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Mesocortical Dopamine Neurons

Lack of Autoreceptors Modulating Dopamine Synthesis

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SUMMARY

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The regulation of dopamine (DA) synthesis in rat mesocortical DA neurons was studied and compared with DA synthesis in nigrostriatal DA neurons. The increase in striatal DA content caused by γ-butyrolactone (GBL) was reversed by activation of nerve-terminal DA autoreceptors by apomorphine. In contrast, the GBL-induced increase in prefrontal cortical DA was unaffected by DA agonists. By using the accumulation of dopa after the administration of the dopa decarboxylase inhibitor Ro4-4602 as an index of DA synthesis, it was demonstrated that the increase in striatal DA following GBL was due to an acceleration of DA synthesis. In contrast, GBL did not increase cortical dopa accumulation. However, GBL completely prevented the rapid decline of DA seen following α-methyltyrosine treatment, indicating that DA turnover had been inhibited in the mesocortical neurons, as has been previously demonstrated with other DA neurons. The monoamine oxidase inhibitor pargyline increased both striatal and cortical DA levels, leading to greatly attenuated dopa accumulation (tyrosine hydroxylation *in vivo*) in both regions. Finally, the DA antagonist haloperidol greatly accelerated striatal dopa accumulation, but effected only a modest change in cortical dopa accumulation, which was completely prevented by the GBL-induced inhibition of impulse flow. These results suggest that transmitter synthesis in the prefrontal cortical terminals of mesocortical DA neurons is subject to end product inhibition and is regulated by changes in neuronal impulse flow. However, the synthesis of DA in these terminals does not seem to be modulated by DA autoreceptors.

INTRODUCTION

A dopaminergic innervation of the rat cerebral cortex has been identified and characterized (see ref. 1). Much interest has recently focused on this DA[1] system because of differences in the pharmacology of mesocortical DA neurons as compared with other DA systems, e.g., the response to chronic neuroleptic treatment (2, 3).

In the nigrostriatal and mesolimbic DA systems, the influence of DA neurons on postsynaptic follower cells is regulated in part by autoreceptors which modulate DA synthesis (4) and DA release (5). Recently synthesis-modulating autoreceptors have also been identified on tuberohypophyseal DA neuron terminals (6).

The study of these synthesis-modulating autoreceptors *in vivo* has been possible following the administration of GBL, which causes a rapid and reversible inhibition of impulse flow in central DA neurons. This pharmacological lesion produces increased DA levels and accelerated dopa accumulation similar to the effects of mechanical lesioning of DA neurons. DA agonists now exert their neuroleptic-reversible effects on DA synthesis by interacting with kainic acid-insensitive (7) autoreceptors on DA nerve terminals (for a review, see ref. 8). The purpose of the present experiments was to study the regulation of DA synthesis in mesocortical DA neurons with special attention directed to the modulation of synthesis by DA autoreceptors. The simultaneous determination of DA, NE, and dopa in brain extracts was accomplished by using reversed-phase, high-performance liquid chromatography coupled with electrochemical detection (9).

MATERIALS AND METHODS

Animals and dissections. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington,

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[1] The abbreviations used are: DA, dopamine; GBL, γ-butyrolactone; NE, norepinephrine; dopa, dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylacetic acid.

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Mass.) weighing 125–260 g were used in all experiments. Rats were killed by decapitation, the brains were rapidly removed and placed on ice, and the olfactory tubercles were removed as previously described (10). Except in initial pilot experiments, the frontal cortex was then dissected as follows. The brain was placed ventral side up in a Lucite brain mold which had transverse channels at 1-mm intervals. By means of razor blades, a 2-mm section was sliced 2–4 mm from the rostral-most part of the cortex. The posterior plane of the slice corresponded to approximately *A* 10,300 in the atlas of König and Klippel (11). From this slice, a wedge was cut between the forceps minor which included most of the prefrontal cortex DA innervation and excluded the adjacent nucleus accumbens (12). This piece of prefrontal cortex weighed 19.1 ± 0.3 mg.

In an effort to obtain the frontal cortical area most enriched for DA relative to NE, several different cortical dissections were initially attempted. Area 1 dissection is described above. Area 2 was taken from the same brain slices as Area 1, but lateral to the forceps minor and dorsal to the nucleus accumbens (13). Area 3 was a cut identical with Area 1, except that it was 1 mm rostral. Area 4 was a lateral cut corresponding to Area 2, except that it was from the slice taken for Area 3, i.e., 1 mm rostral. For Area 5, a freehand cut, posterodorsal to anteroventral, was made rostral to the striatum and olfactory tubercles. From this cut, the dorsal cortex was dissected free of underlying areas (14). Area 6 consisted of the initial cortical cut made for Area 5, with only the forceps minor dissected out. Following the cortical dissection, the striatum was dissected as previously described (15). The entire dissection procedure was accomplished in less than 4 min. Tissue samples were then frozen at −75° for 1–7 days before processing for biochemical analysis.

Sample preparation. DA, NE, and dopa were isolated using an alumina adsorption method (16) modified for batch elution to allow for smaller elution volumes. Brain samples were homogenized in 2 ml of 15% trichloroacetic acid, and the homogenates were centrifuged at 12,000 × *g* for 10 min. Supernatants were transferred to clean tubes containing 200 μl of 0.2 M EDTA and allowed to reach room temperature. The samples were Vortexed during which 600 μl of a 1:1 mixture of 5 N NaOH and 2 M Tris buffer (pH 9) were added to each sample. The samples were then adjusted to pH 8.4–8.5 with NaOH, immediately poured into screw-cap conical test tubes containing 100 mg of Woelm-grade activated alumina, and mixed. Tubes were mixed a second time and the liquid was aspirated off. The alumina was washed with 10 ml of distilled water, and the water was aspirated off. After this water wash was repeated, the alumina was suspended in 1 ml of water and transferred to 1.5-ml Teflon microfuge tubes. These tubes were centrifuged for 1 min (approximately 900 × *g*), and the water phase was aspirated off. After the addition of 300 μl of 0.8 N perchloric acid, the tubes were thoroughly Vortexed and centrifuged for 1 min, and 200 μl of the eluate were transferred to a clean 1.5-ml microfuge tube. In order to remove perchlorate by precipitation as the potassium salt and to make the samples compatible with the liquid chromatography mobile phase (see below), 35 μl of a 4:3 mixture of a buffer (1 M K_2HPO_4 with 1 mM EDTA and 2 mM sodium octanesulfonate) and 5 N KOH, and 15 μl of methanol were added to each tube while Vortexing. The final pH of samples was 1.5–3. Samples were placed on ice or frozen until assayed. Recoveries of DA, NE, and dopa were initially determined using tritium-labeled compounds and scintillation counting. Routinely, the recovery of exogenous DA, NE, and dopa, which had been added to brain tissue homogenates, was determined (see below) with each group of samples processed. Recoveries varied with alumina batch and storage procedure (see below) but were typically between 60% and 41% for NE, dopa, and DA. All values were corrected for the appropriate recoveries.

Liquid chromatography-electrochemical detection. Samples were assayed using reversed-phase liquid chromatography coupled to electrochemical detection, as previously described (9). Briefly stated, 20-μl aliquots of samples were injected onto Ultrasphere-Octyl columns (Altex Scientific Inc., Berkeley, Calif.) operated at a flow rate of 1 ml/min. The mobile phase consisted of a 0.1 M NaH_2PO_4 solution containing 0.08 mM EDTA and 0.2 mM sodium octanesulfonate (previously filtered and degassed), to which a volume of degassed methanol equal to 4% of the buffer volume was admixed. The final solution was pH 3. LC-3 amperometric controllers equipped with glassy carbon electrodes (Bioanalytical Systems, Inc., West Lafayette, Ind.) were used for electrochemical monitoring of the column eluates, with the output fed to a chart recorder.

Drugs and chemicals. Ro4-4602 (seryltrihydroxybenzylhydrazine; Hoffman-LaRoche, Nutley, N. J.), pargyline HCl (Sigma Chemical Company, St. Louis, Mo.), and *dl*α-methyltyrosine methyl ester HCl (Regis Chemical Company, Morton Grove, Ill.) were dissolved in distilled water. Haloperidol (injectable; McNeil Laboratories, Fort Washington, Pa.), and GBL (Matheson, Coleman and Bell, East Rutherford, N. J.) were diluted with distilled water. Apomorphine HCl (Merck and Company, Rahway, N. J.) was dissolved in 0.1% ascorbic acid, as was bromocriptine (Sandoz Pharmaceuticals, East Hanover, N. J.), with the addition of several drops of acetic acid. Alumina (Aluminum oxide, Woelm neutral, activity grade 1, Alupharm Chemicals, New Orleans, La.) was activated as previously described (17), and stored at 100° in a vacuum oven.

Statistics. Comparisons between experimental groups were made using Student's *t*-test. No significant difference was found between control (untreated or vehicle-treated) groups in the various experiments; these groups were subsequently combined for comparison with drug-treated groups.

RESULTS

Of the various frontal cortical dissections described under Materials and Methods, the wedge of prefrontal cortex (Area 1) was found to be the most enriched for DA relative to NE (data not shown); this dissection was used for all other experiments.

GBL, administered 35 min before the animals were killed, dramatically increased the concentration of DA in

the striatum (Fig. 1a), as previously reported (18, 19). This increase in DA was totally prevented by pretreatment with apomorphine, 2 mg/kg. DA levels in the prefrontal cortex were doubled 35 min after GBL administration (Fig. 1b). However, this increase was unaffected by pretreatment with apomorphine, 2 mg/kg. Bromocriptine, 10 mg/kg, which also reverses the GBL-induced increase in DA synthesis in the striatum (20, 21), had no effect on the DA increase in the cortex. Similarly, 60 min after GBL administration, cortical DA levels were doubled but were unaffected by apomorphine at a dosage of either 0.25 mg/kg or 2 mg/kg (Fig. 1b). NE levels were unaffected by GBL at all times in both prefrontal cortex and striatum (data not shown).

To study the mechanism of the GBL-induced increase in prefrontal cortical DA levels, dopa accumulation after treatment with the dopa decarboxylase inhibitor Ro4-4602 was used as an index of tyrosine hydroxylation *in vivo*. Figure 2 shows a typical experiment in which dopa accumulation in the striatum and prefrontal cortex, as well as in the olfactory tubercle, was linear over the 1 hr period studied. Dopa accumulation in the prefrontal cortex over the time period studied appears to reflect tyrosine hydroxylation primarily in DA neurons. This conclusion is based on the relative depletions of DA versus NE following synthesis inhibition in the prefrontal cortex, both 30 min after Ro4-4602 administration (Table 1) and 55 min after administration of the tyrosine hydroxylase inhibitor α-methyltyrosine (Table 2). Dopa accumulation was significantly increased in the striatum following GBL pretreatment (Table 1). In contrast, dopa accumulation in the prefrontal cortex was unaffected by GBL treatment (Table 1).

Although GBL did not affect prefrontal cortical dopa accumulation, GBL pretreatment did attenuate the α-methyltyrosine (250 mg/kg, 55 min before sacrifice)-induced decline of cortical DA (Table 2). The dramatic increase in DA in the prefrontal cortex following GBL

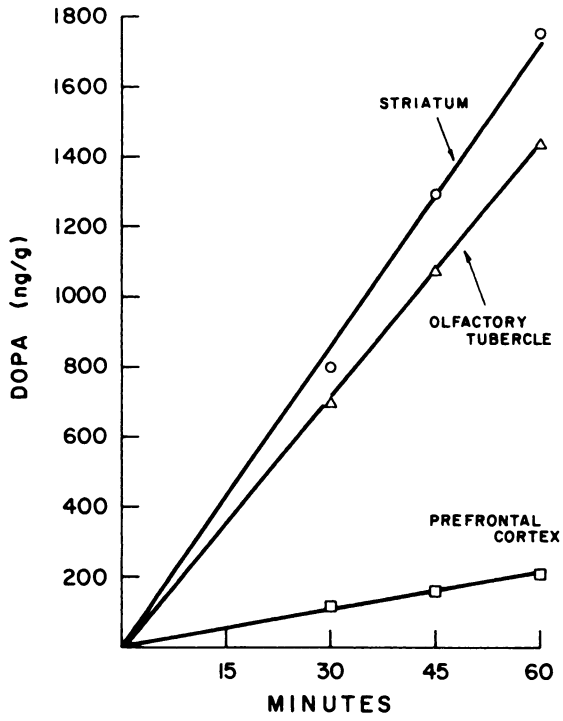

FIG. 2. *Time course of dopa accumulation in the striatum, prefrontal cortex, and olfactory tubercle*

A representative experiment of 60-min dopa accumulation after Ro4-4602 (800 mg/kg, i.p.) administration. Each data point represents the mean value obtained from three or four rats.

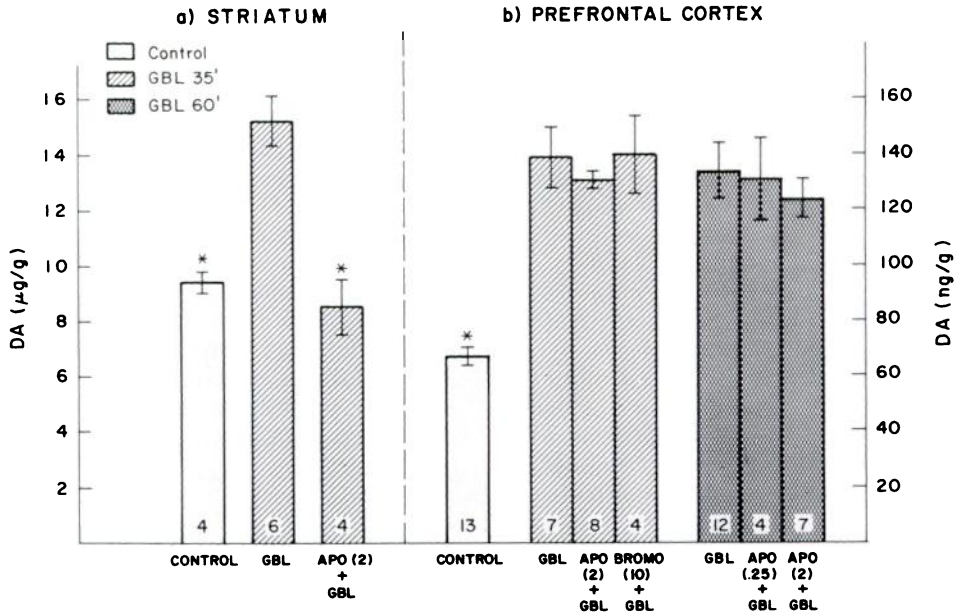

FIG. 1. *Reversal of the GBL-induced increase in DA in the striatum and prefrontal cortex by DA agonists*

GBL (750 mg/kg) was administered either 35 min or 60 min before sacrifice. Apomorphine (APO) was administered 5 min prior to GBL dosing (either 40 min or 65 min before sacrifice). Bromocriptine (*BROMO*) was administered 90 min before sacrifice. Numbers in parentheses indicate the dose of agonist (milligrams per kilogram); all drugs were injected i.p. The *bars* on the columns represent the standard error of the mean and the numbers in the column indicate *N*. *Asterisks* denote DA levels significantly different from animals treated with GBL alone ($p < 0.001$).

TABLE 1

Effect of GBL on dopa accumulation in the striatum and prefrontal cortex

Ro4-4602 (800 mg/kg, i.p.) or vehicle was administered 30 min before sacrifice. In some cases, GBL (750 mg/kg, i.p.) was administered 35 min before sacrifice. Values are means ± standard error of the mean. Numbers in parentheses indicate the *N* for each group.

Brain region	Drug treatment	Dopa	DA	NE
		ng/g	*ng/g*	*ng/g*
Striatum	Vehicle	<10	9,397 ± 400 (4)	111 ± 14 (5)
	Ro4-4602	862 ± 29 (4)	8,053 ± 484 (3)	95 ± 9 (3)
	GBL + Ro4-4602	3,122 ± 214 (4)[a]	12,938 ± 1523 (4)[b]	99 ± 11 (3)
Prefrontal cortex	Vehicle	<25	67 ± 3 (13)	277 ± 15 (13)
	Ro4-4602	150 ± 8 (7)	<20	262 ± 35 (5)
	GBL + Ro4-4602	144 ± 12 (6)	123 ± 17 (5)[c]	307 ± 21 (5)

[a] Dopa accumulation significantly different from that after Ro4-4602 treatment alone ($p < 0.001$).
[b] DA levels significantly different from control values ($p < 0.05$).
[c] DA levels significantly different from control values ($p < 0.001$).

treatment (Fig. 1b) was presumably due to continued DA synthesis while DA release was inhibited. In order to determine further the effects of GBL-induced increases in DA levels on tyrosine hydroxylation *in vivo,* GBL was administered 90 min before sacrifice and Ro4-4602 injected 60 min later (30 min before sacrifice). Although DA levels were doubled as compared with those of control rats (see Table 1), dopa accumulation was unaffected (Table 3). In contrast, the monoamine oxidase inhibitor pargyline (75 mg/kg), given 3 hr before sacrifice, increased prefrontal cortical and striatal DA levels by 43% and 26%, respectively, and dramatically reduced the 30-min accumulation of dopa in both the prefrontal cortex (Table 3) and the striatum (Table 3), as previously reported (18, 19).

Finally, the effect of the DA antagonist haloperidol on dopa accumulation in DA neurons with intact impulse flow was assessed. As previously reported (6, 19), haloperidol (1 mg/kg, 45 min prior to sacrifice) greatly accelerated striatal 30-min dopa accumulation (Fig. 3). This increase was only slightly attenuated by the administration of GBL 35 min before sacrifice. In prefrontal cortex, a smaller but significant increase in dopa accumulation was observed (Fig. 3). This increase was completely prevented when the impulse flow in the mesocortical DA system was inhibited by administration of GBL.

DISCUSSION

As previously reported (18, 19), GBL administration 35 min before sacrifice caused an apomorphine-reversible increase in striatal DA levels (Fig. 1a). GBL also increased prefrontal cortical DA levels (Fig. 1b); this increase was not reversed by apomorphine, 2 mg/kg or by bromocriptine [another DA autoreceptor agonist (20, 21)], 10 mg/kg. These supramaximal doses of agonist completely reverse the GBL-induced increase in DA levels or dopa accumulation in the striatum (18, 21). Similarly, 60 min after GBL administration, the increased cortical DA levels observed were not affected by apomorphine at a dosage of either 0.25 mg/kg or 2 mg/kg (Fig. 1b). Higher doses of apomorphine caused a significant direct inhibition of striatal and cortical tyrosine hydroxylation *in vivo.*[2] In contrast to our data, Pericic and Walters (22) reported that the increase in prefrontal cortical DA which they observed 60 min after GBL administration was reversed by apomorphine pretreatment (2 mg/kg). The reason for this discrepancy is unclear at present.

In order to study further the effect of GBL on mesocortical DA neurons, prefrontal cortical dopa accumulation after GBL was assessed. On the basis of the relative depletions of cortical DA versus NE after synthesis inhibition (Tables 1 and 2), prefrontal cortical dopa accumulation was thought to reflect tyrosine hydroxylation primarily in DA neurons. This is further supported by experiments conducted 30 min after i.v. α-methyltyrosine in which the prefrontal cortical DA content was significantly reduced (53 ± 6% control) whereas NE levels remained unaltered (114 ± 10% control).[3] Although GBL significantly increased striatal dopa accumulation, cortical dopa accumulation was unaffected (Table 1).

Electrophysiological experiments have shown that GBL administration causes cessation of impulse flow both in DA neurons which originate in the substantia nigra (nigrostriatal DA neurons; ref. 18) and in DA cells which originate in the ventral tegmental area (mesocortical and mesolimbic DA neurons[4]). The attenuation of the α-methyltyrosine-induced decline in prefrontal cortical DA by GBL (Table 2) provides additional biochemical evidence that GBL inhibits impulse flow in the mesocortical DA neurons. The decrease in synaptic DA following GBL-induced cessation of impulse flow results in decreased DA autoreceptor modulation of DA synthesis, with a resulting increase in dopa accumulation and DA levels in the striatum and olfactory tubercle (8).

TABLE 2

Effect of GBL on α-methyltyrosine-induced decline of prefrontal cortical DA

GBL (750 mg/kg, i.p.) was administered 60 min before sacrifice. α-Methyltyrosine (250 mg/kg, i.p.) was injected 55 min before sacrifice. Values are means ± standard error of the mean. Control values for DA and NE were 67 ± 3 ng/g and 277 ± 15 ng/g, respectively, and are taken from Table 1. Numbers in parentheses indicate *N*.

Drug treatment	DA, % control	NE, % control
Vehicle	100 ± 5 (13)	100 ± 5 (13)
α-Methyltyrosine	<30	80 ± 4 (4)[a]
GBL + α-methyltyrosine	103 ± 6 (4)	85 ± 8 (4)[a]

[a] NE levels significantly different from control ($p < 0.05$).

[2] M. J. Bannon and R. H. Roth, unpublished observations.
[3] M. J. Bannon, E. B. Bunney, and R. H. Roth, in preparation.
[4] M. C. Nowycky and R. H. Roth, unpublished observations.

TABLE 3

Effects of a monoamine oxidase inhibitor and GBL on DA levels and dopa accumulation in the striatum and prefrontal cortex

Ro4-4602 (800 mg/kg, i.p.) was administered 30 min before sacrifice. GBL (750 mg/kg, i.p.) was administered 90 min before sacrifice. Pargyline (75 mg/kg, i.p.) was injected 180 min prior to sacrifice. Values are means ± standard error of the mean. Numbers in parentheses indicate *N*.

Brain region	Drug treatment	DA	Dopa
		ng/g	*ng/g*
Prefrontal cortex	Ro4-4602[a]	<20	150 ± 8 (7)
	GBL (90 min) + Ro4-4602	133 ± 13 (5)	155 ± 4 (4)
	Pargyline (180 min) + Ro4-4602	96 ± 18 (4)	21 ± 2 (4)[b]
Striatum	Ro4-4602[a]	8,053 ± 483 (3)	862 ± 29 (4)
	Pargyline (180 min) + Ro4-4602	11,794 ± 603 (4)	221 ± 24 (4)[b]

[a] Values taken from Table 1.

[b] Dopa accumulation significantly different from that after Ro4-4602 alone ($p < 0.001$).

Recently, however, it has been reported (6) that although GBL apparently blocks impulse flow in tuberoinfundibular DA neurons, dopa accumulation is not apomorphine-sensitive; i.e., the median eminence DA terminals lack autoreceptors modulating DA synthesis. A similar situation seems to exist in the prefrontal cortex of the rat. It should be noted that the present experiments have not ruled out the possibility of cortical DA autoreceptors which modulate only DA release, as the regulation of DA synthesis and release seem to be largely independent (see ref. 23).

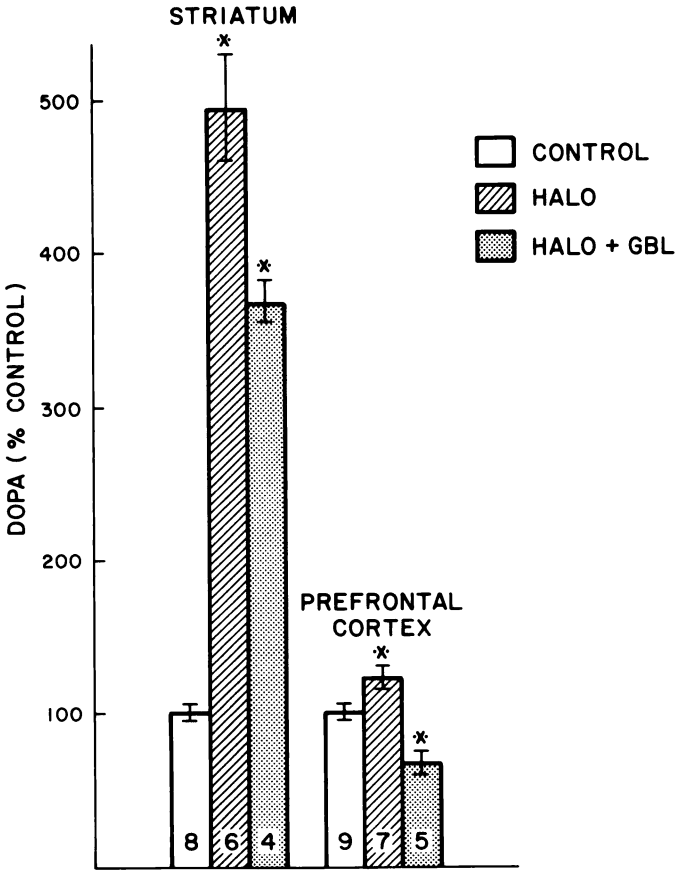

FIG. 3. *Effect of haloperidol on striatal and prefrontal cortical dopa accumulation.*

Ro4-4602 (800 mg/kg, i.p.) was administered to all rats 30 min before sacrifice. GBL (750 mg/kg, i.p.) was injected 35 min before sacrifice. Haloperidol (*HALO*; 1 mg/kg, i.p.) was administered 45 min before sacrifice. The *bars* on the columns represent the standard error of the mean; the numbers in the columns indicate *N*. Values are expressed as percentage of control. Control dopa values were 784 ± 48 ng/g for the striatum and 144 ± 7 ng/g for the cortex. *Asterisks* denote dopa accumulation significantly different from that after Ro4-4602 alone ($p < 0.05$).

The continuation of DA synthesis along with the inhibition of DA release is presumably the cause of the increase in cortical DA seen following GBL treatment (Fig. 1b). Cortical dopa accumulation was unaltered in animals in which DA levels were elevated by GBL (Tables 1 and 3). Striatal dopa accumulation of near-control values has been previously reported in rats pretreated with GBL 90 min before sacrifice (18, 19). Although GBL-induced increased cortical DA does not affect dopa accumulation, the monoamine oxidase inhibitor pargyline dramatically depresses cortical and striatal dopa accumulation (Table 3), indicating that end product inhibition is a regulatory mechanism in these neurons. Similar effects of monoamine oxidase inhibitors are seen in every DA system studied (6, 18, 19).

Administration of the DA antagonist haloperidol greatly accelerated striatal dopa accumulation (Fig. 3), as previously reported (6, 19). In contrast, a very modest increase in prefrontal cortical dopa accumulation was seen. These results are consistent with data previously obtained in primates (24). DA antagonists can influence dopaminergic function through multiple sites of action (8). The haloperidol-induced increase in striatal dopa accumulation is presumably due to (a) the blockade of post-synaptic DA receptors with subsequent activation of DA neurons through neuronal feedback mechanism, and (b) the blockade of terminal DA autoreceptors. GBL only somewhat attenuated the effect of haloperidol on striatal dopa accumulation; although it blocks the impulse flow-mediated effects of haloperidol, like haloperidol, GBL accelerates dopa accumulation by preventing the stimulation by DA of striatal DA autoreceptors. As the haloperidol-induced increase in prefrontal cortical dopa accumulation was completely prevented by the elimination of impulse flow by GBL (Fig. 3), the haloperidol effect appears to have been mediated by a neuronal feedback mechanism. The fact that GBL did not accelerate cortical dopa accumulation is evidence of the lack of synthesis-modulating autoreceptors, as discussed above.

The dopaminergic innervation to the cerebral cortex has been well characterized (1). However, it is at present

unclear whether the mesolimbic and mesocortical DA systems, both of which originate primarily within the ventral tegmental area, constitute distinct neuronal populations. It has been shown that, in response to either lesioning of a ventral tegmental area input from the habenula or to electric foot-shock stress, the mesocortical system is activated (an increase in the cortical DOPAC to DA ratio is seen) whereas the mesolimbic system is not (25, 26). This difference may be explained in part by the absence of cortical synthesis-modulating nerve-terminal DA autoreceptors which would tend to dampen DA release (DOPAC/DA) for a given impulse flow. Similarly, if tolerance to chronic neuroleptic treatment is due to the development of receptor supersensitivity (27), including autoreceptor supersensitivity (7, 28, 29), then the relative lack of mesocortical DA system tolerance relative to the mesolimbic and nigrostriatal systems (2, 3) may also be explained in part by the absence of autoreceptors.

In conclusion, the terminals of mesocortical DA neurons projecting to the rat prefrontal cortex lack synthesis-modulating DA autoreceptors. In addition, these neurons appear to have a fast DA turnover rate, to have a modest response to neuroleptic-induced increase in impulse flow, and to exert control over DA synthesis by normal end product inhibition.

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