initial rate of loss of DOPAC is the product of the fractional rate of elimination and the steady-state concentration (Karoum *et al.*, 1977).

Linear regression analysis of the data gave r = 0.51, $P < 0.01$, d.f. = 34 and r = 0.93, $P < 0.001$, d.f. = 40 for ventral tegmentum and nucleus accumbens respectively.

(range 38 to 100%) and nucleus accumbens (range 31 to 81%) (Table 3). Thioridazine, clozapine and sulpiride, a group of neuroleptic drugs thought to act preferentially on the mesolimbic system (Bartholini, 1976) caused similar elevations in both the somatodendritic and nerve terminal regions. Chlorpromazine acted in a biphasic manner. At small doses chlorpromazine produced a significant rise in DOPAC levels in ventral tegmentum, while DOPAC levels in nucleus accumbens remained unchanged. However, with a 10 fold increase in the dose of chlorpromazine, DOPAC levels in ventral tegmentum were unchanged relative to control, while DOPAC levels in nucleus accumbens were elevated significantly. The actions of the neuroleptic drugs on DOPAC concentrations are summarized in Table 3.

Action of γ-butyrolactone

The hypnotic drug, γ-butyrolactone has been shown to inhibit the firing rate of dopaminergic neurones and increase the level of DOPAC in the corpus striatum (Walters & Roth, 1972; Roth, Murrin & Walters, 1976). We have confirmed the latter finding by examining DOPAC levels in corpus striatum after the administration of γ-butyrolactone (750 mg/kg). DOPAC levels were significantly elevated at 3 h ($P < 0.001$) but were not significantly changed at 2 h: DOPAC concentrations at 2 and 3 h were 1728 ± 166 ($n = 4$) and 1860 ± 138 ($n = 5$) ng/g wet wt. respectively, relative to a control value of 1389 ± 39 ng/g wet wt. ($n = 10$).

By contrast, γ-butyrolactone (750 mg/kg) caused a significant decrease in the concentration of DOPAC in ventral tegmentum, when administered for 0.5 and 1 h, but had no significant effect in nucleus accumbens. DOPAC levels in ventral tegmentum had returned to control at longer time intervals (Figure 1).

Discussion

There is favourable evidence to support a role for dopamine in synaptic transmission in ventral tegmentum (Beart & Kuppers, 1978; Beart, Kuppers & Gundlach, unpublished observations). DOPAC was

Table 2 Effect of various drugs on DOPAC in ventral tegmentum and nucleus accumbens

	DOPAC (% control)	
	Ventral tegmentum	*Nucleus accumbens*
Control	100 ± 7	100 ± 6
Reserpine (5 mg/kg, 1 h)	224 ± 8*	162 ± 8*
Apomorphine (1 mg/kg, 0.5 h)	103 ± 13	95 ± 6
Amphetamine (2.5 mg/kg, 1 h)	80 ± 4	81 ± 4
Probenecid (200 mg/kg, 2 h)	128 ± 7*	103 ± 7
Tropolone (25 mg/kg, 1 h)	127 ± 14	94 ± 6

Control values: 382 ± 28 and 1650 ± 100 ng/g wet wt. for ventral tegmentum and nucleus accumbens respectively. All values are the mean ± s.e. mean of 4 to 24 determinations. *$P < 0.05$.

Table 3 Effect of various neuroleptic drugs on DOPAC in ventral tegmentum and nucleus accumbens

	DOPAC (% control)	
	Ventral tegmentum	*Nucleus accumbens*
Control	100 ± 7	100 ± 6
Thioridazine (20 mg/kg, 2 h)	138 ± 13*	161 ± 16*
Clozapine (40 mg/kg, 2 h)	197 ± 4*	169 ± 6*
Sulpiride (200 mg/kg, 1 h)	166 ± 21*	181 ± 25*
Haloperidol (1 mg/kg, 1.5 h)	200 ± 6*	136 ± 10*
Pimozide (5 mg/kg, 2 h)	180 ± 11*	131 ± 5*
Chlorpromazine (0.5 mg/kg, 2 h)	144 ± 14*	116 ± 8
Chlorpromazine (1.0 mg/kg, 2 h)	114 ± 9	101 ± 9
Chlorpromazine (5.0 mg/kg, 2 h)	113 ± 4	173 ± 7*

Control values: 382 ± 28 and 1650 ± 100 ng/g wet wt. for ventral tegmentum and nucleus accumbens respectively. All values are the mean ± s.e. mean of 4 to 24 determinations. *$P < 0.02$.

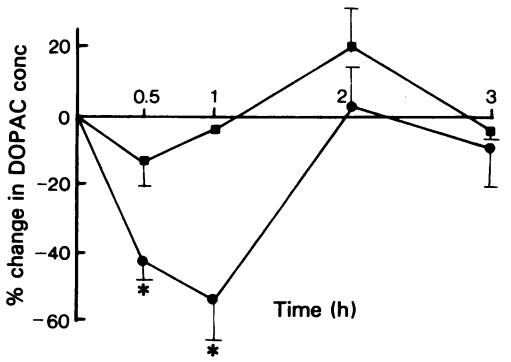

Figure 1 Effect of γ-butyrolactone (750 mg/kg, i.p.) on DOPAC concentrations in ventral tegmentum (●) and nucleus accumbens (■). Control DOPAC concentrations were 432 ± 37 (n = 10) and 1450 ± 93 (n = 16) ng/g wet wt. for ventral tegmentum and nucleus accumbens respectively. *$P < 0.025$. Values are expressed as percentage change relative to control and are the mean ± s.e. mean of 4 to 8 observations.

present in ventral tegmentum and the concentration was altered by drug treatment, being elevated by neuroleptic drugs and decreased after inhibition of deamination of dopamine by pargyline. Thus, dopamine release in ventral tegmentum is altered in response to drugs, further indicating an involvement of dopamine in regulatory function in this somatodendritic region.

Although DOPAC present in both the somatodendritic and nerve terminal regions of the mesolimbic dopaminergic pathway showed many similarities in response to drug treatments (Tables 2 and 3), dopamine catabolism did differ in some respects in ventral tegmentum relative to nucleus accumbens. DOPAC turnover in ventral tegmentum was much slower than in nucleus accumbens. Our values for DOPAC $T_{\frac{1}{2}}$ in ventral tegmentum (11.5 min) and nucleus accumbens (4.2 min) are similar to values reported by other workers. Karoum, Neff & Wyatt (1977) found a $T_{\frac{1}{2}}$ for DOPAC in midbrain of 6.2 min, and Westerink & Korf (1976a) reported a value of 9.5 min in mesolimbic tissue containing both the olfactory tubercle and nucleus accumbens. The higher turnover of DOPAC in nucleus accumbens may reflect a higher level of synaptic activity in nerve terminal regions due to a greater number of synapses and/or a relatively more frequent involvement in synaptic transmission than at somatodendritic synapses. The relative inability of the COMT inhibitor, tropolone, and of probenecid, which blocks the efflux of acid metabolites, to alter DOPAC levels indicated there was normally a low conversion of DOPAC to HVA and very little efflux of DOPAC via a probenecid-sensitive transport system in mesolimbic regions (Murphy *et al.*, 1969; Roffler-Tarlov *et al.*, 1971). Reserpine treatment produced a greater elevation of DOPAC levels in ventral tegmentum relative to nucleus accumbens (124% and 62% above control levels respectively) and this difference may indicate a closer proximity of reaccumulated somatodendritic dopamine to deamination sites. Thus our results indicate the presence of vesicular storage sites in ventral tegmentum and suggest a high level of catabolism of this released dopamine (also Beart & Kuppers, 1978).

Neuroleptic drugs had similar effects on the mesolimbic system, and generally the somatodendritic and nerve terminal regions responded similarly to the neuroleptic drugs used. Elevation of DOPAC concentration in ventral tegmentum suggests that this brain region may be a site of action of neuroleptic drugs, in addition to nucleus accumbens and other brain regions. The increase in DOPAC levels following neuroleptic drugs is presumably the result of a compensatory increase in dopamine synthesis and release caused by blockade of dopamine receptors (Iversen, 1975), and therefore our data may indicate that dopamine receptors are present in the ventral tegmentum. Westerink & Korf (1976b) have found that neuroleptic drugs can increase the concentrations of DOPAC and HVA in substantia nigra, suggesting the presence of dopamine receptors in this somatodendritic region. These authors also suggest that there may be differential responses to neuroleptic drugs in somatodendritic and nerve terminal regions of the nigrostriatal pathway, and our results with chlorpromazine may relate to such a phenomenon.

γ-Butyrolactone has been widely studied because it has a unique ability to inhibit the firing rate of dopaminergic neurones (Roth *et al.*, 1976). Subsequent biochemical changes include an increase in the level of dopamine and DOPAC in corpus striatum (Walters & Roth, 1972). Our experiments confirmed that γ-butyrolactone produced an increase in DOPAC levels in the striatum, although not to the extent described by Walters & Roth (1972). The DOPAC concentration in ventral tegmentum was by contrast decreased by 50% 1 h after γ-butyrolactone, while there was a trend for DOPAC levels to increase in nucleus accumbens (see Figure 1). This response in the somatodendritic region of mesolimbic dopaminergic neurones is remarkably similar to the 40% decrease in dopamine synthesis in the substantia nigra observed by Hefti, Lienhart & Lichtensteiger (1976). Our data demonstrate an apparent difference in the regulation of catabolism in the somatodendritic and nerve terminal regions, and further indicate that γ-butyrolactone may be a useful pharmacological tool for revealing such differences (Hefti *et al.*, 1976).

Our findings provide new evidence that dopaminergic neurones can release their transmitter at both axonal and somatodendritic sites, and reveal that drugs

can alter dopamine release in the somatodendritic region of mesolimbic neurones. The ability of neuroleptic drugs to alter dopamine catabolism in ventral tegmentum suggests that this somatodendritic area is an important site of action of these drugs. There is evidence for the existence of mechanisms regulating the function of dopaminergic neurones in the substantia nigra (Korf *et al.*, 1976; Westerink & Korf, 1976b; Geffen *et al.*, 1976) and the mesolimbic pathway therefore appears similar to the nigrostriatal system. The relative importance of biochemical changes in somata and dendrites (and nerve collaterals) in relation to these mechanisms is at present difficult to assess, but available evidence suggests that dendritic processes are more important (Geffen *et al.*, 1976; Hefti *et al.*, 1976). Many similarities exist between the dopamine catabolism in somatodendritic and nerve terminal regions of mesolimbic dopaminergic neurones, but differences in regulation certainly do occur in at least some functional states and can be demonstrated upon suitable pharmacological treatments.

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