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Effects of Drugs on Regional Brain Concentrations of Dopamine and Dihydroxyphenylacetic Acid[1]

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ABSTRACT

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A radioenzymatic assay sensitive to less than 1 ng of dopamine and dihydroxyphenylacetic acid (DOPAC) was developed and employed to measure the effects of several drugs on the quantities of these compounds in the striatum, olfactory tubercle, hypothalamus and median eminence, regions which contain the terminals of nigrostriatal, mesolimbic, incertohypothalamic and tuberoinfundibular dopaminergic neurons, respectively. Pretreatment of rats with a monoamine oxidase inhibitor (pargyline) increased dopamine and decreased DOPAC concentrations in all brain regions. Neuroleptics (haloperidol, thioridazine) did not alter the dopamine concentrations in any brain region, but increased DOPAC concentrations in the striatum, olfactory tubercle and hypothalamus, but not in the median eminence. Three days of treatment with estradiol benzoate did not alter dopamine or DOPAC concentrations in any brain region. Reserpine markedly reduced the dopamine concentration in all four brain regions; it also caused a prolonged increase in the concentration of DOPAC in the striatum, transient increases in the olfactory tubercle and hypothalamus, but was without effect on DOPAC concentrations in the median eminence. Since treatment with neuroleptics and estradiol, which have been shown to increase the α-methyltyrosine-induced decline of dopamine in the median eminence, are without effect on DOPAC concentrations in this brain region, it is suggested that changes in concentrations of this metabolite cannot be used to estimate the activity of tuberoinfundibular dopaminergic neurons.

There are several distinct dopaminergic neuronal systems in the brain (Ungerstedt, 1971). Nigrostriatal neurons, which have cell bodies located in pars compacta of substantia nigra (A8 and A9 in the rat brain) and axons that project ipsilaterally to the caudate nucleus and putamen (striatum), make up the largest and most thoroughly studied system. Mesolimbic neurons, which originate from cell bodies located in the ventromedial tegmentum (A10 in rat brain), have axons which project to nucleus accumbens, bed nucleus of stria terminalis and olfactory tubercle. Other major dopaminergic neurons comprising the mesocortical system also originate in areas A9 and A10 of the rat brain; they terminate in the frontal, cingulate and entorhinal cortices (Lindvall *et al.*, 1974). In addition to these long ascending dopaminergic neurons there are several groups of dopaminergic neurons located within the hypothalamus. The tuberoinfundibular neurons have cell bodies in the arcuate nucleus (A12) and short axons which terminate in the external layer of the median eminence (Björklund *et al.*, 1973). The incertohypothalamic neuronal system is composed of dopamine neurons in distinct regions of the hypothalamus (designated A11, A13 and A14 in rat brain); they are believed to give rise to short axons which are restricted to the hypothalamus (Björklund *et al.*, 1975).

Changes in functional states of dopamine nerves in the central nervous system are generally reflected in changes in the turnover of dopamine in brain regions containing the terminals of these neurons. This is particularly true for the striatum and olfactory tubercle which contain the terminals of the nigrostriatal and mesolimbic neurons and relatively large quantities of dopamine. A variety of methods have been employed including measurements of the rate of conversion of radioactive tyrosine to dopamine (Nybäck and Sedvall, 1968), the rate of accumulation of dihydroxyphenylalanine after the administration of a decarboxylase inhibitor (Carlsson, 1975) and the rate of decline of dopamine after inhibition of synthesis with α-methyltyrosine (Corrodi *et al.*, 1967; Waldmeier and Maitre, 1976). With the latter technique it has been demonstrated that all dopaminergic neurons in the brain do not respond in the same way to pharmacological and endocrinological manipulations (Moore and Gudelsky, 1977). For example, neuroleptics produce a prompt increase in the α-methyltyrosine-induced decline of dopamine in the striatum and olfactory tubercle, but increase dopamine turnover in the median eminence only after a prolonged latent period (Fuxe *et al.*, 1975; Gudelsky and Moore, 1977).

In order to examine the regulation of dopaminergic neurons comprising the tuberoinfundibular and incertohypothalamic systems we have explored the possibility of obtaining an index of dopaminergic nerve activity by a method that does not employ α-methyltyrosine. It has been suggested that the tissue

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concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) reflects the amount of dopamine that is released, recaptured by dopamine nerve terminals and then oxidatively deaminated by intraneuronal monoamine oxidase (Roth *et al.*, 1976). These investigators demonstrated that changes in DOPAC concentrations, at least in the striatum, olfactory tubercle and nucleus accumbens, can provide an index of activity of nigrostriatal and mesolimbic dopaminergic neurons, an increased concentration of DOPAC being associated with increased activity and a decreased concentration associated with reduced activity. It is not known if the same relationship holds true for tuberoinfundibular and incertohypothalamic neurons. Since there are only small amounts of dopamine and DOPAC in the hypothalamus and median eminence a radioenzymatic assay capable of measuring less than a nanogram of dopamine and DOPAC was developed and employed to quantify the effects of several drugs on the concentrations of these compounds in the striatum, olfactory tubercle, hypothalamus and median eminence of rat brain, regions which contain the terminals of the nigrostriatal, mesolimbic, incertohypothalamic and tuberoinfundibular neuronal systems, respectively.

Methods

Animals and drug treatments. Male Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.) weighing 200 to 300 g were housed in an animal room maintained at 21 ± 1°C. They were fed water and Wayne Lablox *ad libitum*. Lights were off from 19:00 to 07:00 hr, and animals were sacrificed by decapitation between 09:00 and 11:00 hr at various times after injection of drugs or their vehicles. Haloperidol (McNeil Laboratories, Fort Washington, Pa.) was dissolved in 0.3% tartaric acid and thioridazine (Sandoz Pharmaceuticals, East Hanover, N.J.) was dissolved in 1.5% tartaric acid. Pargyline HCl (Regis Chemical Co., Morton Grove, Ill.) was dissolved in 0.9% saline, estradiol benzoate (ICN Pharmaceuticals, Cleveland, Ohio) was dissolved in corn oil and probenecid (Merck, Sharp and Dohme Research Laboratories, West Point, Pa.) was dissolved in 0.1 N NaOH and the solution was neutralized to pH7 with 0.1 N HCl.

Tissue dissections and extraction. Brains were quickly removed from the skull and placed on a cold plate (Thermoelectrics Unlimited, Inc., Wilmington, Del.) during the dissections. The median eminence was removed from the hypothalamus with the aid of a dissecting microscope and fine scissors as described by Cuello *et al.* (1974). The olfactory tubercles (9–11 mg wet weight) were then removed as described by Horn *et al.* (1974) and the head of the striata (35–40 mg) and hypothalamus (40–46 mg) were dissected essentially as described by Glowinski and Iversen (1966). The median eminence was homogenized in 40 μl of ice-cold 0.2 N perchloric acid containing 10 mg/100 ml of ethylene glycol bis (β-aminoethyl ether)-N,N′-tetraacetic acid (EGTA). The other tissues were weighed and homogenized in different volumes of the same perchloric acid solution (striata, 50 μl/mg; olfactory tubercle, 40 μl/mg; hypothalamus, 10 μl/mg). For each brain, these operations could be completed by two individuals working together in less than 4 min. The ice-cold homogenates were centrifuged for 15 sec at room temperature in a Beckman 152 Microfuge, and 20 μl of the resulting supernatant were analyzed for dopamine and DOPAC as described below. The homogenate pellets were analyzed for protein as described by Lowry *et al.* (1951), and the tissue concentrations of DOPAC and dopamine are reported on the basis of the protein content, *i.e.*, nanograms of DOPAC or dopamine/per milligram of protein.

Dopamine and DOPAC assays. Dopamine and DOPAC were assayed using a radioenzymatic procedure in which these compounds were incubated with partially purified catechol O-methyltransferase and S-adenosyl-L-[*methyl*-^{3}H] methionine and the resulting radioactive methylated products (3-methoxytyramine and homovanillic acid, HVA) were separated by organic extraction and thin-layer chromatography.

Twenty microliters of perchloric acid extracts of brain tissue or standards (0.5–3 ng of dopamine, as the hydrochloride, and 0.06 to 1 ng of DOPAC, both obtained from Sigma Chemical Co., St. Louis, Mo.) were added to 15-ml conical centrifuge tubes kept in ice. Fifty microliters of freshly prepared "incubation mix" was then added to each tube. This mixture (for 30 assays) contained: 100 μl of 20 mM sodium EGTA, pH 7.2; 500 μl of catechol O-methyltransferase prepared as described previously (Moore and Phillipson, 1975); 300 μl of S-adenosyl-L-[*methyl*-^{3}H] methionine (9–10 Ci/mmole, 250 μCi/ml, New England Nuclear Corp., Boston, Mass.); 100 μl of freshly prepared pargyline HCl, 16 mg/ml, in 10% mercaptoethanol; 650 μl of 1 M Tris containing 3 mM $MgCl_2$, pH 10.4. The mixture was incubated at 37°C for 60 min.

Dopamine was estimated by quantifying the 3-[^{3}H]-methoxytyramine in the incubation mixture. Twenty microliters of the incubation mixture was added to 15-ml centrifuge tubes containing 30 μl of solution consisting of 5 volumes of 0.45 M borate buffer (pH 10) and 1 volume of a methoxyamine carrier (0.25 mg of each of the following compounds per ml of 0.1 N HCl: 3-methoxytyramine HCl, normetanephrine HCl, metanephrine HCl, HVA; all compounds obtained from Sigma). Then, in quick succession were added 20 μl of 1.5% tetraphenyl boron and 500 μl of toluene- isopentyl alcohol (3:2). The tubes were centrifuged and 475 μl of the organic phase was transferred to 5-ml centrifuge tubes containing 250 μl of 0.45 M borate buffer, pH 10. After vortexing and centrifugation, 400 μl of the organic phase was transferred to 10-ml centrifuge tubes with specially prepared pointed tips containing 40 μl of 0.1 N HCl. After vortexing and centrifugation, the organic phase was aspirated and the acid phase washed with 250 μl of ethylacetate saturated with water. After aspiration of the ethylacetate, 30 μl of the acid phase was spotted on thin-layer chromatography plates precoated with silica gel (LK6D, Linear-K, Whatman, Clifton, N.J.). The chromatographs were developed for 1.5 hr with methylamine-ethanol-chloroform (5:18:40). The spots were visualized after spraying with phenol reagent (Folin and Ciocalteu, Harleco, Gibbstown, N.J.) diluted 1:1 with water. The 3-methoxytyramine spot (R_f 0.78–0.82) was scraped into scintillation vials and extracted with 0.5 ml of acetic acid–ethylacetate–water (3:3:1) for 30 min. After addition 10 ml of toluene–ethanol (7:3) with 0.5% 2.5-diphenyloxazole (PPO), radioactivity was determined in a Beckman LS100 scintillation counter (21% efficiency).

DOPAC was estimated by quantifying the [^{3}H]-HVA in the incubation mixture. Fifty microliters of the incubation mixture was added to 15-ml centrifuge tubes and then were added: 10 μl of 1 N perchloric acid; 10 μl of HVA (0.25 mg/ml of 0.1 N HCl); 10 μl of saturated solution of KCl; 220 μl of ethylacetate saturated with water. After vortexing and centrifugation, 160 μl of the organic phase was transferred to 5-ml centrifuge tubes containing 300 μl of 0.2 Tris, pH 8.6, and 300 μl of ethylacetate. After vortexing and centrifugation, the organic phase was aspirated and the aqueous phase washed again with 200 μl of ethylacetate. After aspiration of the organic phase, 30 μl of 3 N HCl and 100 μl of ethylacetate were added. After vortexing and centrifugation, 60 μl of the organic phase was spotted on thin-layer chromatography plates (LK6D, Linear-K, Whatman). The chromatographic plates were developed for 1.5 hr with isopropanol–ammonium hydroxide–water (8: 1:1). The HVA spots (R_f 0.42–0.48) were visualized after spraying with phenol reagent diluted 1:1 with water, scraped into scintillation vials and extracted for 30 min with 0.5 ml of acetic acid–ethylacetate–water (3:3:1). After adding 10 ml of toluene–ethanol (7:3) with 0.5% PPO, radioactivity was determined in a Beckman LS100 scintillation counter.

The sensitivites of the assays varied slightly from day to day depending on the counts per minute of the blanks, which consisted of 10 μl of 0.2 N perchloric acid containing 10 mg/100 ml of EGTA carried through the entire assay procedure. In a series of five experiments the dopamine blank (3-methoxytyramine spot) contained 192 ± 23 cpm and the DOPAC blank (HVA spot) contained 551 ± 23 cpm. In these assays 1 ng of dopamine and 1 ng of DOPAC produced 4533 ± 249 and 6556 ± 284 cpm, respectively. The assays were linear at least up to 3 ng of dopamine and 1 ng of DOPAC, which were the highest amounts of standards employed. There was no interference of norepinephrine or DOPAC in the dopamine assay nor of norepinephrine or dopamine in the DOPAC assay. Internal standards were not routinely run, but preliminary experiments revealed standards of dopamine and DOPAC added to hypothalamic extracts gave the same counts per minute as standards added to the perchloric acid mixture.

Statistical analysis. Changes in dopamine or DOPAC concentrations induced by neuroleptics (figs. 2 and 3) and by reserpine (Fig. 5) were analyzed statistically by analysis of variance and the least significant difference test (Sokal and Rohlf, 1969); effects of drug treatments depicted in figures 1 and 4 and table 2 were examined using Student's *t* test.

Results

The dopamine and DOPAC concentrations in brain regions which contain terminals of nigrostriatal (striatum), mesolimbic (olfactory tubercle), incertohypothalamic (hypothalamus) and tuberoinfundibular (median eminence) neurons are summarized in table 1. Values for dopamine are similar to those reported previously by our laboratory (Gudelsky and Moore, 1976; Gudelsky *et al.*, 1977) and by others (Brownstein *et al.*, 1974; Selmanoff *et al.*, 1976). Values for DOPAC are similar to those reported by others for the striatum (Karoum *et al.*, 1977; Argiolas *et al.*, 1977) and olfactory tubercle (Wilk *et al.*, 1975; Roth *et al.*, 1976) using fluorometric, gas chromatographic or radioenzymatic procedures. To our knowledge DOPAC concentrations in the hypothalamus and median eminence have not been reported previously.[2]

Dopamine and DOPAC concentrations in various brain regions after administration of monoamine oxidase (MAO) inhibitor. There have been numerous reports that MAO inhibitors increase the concentration of dopamine and reduce the concentration of DOPAC in regions containing terminals of nigrostriatal (striatum) and mesolimbic (nucleus accumbens) neurons (Roth *et al.*, 1976; Argiolas *et al.*, 1977; Karoum *et al.*, 1977). The results summarized in figure 1 demonstrate that similar effects also occur in regions containing terminals of tuberoinfundibular and incertohypothalamic neurons; 1 hr pretreatment with pargyline significantly increased the dopamine and reduced the DOPAC concentrations in all brain regions. In the hypothalamus pargyline reduced the DOPAC concentration to less than the sensitivity of the assay.

Dopamine and DOPAC concentrations in various brain regions after administration of neuroleptics. Neuroleptics increase the activity of dopaminergic nerves in the nigrostriatal and mesolimbic systems (Bunney *et al.*, 1973). This is reflected in an increased turnover of dopamine, and thus in the concentration of DOPAC in the terminal regions of these neurons (see review by Moore and Kelly, 1978). The results summarized in figure 2 are consistent with previous studies and show that a relatively high dose of haloperidol (2.5 mg/kg) does not alter the concentration of dopamine in any brain region but increases the concentration of DOPAC not only in the striatum and olfactory tubercle but also in the hypothalamus. In contrast, haloperidol does not alter the DOPAC concentration in median eminence. The increase in DOPAC concentrations is most marked in the striatum, increasing to 3 times control values and lasting for at least 8 hr. The increase in the olfactory tubercle lasted for the same duration but was less pronounced (less than 2 times control) and was followed by a slight but significant decrease at 16 hr. In the hypothalamus the increase in DOPAC was less pronounced being only 1.5 times control. The duration of the effect of 2.5 mg/kg of haloperidol on increased DOPAC

[2] After submission of this manuscript a paper by Argiolas *et al.* (Life Sci. **22:** 461-466, 1978) appeared in which the medial basal hypothalamus was reported to contain a total of 2.86 ng of DOPAC.

TABLE 1

Dopamine and DOPAC concentrations in various brain regions of nontreated rats

Values represent means ± S.E. determined in brain regions of 26 to 35 nontreated male rats.

	Dopamine	DOPAC	DOPAC/Dopamine × 100
	ng/mg protein		
Striatum	98.4 ± 2.8	12.0 ± 0.4	12.1
Olfactory tubercle	65.0 ± 1.7	11.4 ± 0.5	17.6
Hypothalamus	3.9 ± 0.2	0.72 ±0.05	18.4
Median eminence	106 ± 4.0	5.8 ± 0.5	5.5

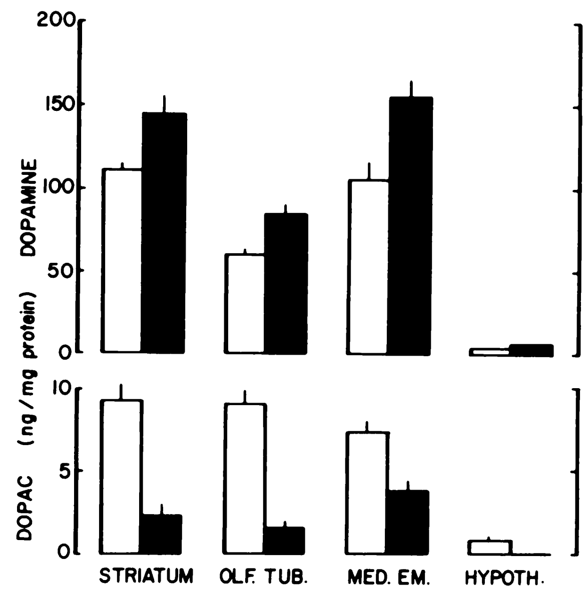

Fig. 1. Effects of a monoamine oxidase inhibitor on dopamine and DOPAC concentrations in various regions of rat brain. Pargyline (75 mg/kg) or saline was injected i.p. 1 hr before sacrifice. The height of each column and the vertical line represent the mean and 1 S.E. of dopamine and DOPAC concentrations determined from six animals. Open columns represent values in animals injected with saline and solid columns represent animals injected with pargyline. In all regions the concentrations of dopamine and DOPAC in pargyline-treated animals were significantly different from saline-treated animals ($P < .05$).

concentration in the striatum and olfactory tubercle is consistent with other indices of increased dopamine turnover (*e.g.*, α-methyltyrosine-induced decline of dopamine after 2.5 mg/kg of haloperidol; Gudelsky and Moore, 1977). In the median eminence this dose of haloperidol caused a delayed increase in the turnover of dopamine (*i.e.*, no effect at 2 and 8 hr but a significant increase at 16 and 24 hr; Gudelsky and Moore, 1977). In the present study this haloperidol-induced delayed increase in dopamine turnover was not reflected in an increased concentration of DOPAC.

A similar pattern of increased DOPAC concentrations with no change in dopamine in striatum, olfactory tubercle and hypothalamus was also observed after the administration of thioridazine (16 mg/kg), although the response to this drug was less sustained than with haloperidol (fig. 3). Again, the DOPAC concentration in the median eminence was not altered at any time after the administration of thioridazine whereas the α-methyltyrosine-induced decline of dopamine was increased 16 hr after the administration of the same dose of this neuroleptic (Gudelsky and Moore, 1977).

Dopamine and DOPAC concentrations in various brain

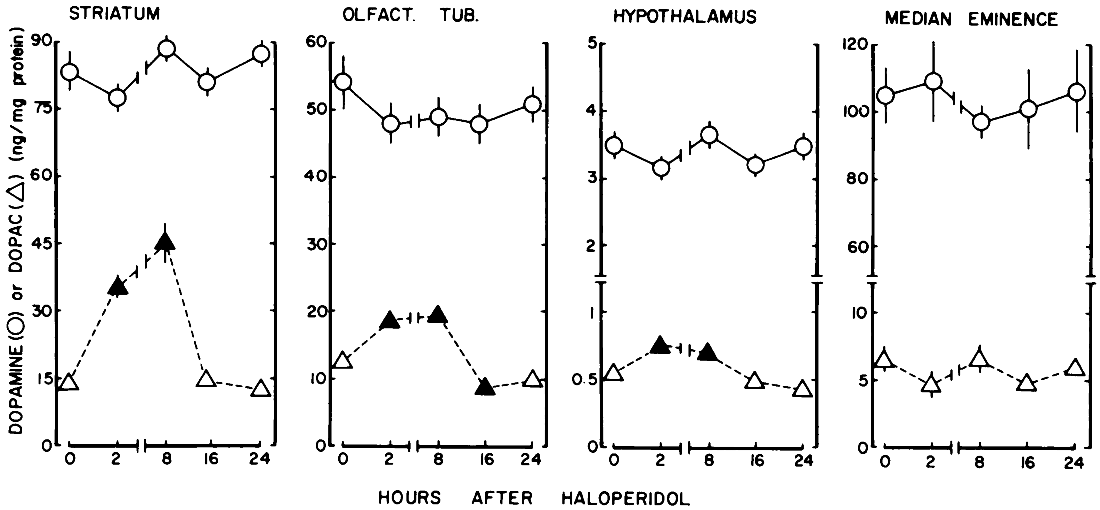

Fig. 2. Time course of the effects of haloperidol (2.5 mg/kg s.c.) on dopamine and DOPAC concentrations in various regions of rat brain. Each symbol represents the mean and the vertical line ± 1 S.E. of dopamine (○) and DOPAC (△) concentrations determined in five to eight animals. Where no S.E. is indicated the value is less than the radius of the symbol. Solid symbols represent those values from haloperidol-treated animals that are significantly different ($P < .05$) from controls.

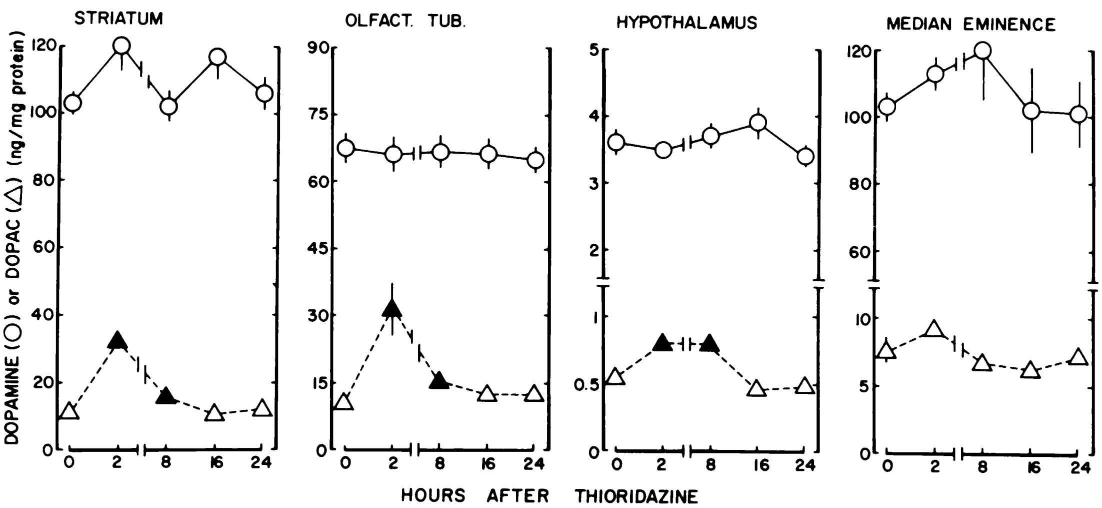

Fig. 3. Time course of the effects of thioridazine (16 mg/kg s.c.) on dopamine and DOPAC concentrations in various regions of rat brain. Each symbol represents the mean and vertical line ± 1 S.E. of dopamine (○) and DOPAC (△) concentrations determined in five to eight animals. Where no S.E. is indicated the value is less than the radius of the symbol. Solid symbols represent those values from thioridazine-treated animals that are significantly different ($P < .05$) from controls.

regions after estrogen pretreatment. Fuxe *et al.* (1969) demonstrated that estrogen pretreatment increased the α-methyltyrosine-induced loss of fluorescence in terminals of tuberoinfundibular neurons. Subsequently Eikenburg *et al.* (1977) showed that treatment of male rats with estradiol benzoate (25 μg/kg) for 3 or 5 days significantly increased the α-methyltyrosine-induced depletion of dopamine in the median eminence, but not in the striatum. On the other hand, as depicted in figure 4, estrogen treatment for 3 days failed to alter the dopamine or DOPAC concentrations in any brain region.

Dopamine and DOPAC concentrations in various brain regions after the administration of reserpine. Reserpine treatment is thought to expose catecholamines to the actions of intraneuronal MAO by disrupting the protective binding capability of storage vesicles. Consequently, the tissue concentration of catecholamines declines while that of DOPAC increases (Roffler-Tarlov *et al.*, 1971). The time course of the effect of an acute injection of reserpine on the concentrations of dopamine and DOPAC in various brain regions is depicted in figure 5.

Reserpine caused a pronounced and fairly rapid drop in the concentration of dopamine in all four brain regions, but the decline in the median eminence was much faster ($T_{1/2}$ of 13 min) than in the other regions ($T_{1/2}$ of 32-40 min). The dopamine concentration remained low over the duration of the experiment but appeared to increase slightly by 24 hr, particularly in the hypothalamus and median eminence. The DOPAC concentration increased to approximately 200% of control in the striatum within 20 min after injection of reserpine and remained significantly elevated for at least 11 hr. There were also smaller and very brief increases of DOPAC concentrations in olfactory tubercle and hypothalamus (significant only at 20 min). Except for a slight decrease in the olfactory tubercle at 4 hr the concentration of DOPAC in all brain regions did not significantly decline, and in the median eminence the concentration of DOPAC did not differ from control at any time after reserpine.

DOPAC concentrations in various brain regions after the administration of probenecid. Karoum *et al.* (1977)

examined the importance of probenecid-sensitive transport systems for the elimination of HVA and DOPAC from the brain. They concluded that in some brain regions (*e.g.*, hypothalamus and midbrain) but not in others (*e.g.*, striatum) a fraction of the DOPAC formed is transported out of the brain by a probenecid-sensitive mechanism. The results summarized in table 2 are consistent with their findings; probenecid significantly increased the DOPAC concentration in olfactory tubercle, hypothalamus and median eminence, but not in the striatum.

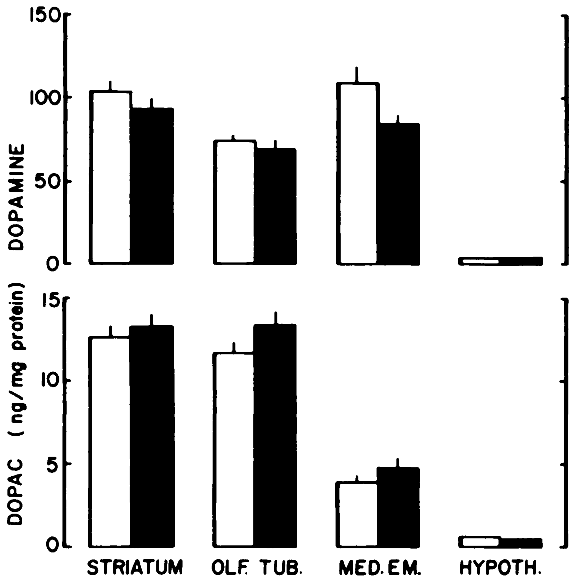

Fig. 4. Effects of estrogen treatment on dopamine and DOPAC concentrations in various regions of rat brain. Rats were injected once daily with estradiol benzoate (25 μg/kg s.c.; solid columns) or corn oil vehicle (open columns) for 3 days and the animals were sacrificed 24 hr after the last injection. The height of each column and the vertical line represent the mean and 1 S.E. of dopamine and DOPAC concentrations determined in six to seven rats There were no significant differences (P < .05) in dopamine or DOPAC concentrations in brain regions of estrogen- or vehicle-treated animals.

Discussion

Efforts have been made to determine what factors influence the activity of tuberoinfundibular dopaminergic neurons by measuring the turnover of dopamine in the median eminence. To date, this has been estimated only by determining the rate of decline of dopamine after blockade of synthesis with α-methyltyrosine (see review, Moore *et al.*, 1978). The present study was initiated to determine whether the concentration of DOPAC in the median eminence could also be used to obtain a biochemical index of tuberoinfundibular nerve activity. Roth *et al.* (1976) reported that changes in the activity of dopamine nerves terminating in the striatum and olfactory tubercle were associated with changes in the concentration of DOPAC, which is believed to reflect the amount of dopamine released, recaptured and deaminated by MAO in the terminals of the nigrostriatal and mesolimbic dopaminergic neurons, respectively. Because the rat median eminence weighs less than 0.2 mg and contains only 2 to 3 ng of dopamine (Gudelsky and Moore, 1976), it was necessary to employ a sensitive procedure for measuring DOPAC in the tissue.

Fluorometric assays were initially employed to measure DOPAC but the lack of sensitivity of these methods limited the

TABLE 2

Effect of probenecid on DOPAC concentrations in various regions of rat brain

Rats were sacrificed 1 hr after an i.p. injection of probenecid (200 mg/kg) or its vehicle. Values represent the mean ± 1 S.E. as determined from six vehicle- and six probenecid-treated animals.

	DOPAC	
	Vehicle	Probenecid
	ng/mg protein	
Striatum	13.5 ± 1.4	13.0 ± 1.1
Olfactory tubercle	9.9 ± 0.9	13.8 ± 1.5*
Hypothalamus	0.81 ± 0.10	2.39 ± 0.26*
Median eminence	4.2 ± 1.0	5.9 ± 0.5*

* Values from probenecid-treated animals that are significantly greater (P < .05) than those from vehicle-treated animals.

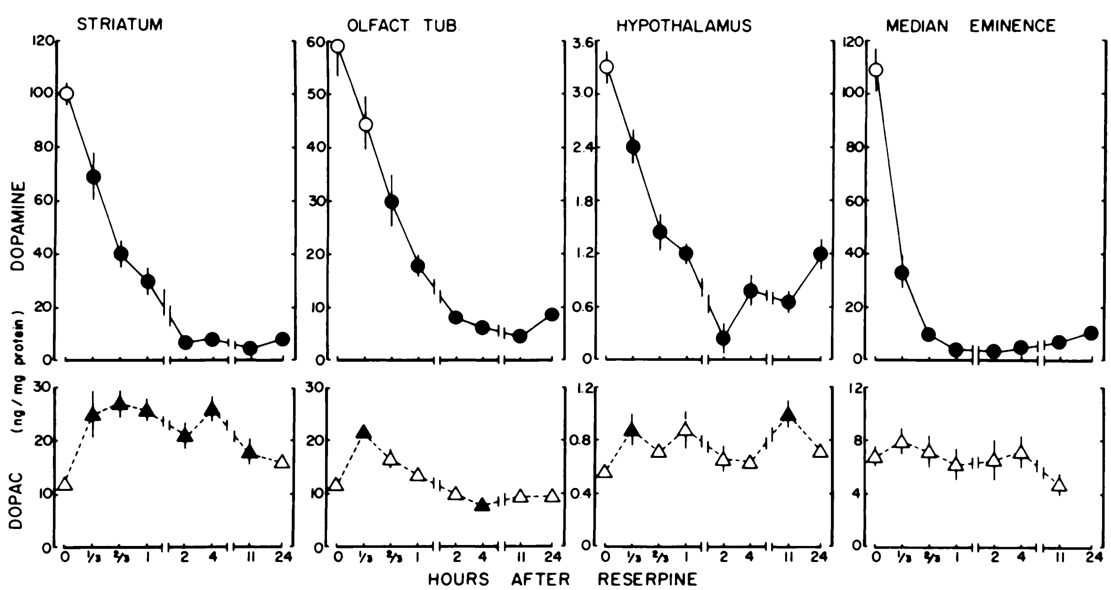

Fig. 5. Time course of effects of reserpine (2 mg/kg i.p.) on dopamine and DOPAC concentrations in various regions of rat brain. Each symbol represents the mean and vertical lines ± 1 S.E. of dopamine (○) and DOPAC (△) concentrations in five to seven animals. Where no S.E. is indicated the value is less than the radius of the symbol. Solid symbols represent those values from reserpine-treated animals that are significantly different (P < .05) from controls.

analysis to brain regions containing relatively large amounts of dopamine and its metabolites, *e.g.*, striatum, olfactory tubercle, nucleus accumbens and cerebral cortex (Roth *et al.*, 1976; Waldmeier and Maitre, 1976; Westerink and Korf, 1976a,b). Gas chromatographic and mass fragmentographic procedures have been developed which permit the quantification of DOPAC in less than milligram amounts of brain tissue (Wilk *et al.*, 1975; Karoum, *et al.*, 1977) or in blood (Bacopoulas *et al.*, 1977). Radioenzymatic procedures have also been developed which are sensitive enough to analyze DOPAC in substantia nigra (Kebabian *et al.*, 1977; Argiolas *et al.*, 1977) and, as described in the present study, in the hypothalamus and median eminence.

The responses of tuberoinfundibular neurons to some drugs resemble those of other dopaminergic neurons in the brain. For example, MAO inhibitors caused a similar increase in dopamine and a decrease in DOPAC concentrations in all brain regions examined. On the other hand, not all dopaminergic neurons responded in the same manner to the administration of dopaminergic antagonists (neuroleptics). Haloperidol and thioridazine did not alter the dopamine concentration in any brain region, but as reported previously (see review, Moore and Kelly, 1978) these drugs increased DOPAC concentrations in the striatum and olfactory tubercle. As demonstrated in figures 2 and 3 these drugs also caused a small but significant increase of DOPAC in the hypothalamus. At no time, however, did they alter the DOPAC concentration in the median eminence. It has been demonstrated previously by histochemical procedures (Fuxe *et al.*, 1975) and by chemical measurements (Gudelsky and Moore, 1977) that although neuroleptics increase the rate of α-methyltyrosine-induced decline of dopamine in the striatum and median eminence, the time course of this effect is different for the two regions. The increased turnover of dopamine in the striatum occurs shortly after the administration of the neuroleptic, whereas this effect is delayed for hours in the median eminence. Furthermore, the neuroleptic-induced increase of dopamine turnover is absent in the median eminence, but not in the striatum, of hypophysectomized rats (Fuxe and Hökfelt, 1974; Gudelsky and Moore, 1977). Since injections of prolactin also cause a delayed increase of dopamine turnover in the median eminence (Hökfelt, and Fuxe, 1972; Gudelsky *et al.*, 1976), it has been postulated that the neuroleptic-induced increase of dopamine turnover in the median eminence is dependent upon the ability of these drugs to increase the plasma concentration of prolactin (Fuxe *et al.*, 1975; Gudelsky and Moore, 1977). This has led to the proposal (for recent discussion see Moore *et al.*, 1978) that dopaminergic neurons comprising the nigrostriatal and mesolimbic system are regulated, in part, by a neuronal feedback loop, whereas tuberoinfundibular neurons are regulated by a hormonal (prolactin-mediated) feedback loop. The latter process involves the action of prolactin on neurons in the medial basal hypothalamus, possibly on the cell bodies of the tuberoinfundibular dopaminergic neurons themselves, such that an increase in the blood concentration of prolactin causes a selective increase in the activity of tuberoinfundibular neurons (Gudelsky *et al.*, 1978). Any procedure that increases blood levels of prolactin (*e.g.*, administration of exogenous prolactin, neuroleptics, estrogen) also increases dopamine turnover in the median eminence but not in other brain regions (Moore *et al.*, 1978). In the present study, the increased activity of tuberoinfundibular neurons, as evidenced by the increased rate of the α-methyltyrosine-induced decline of dopamine, is not reflected in an increased concentration of DOPAC in this region. This is clearly depicted after administration of neuroleptics and also after estradiol benzoate administration. Administration of this hormone increases the α-methyltyrosine-induced decline of dopamine in the median eminence but not in other brain regions (Fuxe *et al.*, 1969; Eikenburg *et al.*, 1977). In the present study estradiol pretreatment clearly did not alter the concentration of DOPAC in the median eminence.

Tuberoinfundibular neurons also differ slightly from other dopaminergic neurons in their response to reserpine. This drug disrupts synaptic vesicles causing stored catecholamines to leak from these protective organelles into the cytoplasm where they are oxidatively deaminated by intraneuronal MAO. It is not unexpected therefore that reserpine would reduce dopamine and increase DOPAC concentrations in the brain. Reserpine caused a rapid decline of dopamine in all four brain regions examined; the rate of dopamine loss was much faster in the median eminence than in the other three regions. Reserpine caused a rapid increase in the DOPAC concentration in the striatum; similar effects have been reported previously. Guldberg and Broch (1971) reported a rapid but transient increase in the DOPAC concentration in the striatum after reserpine, but the results presented in figure 5 and those reported by Roffler-Tarlov *et al.* (1971) and Fadda *et al.* (1977) indicate that the increase of DOPAC is long lasting. This suggests that dopamine continues to be synthesized, possibly at an increased rate (Koe, 1974) and metabolized to DOPAC long after the stored dopamine has been depleted. In the olfactory tubercle and hypothalamus, however, reserpine caused only a transient increase in the DOPAC concentration while in the median eminence the DOPAC concentration was not altered after reserpine.

It has been noted previously that probenecid increases the DOPAC concentration in some brain regions and not in others. For example, several investigators have noted that probenecid does not alter DOPAC concentrations in the striatum but increases this metabolite in the hypothalamus, midbrain and olfactory tubercle (Roffler-Tarlov *et al.*, 1971; Guldberg and Broch, 1971; Karoum *et al.*, 1977; Wilk *et al.*, 1975; Elchisak *et al.*, 1977). The results summarized in table 2 are consistent with these previous reports and indicate that the median eminence can be added to the list of those regions in which a fraction of DOPAC is transported out of the brain by a probenecid-sensitive mechanism. The inability of probenecid to increase DOPAC concentrations in the striatum may reflect the lack of an acid transport system in this brain region. This may account for differences in drug-induced changes in striatum and in the olfactory tubercle and hypothalamus, regions that do appear to have a probenecid-sensitive acid transport system. For example, there was a greater increase of DOPAC after neuroleptics and a more prolonged increase of DOPAC after reserpine in the striatum than in the olfactory tubercle. These differences may merely reflect differences in the rates at which DOPAC can be removed from these brain regions by an acid transport system.

In summary, the present results demonstrate that the effect of drugs on DOPAC concentrations varies in different brain regions. As has been demonstrated previously (Roth *et al.*, 1976) an increased concentration of DOPAC with no change in dopamine appears to reflect an increase in dopaminergic nerve activity, this is especially clear after the administration of neuroleptics. The same relationship appears to hold for the hypothalamus but in the median eminence the DOPAC concentration does not appear to reflect changes in dopamine turnover. Pharmacological and endocrinological manipulations that have been shown previously to increase α-methyltyrosine-induced decline of dopamine in the median eminence are without effect on DOPAC concentration in this brain region.

It is possible that tuberoinfundibular neurons handle DOPAC differently from the other dopaminergic neurons. One obvious difference is that the concentration of DOPAC represented as a percentage of dopamine is lower in the median eminence (5.5%) than in the terminal regions of the other dopamine neurons (12-18.5%, table 1). This could be the result of any one of a number of factors. For example, there may be less MAO activity or less dopamine uptake capability in the terminals of tuberoinfundibular neurons than in terminals of other dopamine neurons. On the other hand, there may be a greater transport of DOPAC out of tuberoinfundibular neurons or a greater conversion of this metabolite to HVA. Unlike other dopaminergic neurons in the central nervous system, the terminals of the tuberoinfundibular neurons do not form synapses, but terminate adjacent to perivascular spaces in the external layer of the median eminence (Ajika and Hökfelt, 1973). It is currently believed that dopamine is released from the tuberoinfundibular neurons directly into the hypophyseal portal blood and transported to the anterior pituitary where it activates receptors on lactotrophs to inhibit the release of prolactin (MacLeod, 1974). If dopamine released from tuberoinfundibular neurons is quickly transported away from the terminals by the blood, there may be little opportunity for a neuronal uptake transport system to capture the released dopamine and thus permit intraneuronal MAO to convert it to DOPAC. In support of this proposal it has been demonstrated recently with *in vitro* experiments that the median eminence has a less effective uptake system for dopamine than other brain regions (M. K. Selmanoff, personal communication).

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