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Dopaminergic Neurons: Effects of Electrical Stimulation on Dopamine Biosynthesis

L. CHARLES MURRIN AND ROBERT H. ROTH

Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06520

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SUMMARY

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Electrical stimulation of the medial forebrain bundle was employed in order to study the effects of increased impulse flow on dopamine synthesis. Stimulation at frequencies ranging from 5 to 30 Hz resulted in an increase in the specific activity of dopamine isolated from the neostriatum 20 min after intravenous administration of [^{3}H]tyrosine. The optimal frequency for this effect was 15 Hz. Similar results were found in the olfactory tubercles. Phenylalanine was also found to be a precursor for neostriatal dopamine. The increase in neostriatal dopamine specific activity following labeled precursor administration continued for 15 min after stimulation. About 99% of the total fraction of labeled catecholamines found in the neostriatum 20 min following tyrosine administration was identified as dopamine by Amberlite CG-120 column chromatography. Dopa accumulation following dopa decarboxylase inhibition was also increased by electrical stimulation. This increased accumulation of dopa continued for at least 15 min after stimulation. These data are discussed in terms of the ability of increased impulse flow to increase dopamine synthesis by altering the activity of tyrosine hydroxylase in dopaminergic neurons.

INTRODUCTION

It has been appreciated for many years that increases in impulse flow in the mammalian sympathetic nervous system produced by electrical stimulation lead to stimulus-dependent increases in norepinephrine release (1-3) and synthesis (4-8). The increase observed in norepinephrine synthesis has been shown to be mediated through an increase in the activity of tyrosine hydroxylase (5, 8), the rate-limiting enzyme (9). Similarly, increases in impulse flow in several central monoaminergic neurons due to electrical stimulation have been shown to result in an increase in transmitter release and metabolism. This appears to be the case for central serotonergic (10-14), noradrenergic (15-17), and dopaminergic neurons (18-22).

It has been shown by several investigators that antipsychotic drugs increase the accumulation of dopamine metabolites (23-25) and increase dopamine synthesis and turnover (26-31) in the neostriatum. Similar results were reported in the olfactory tubercles (24, 32). Carlsson and Lindqvist (23) suggested that this was due to the ability of antipsychotic drugs to

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block postsynaptic dopamine receptors and thus bring about a compensatory increase in the rate of firing of the dopaminergic neurons via a neuronal feedback loop. This idea is supported by the findings that lesions of the nigro-neostriatal pathway block these drug effects (33–35) and that the antipsychotic drugs do bring about an increase in the rate of firing of the dopaminergic cell bodies in the substantia nigra (36).

Nevertheless, there has been no direct study of the effects of a controlled increase in impulse flow in the dopaminergic neurons on dopamine metabolism. In this study we have investigated the effects of stimulation of central dopaminergic neurons upon dopamine synthesis. Part of this work has been presented previously in abstract form (37).

METHODS

Electrical stimulation experiments. Male Sprague-Dawley rats (Charles River Laboratories) weighing 220–300 g were anesthetized with chloral hydrate (400 mg/kg intraperitoneally) and were placed in a David Kopf stereotaxic holder. A small burr hole was drilled in the skull, and a coaxial electrode (NE-100, David Kopf Instruments) was lowered into the brain so that the position of the electrode tip corresponded approximately to the coordinates A 2800 μm, L 1.4 mm, V −3.0 mm in the König and Klippel rat atlas (38). Square-wave electrical pulses were delivered from a BBS-1 electrical stimulator (Electronic Instruments, Inc., Northford, Conn.). Pulses were 200 μamp (1.5-msec duration monophasic) or 400 μamp (3.0-msec duration biphasic) unless otherwise noted. Similar results were obtained with monophasic and biphasic pulses. Frequency and length of stimulation were varied as noted. Stimulation parameters were continuously monitored by means of an oscilloscope. Stimulation parameters used were considerably less than those necessary to produce tissue damage (39), and no evidence of tissue damage was found. At the end of the stimulations the brain sections needed for assay were rapidly dissected, frozen on Dry Ice, weighed, and stored at −20°.

The olfactory tubercles were dissected as follows. The olfactory bulb was removed, usually in the process of removing the brain from the skull cavity, and the olfactory tract anterior to the olfactory tubercles was removed. The olfactory tubercles were dissected with pointed spatulas, using the tractus olfactorius lateralis as the lateral boundary, the anterior commissure (pars anterior) as the dorsal boundary, and the midline as the medial boundary. The posterior boundary was defined by a line just anterior to the optic chiasm. This dissection included a small portion of the nucleus accumbens. The average wet weight of the olfactory tubercles was 15.8 ± 0.4 mg (n = 22). The neostriata were dissected as described by Bunney *et al.* (36). In some experiments two or more neostriata or olfactory tubercles were pooled for assay. Stimulated tissues were always compared with the contralateral unstimulated tissues. Brain stems were fixed in 5% glutaraldehyde, sectioned, stained with cresyl violet, and examined by light microscopy to verify electrode placement.

Purification of [^{3}H]tyrosine and [^{3}H]-phenylalanine. L-[3′,5′-^{3}H]Tyrosine (50 Ci/mmole; New England Nuclear Corporation) and L-[2,3-^{3}H]phenylalanine (40 Ci/mmole; Schwarz/Mann) were purified over alumina columns as described by Boadle-Biber *et al.* (40) to remove catechol contaminants. The column effluent was adjusted in volume to give the desired amount of radioactivity, usually about 200 μCi/0.8 ml. Tyrosine and phenylalanine were injected via the tail vein immediately or 2 min before the beginning of electrical stimulation.

Endogenous dopamine assay. Tissues were homogenized in 2 ml of 15% trichloracetic acid, the homogenizer was washed with 1 ml of trichloracetic acid, and the wash was combined with the homogenate. The samples were centrifuged for 20 min at 12,000 × g and 4°. Dopamine was isolated from the supernatant by the alumina column technique of Boadle-Biber *et al.* (40) and assayed fluorometrically according to a modification of the method of Laverty and Taylor (41) as described by Walters and Roth (42).

Tyrosine assay. The sample eluate plus

the first 10 ml of H_2O wash from the alumina column procedure for isolating dopamine were saved for assay of tyrosine. Tyrosine was isolated from this sample by means of a Dowex 50W-X4 cation-exchange resin column and assayed as described by Walters and Roth (42).

Dopa accumulation experiments. In these experiments the dopa decarboxylase inhibitor Ro 4-4602 (N^1-*dl*-seryl-N^2-(2,3,4)-trihydroxybenzylhydrazine HCl) was administered in a dosage of 800 mg/kg intraperitoneally. Dopa was isolated and assayed according to Kehr *et al.* (43) with the modifications of Walters and Roth (44).

Separation of dopamine and norepinephrine. Dopamine and norepinephrine were separated according to a modification of the method of Stjärne and Lishajko (45). Amberlite CG-120, 200–400 mesh (Mallinckrodt) was washed with 2 N NaOH, resuspended in distilled H_2O, and washed thoroughly to remove fine particles. It was then resuspended in 3 N HCl overnight, washed thoroughly the next day with H_2O, dried at 70°, and stored until use. At the time of the assay, the resin was washed three times with 80–100 ml of H_2O and resuspended in H_2O, and 16 × 0.4 cm columns of the resin were prepared. The columns were washed overnight with 1.0 M sodium acetate buffer, pH 6.0. The next day the columns were resuspended in H_2O and washed with H_2O for 1 hr, and the column height was then adjusted to 14 cm. Tissue samples were prepared as for the isolation of dopamine. Aliquots of alumina column eluates were taken so that they contained at least 300–500 cpm. To each sample were added 50 μg of norepinephrine and 100 μg of dopamine as nonradioactive carriers. The sample was adjusted to pH 4–5 with 0.5 N KOH. The samples were then chilled for several minutes and centrifuged at low speed to remove any $KClO_4$ precipitate. All samples to be compared were adjusted to approximately the same volume with H_2O and then applied to the columns. When the sample had run onto the column, the column was washed with 5 ml of H_2O, followed by 5 ml of 1.0 M sodium acetate buffer, pH 4.0, and then two 5-ml H_2O washes. After the last wash the column was filled with H_2O and gradient elution was started. The mixer contained 25 ml of 0.25 N HCl, and the reservoir contained 100 ml of 2.0 N HCl. Fractions of 2 ml were collected, at a flow rate adjusted to about 2 ml/15 min. Fractions were assayed for native fluorescence, with activation at 285 nm and emission at 335 nm (uncorrected).

Radioactivity determinations. Radioactivity was determined by counting in a Packard Tri-Carb liquid scintillation counter. The scintillation fluids used were Aquasol (New England Nuclear Corporation); 1 liter each of toluene, ethanol, and dioxane plus 240 g of naphthalene, 15 g of 2,5-diphenyloxazole, and 0.3 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene; and 2 liters of toluene, 1 liter of Triton X-100, 5.5 g of 2,5 diphenyloxazole, and 0.3 g of 1,4 - bis[2 - (4 - methyl - 5 - phenyloxazolyl)] - benzene. All scintillation fluids were checked for efficiency using internal and external standards, and results were corrected for efficiency unless otherwise noted.

Statistics. Statistical comparisons were made using Student's *t*-test (46).

RESULTS

Effect of stimulation frequency on dopamine specific activity. Electrical stimulation of the nigro-neostriatal pathway for 20 min after intravenous administration of [^{3}H]tyrosine caused an increase in the specific activity of the dopamine isolated from the ipsilateral neostriatum (Table 1). A maximal increase of 88% in the incorporation of label from [^{3}H]tyrosine into dopamine was obtained at a frequency of 15 Hz. The increased specific activity of dopamine observed in stimulated neostriata was significantly different, at all frequencies tested, from the specific activity of dopamine in the contralateral unstimulated neostriata. Sham stimulations were carried out by lowering the electrode into position for 20 min without passing any current. Sham stimulations produced no significant increase in dopamine specific activity.

Stimulation of the zona compacta of the substantia nigra produced a similar increase in the total activity of the dopamine fraction from the neostriatum. In these

experiments the electrode tip was situated about midway between the lateral and medial edges of the zona compacta and just dorsal to the cell bodies. Twenty-minute stimulations at 15 Hz with 400 μamp of monophasic current produced a 52% increase in total activity of the dopamine fraction isolated from stimulated neostriata compared with contralateral unstimulated neostriata.

Stimulations as described resulted in at least partial stimulation of the mesolimbic dopaminergic neurons. Assay of the dopamine fraction isolated from olfactory tubercles showed a stimulus-induced increase in total catechol radioactivity following stimulation (Table 2), similar to results seen in the neostriatum. A maximum increase of 69% in total activity was seen following 20 min of stimulation at 15 Hz. Again, sham stimulations produced no significant change in total activity of the dopamine fraction.

Stimulation of the nigro-neostriatal pathway had no effect on the wet weight of the neostriatum or the endogenous levels of dopamine and tyrosine (Table 3). Moreover, stimulation caused no increase in the specific activity of tyrosine on the stimulated side, and in some cases produced a decrease in tyrosine specific activity (Table 4).

Phenylalanine as a precursor for dopamine. The possibility that phenylalanine

TABLE 1

Effect of stimulation frequency on dopamine specific activity in rat neostriatum

The medial forebrain bundle was stimulated for 20 min as described in METHODS with monophasic pulses of 1.5-msec duration and 200 μamp, frequency as noted. Stimulations were begun 2 min after injection of 200 μCi of [^{3}H]tyrosine via the tail vein. Values are the means and standard errors of the number of samples shown.

Frequency	Specific activity		*n*	Change[a]
	Control	Stimulated		
Hz	*dpm/ng dopamine*			%
5	4037 ± 455	6389 ± 467[b]	5	+62 ± 8
10	3510 ± 179	5861 ± 555[b]	6	+63 ± 15
15	4179 ± 614	7916 ± 1261[b]	4	+88 ± 8
30	4782 ± 429	8554 ± 580[b]	6	+82 ± 10
Sham	4625 ± 1314	4712 ± 1372[c]	3	+1 ± 8

[a] Change of each stimulated sample compared with its contralateral control.
[b] Significantly different from control; paired *t*-test, $p < 0.01$.
[c] Not significantly different from control.

TABLE 2

Effect of stimulation frequency on total activity in dopamine fraction from olfactory tubercles

The medial forebrain bundle was stimulated for 20 min as described in METHODS with monophasic pulses of 1.5-msec duration and 200 μamp, frequency as noted. Stimulations were begun 2 min after injection of 200 μCi of [^{3}H]tyrosine via the tail vein. Values are the means and standard errors of the number of samples shown.

Frequency	Total activity		*n*	Change[a]
	Control	Stimulated		
Hz	*dpm/tubercle*			%
5	1779 (941; 2617)	2121 (942; 3300)	2	+13 (0; 26)
10	1107 ± 126	1159 ± 158[b]	6	+5 ± 5
15	352 ± 62	548 ± 90[c]	7	+69 ± 16
20	296 ± 90	481 ± 133[c]	4	+65 ± 14
30	1187 ± 234	1675 ± 434[b]	3	+38 ± 8
Sham	631 ± 62	615 ± 49[b]	12	0 ± 4

[a] Change of each stimulated sample compared with its contralateral control.
[b] Not significantly different from control; paired *t*-test.
[c] Significantly different from control; paired *t*-test, $p < 0.05$.

TABLE 3

Effect of stimulation on neostriatal weight and endogenous dopamine and tyrosine levels

The nigro-neostriatal pathway was stimulated as described in METHODS for 20 min with 200-μamp monophasic electrical pulses of 1.5-msec duration and various frequencies. Dopamine and tyrosine were assayed as described in METHODS. Values are the means and standard errors of the number of samples shown.

Neostriata	Wet weight	*n*	Dopamine	*n*	Tyrosine	*n*
	mg		*μg/g, wet wt*		*μg/g, wet wt*	
Control	58 ± 1	20	10.1 ± 0.3	20	16.3 ± 1.3	7
Stimulated	57 ± 1	20	10.5 ± 0.4	20	15.7 ± 1.3	7

TABLE 4

Effect of stimulation frequency on tyrosine specific activity in neostriatum

The nigro-neostriatal pathway was stimulated for 20 min as described in METHODS with monophasic pulses of 1.5-msec duration and 200 μamp, frequency as noted. Stimulations were begun 2 min after injection of 200 μCi of [^{3}H]tyrosine via the tail vein. Values are the means and standard errors of the number of samples shown.

Frequency	Specific activity		*n*	Change[a]
	Control	Stimulated		
Hz	*dpm/μg tyrosine*			%
5	15,469 ± 4,337	14,277 ± 4,220[b]	4	−9.4 ± 3.5
10	16,539 ± 4,174	15,283 ± 4,004[c]	7	−8.9 ± 4.9
30	15,888 ± 3,521	11,201 ± 2,585[b]	5	−30.0 ± 3.7

[a] Change of tyrosine specific activity in stimulated neostriata compared with the contralateral controls.
[b] Significantly different from control; paired *t*-test, $p < 0.025$.
[c] Not significantly different from control; paired *t*-test.

could serve as a precursor for dopamine was examined by injecting 350 μCi of [^{3}H]phenylalanine via the tail vein immediately before beginning stimulation. It was found that phenylalanine could serve as a precursor for dopamine in the neostriatum (Table 5). Stimulation of the nigro-neostriatal pathway for 20 min at 15 Hz produced a significant increase (+138%) in the total radioactivity of the dopamine fraction found in the ipsilateral neostriatum after phenylalanine administration.

Identity of catecholamines in dopamine fraction. Since the dopamine fraction isolated from neostriata as described in METHODS would also contain norepinephrine, we examined the composition of the dopamine fraction using an Amberlite CG-120 column technique. Animals were stimulated for 20 min at 15 Hz after an intravenous injection of 200–350 μCi of [^{3}H]tyrosine. Using the chromatographic procedure described in METHODS, norepinephrine and dopamine were clearly separated (Fig. 1). In control preparations it was found that dopamine accounted for 98.9% ± 0.4% of the radioactivity in the catecholamine peaks while norepinephrine accounted for 1.27% ± 0.4% of the radiolabel. Stimulation produced no change in the percentage of label in the two fractions compared with control preparations.

When [^{3}H]phenylalanine was used as precursor, the dopamine peak from control preparations was found to contain 80% of the catecholamine label while norepinephrine contained 20% (Table 5). In calculating these values, the counts per minute in the norepinephrine fraction were doubled on the assumption that conversion of dopamine to norepinephrine resulted in the loss of one-half the label from the [1,2-^{3}H]dopamine that had been formed. Electrical stimulation of the nigro-neostriatal pathway produced an increase in radioactivity only in the dopamine peak (Fig. 2 and Table 5), so that in stimulated preparations the dopamine fraction contained 93% of the total radioactivity while the

TABLE 5

Phenylalanine as precursor of dopamine in neostriatum, and effect of stimulation

The nigro-neostriatal pathway was stimulated as described in METHODS for 20 min with 400-μamp biphasic pulses of 3.0-msec duration and 15 Hz immediately following injection of 350 μCi of [^{3}H]phenylalanine via the tail vein. The dopamine fraction was isolated by alumina column chromatography as described in METHODS. Aliquots from seven dopamine fractions were combined to give the control and stimulated samples analyzed by the Amberlite CG-120 chromatographic procedure described in METHODS. Values are the means and standard errors (where applicable) of the number of samples shown.

Treatment	Dopamine (n = 12)	Total activity in alumina column fractions (n = 1)	
		Norepinephrine[a]	Dopamine
	dpm/mg, wet wt	*cpm*	*cpm*
Control	24.1 ± 2.0	44.8 (20%)	176.8 (80%)
Stimulated	55.5 ± 7.2[b]	42.4 (7%)	520.7 (93%)
Change[c]	+138% ± 26%	−5%	+195%

[a] Norepinephrine values were corrected for ^{3}H loss as described in the text.

[b] Significantly different from control; $p < 0.0005$.

[c] Change of stimulated side compared with its contralateral control.

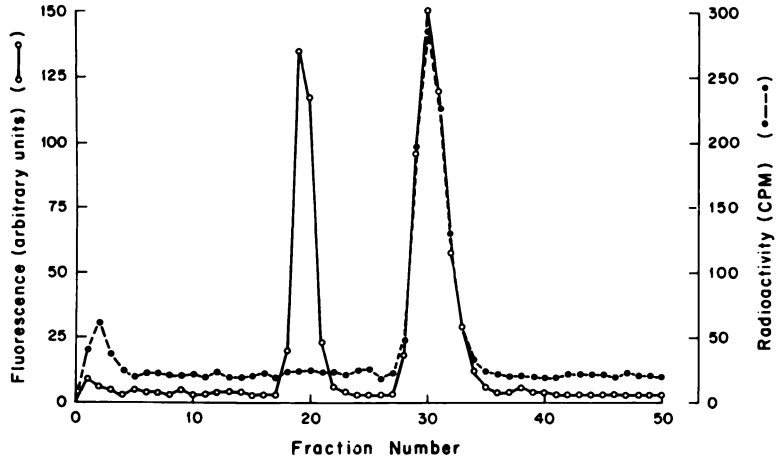

FIG. 1. *Amberlite CG-120 chromatography of dopamine fraction from neostriatum with [^{3}H]tyrosine as precursor*

Immediately following injection of 200–350 μCi of [^{3}H]tyrosine via the tail vein, the nigro-neostriatal pathway was stimulated as described in METHODS for 20 min with 15-Hz, 400-μamp biphasic pulses of 3.0-msec duration. At the end of stimulation the animals were killed, and the dopamine fraction isolated from the neostriata by alumina column chromatography was further chromatographed on Amberlite CG-120 resin as described in METHODS. As nonradioactive carriers, 50 μg of norepinephrine and 100 μg of dopamine were added to each sample. The graph is the result of one experiment with the control neostriata pooled from three rats. ○——○, fluorescence of each fraction in arbitrary units, with the first peak (fractions 17–22) representing norepinephrine and the second peak (fractions 27–35) representing dopamine; ●- - -●, counts per minute (uncorrected) of the fractions.

norepinephrine fraction contained 7%.

Time courses of increase in dopamine specific activity due to stimulation. The specific activity of dopamine increased linearly for at least 30 min following intravenous injection of [^{3}H]tyrosine (Fig. 3). The dopamine specific activity at each time point in control preparations was significantly different from the dopamine specific activity at the other time points (p <0.025). Electrical stimulation at 15 Hz produced a significant increase in dopamine specific activity for up to 20 min (Fig. 3). The specific activities of dopamine in

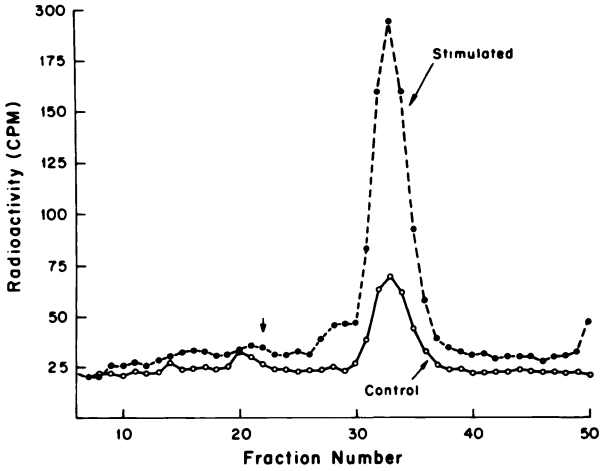

FIG. 2. *Amberlite CG-120 chromatography of radioactivity in dopamine fraction from neostriatum with [^{3}H]phenylalanine as precursor*

Immediately following the injection of 350 μCi of [^{3}H]phenylalanine via the tail vein, the nigro-neostriatal pathway was stimulated as described in METHODS for 20 minutes with 15-Hz, 400-μamp biphasic pulses of 3.0-msec duration. At the end of stimulation the animals were killed and the alumina column dopamine fraction of the neostriata was chromatographed on Amberlite CG-120 as described in METHODS. As nonradioactive carriers, 50 μg of norepinephrine and 100 μg of dopamine were added to each sample. Lines represent counts per minute (uncorrected) of fractions from stimulated (●- - -●) and the contralateral unstimulated (O——O) neostriata. Each line is the result of Amberlite CG-120 chromatography of one sample made up from seven neostriata. The arrow points to the peak of the norepinephrine fluorescence in both samples (fraction 22). The peak of the dopamine fluorescence coincided with the peak of radioactivity at fraction 33 in both samples.

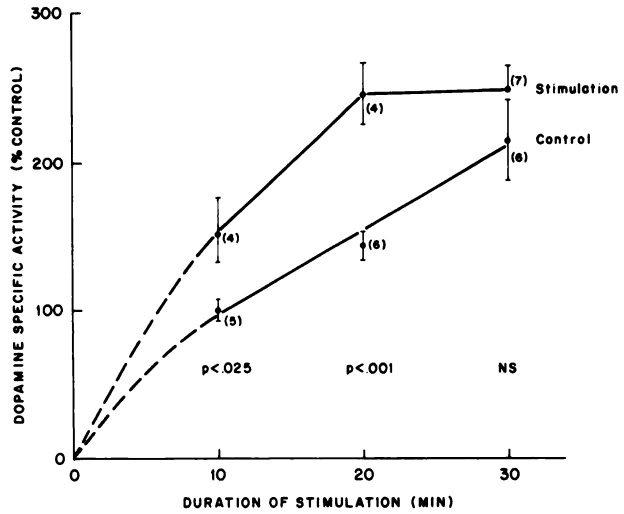

FIG. 3. *Effect of stimulation time on dopamine specific activity in neostriatum*

The nigro-neostriatal pathway was stimulated as described in METHODS for various lengths of time with biphasic pulses of 3.0-msec duration, 400 μamp, 15 Hz. Stimulations were begun immediately following injection of 200 μCi of [^{3}H]tyrosine via the tail vein. Dopamine specific activities (disintegrations per minute per nanogram of dopamine) are expressed as percentage of the dopamine specific activity of the 10-min control (4595 ± 415 dpm/ng of dopamine; n = 4). Numbers in parentheses refer to the number of samples. p values resulted from comparison of stimulated and control samples at each time point. NS, not significantly different. Control line linear regression analysis: r^2 = 0.9828.

TABLE 6

Dopamine specific activity in neostriatum after stimulation

The nigro-neostriatal pathway was stimulated as described in METHODS for 20 min with biphasic pulses of 3.0-msec duration, 400 μamp, 15 Hz. Immediately after the end of stimulation 200 μCi of [^{3}H]tyrosine were injected via the tail vein. The animals were decapitated 15 and 30 min after stimulation, and the neostriata were assayed for dopamine as described in METHODS. Values are the means and standard errors of the number of samples shown.

Time after Stimulation	Dopamine specific activity		*n*	Change[a]
	Control	Stimulated		
min	*dpm/ng dopamine*			%
15	6010 ± 930	9490 ± 380[b]	4	+70 ± 28
30	5690 ± 750	7770 ± 1020[c]	4	+42 ± 28

[a] Change of each stimulated sample compared with its contralateral control.

[b] Significantly different from control; $p < 0.01$.

[c] Not significantly different from control.

preparations stimulated for 10 and 20 min were significantly different from each other ($p < 0.025$). Stimulation for a total of 30 min produced no further significant increase in dopamine specific activity compared with 20 min of stimulation.

Post-stimulation increase in dopamine specific activity. In some experiments a 15-Hz stimulation was applied to the nigro-neostriatal pathway for 20 min, immediately followed by intravenous injection of 200 μCi of [^{3}H]tyrosine. The animals were killed 15 and 30 min after stimulation, and the specific activity of neostriatal dopamine was determined. At 15 minutes after stimulation the specific activity of the dopamine in the stimulated neostriata was significantly different ($p < 0.01$) from the specific activity of the dopamine in contralateral unstimulated neostriata (Table 6). By 30 min after stimulation the dopamine specific activities in control and stimulated preparations were not significantly different.

Time course of dopa accumulation with stimulation. The accumulation of dopa in the neostriatum following administration of the decarboxylase inhibitor Ro 4-4602 (800 mg/kg intraperitoneally) was linear for 60 min (44) in untreated animals and for at least 30 min in chloral hydrate-treated animals (data not shown). When Ro 4-4602 was injected 30 min before death and stimulations were applied for the last 20 min or for the full 30 min, a highly significant ($p < 0.0005$) increase in dopa accumulation was found on the stimulated side compared with the contralateral unstimulated side (Table 7). Similarly, treatment with Ro 4-4602 for 20 min concomitant with 20 min of stimulation gave a highly significant increase in dopa accumulation (+169%) in the stimulated neostriata.

Post-stimulation dopa accumulation. Rats were stimulated for 20 min at 15 Hz, and immediately after the end of stimulation Ro 4-4602 (800 mg/kg) was injected intraperitoneally. Fifteen minutes later the animals were decapitated and the neostriata were assayed for dopa. Dopa accumulation on the stimulated side was significantly greater (+175%) than dopa accumulation on the contralateral unstimulated side (Table 7).

Frequency response of dopa accumulation. A study of the frequency response of dopa accumulation was carried out. Twenty-minute treatment with Ro 4-4602 with a 20-min stimulation was used. The greatest increase in dopa accumulation due to stimulation was found with a frequency of 30 Hz (Table 8). All frequencies tested, including 15 Hz, produced significant increases in dopa accumulation on the stimulated side compared with the unstimulated side.

DISCUSSION

Short-term electrical stimulation of chemically defined neuronal systems is a useful technique for studying the effects of

increased impulse flow on the regulation of neurotransmitter release, catabolism, and synthesis. This technique has been used extensively in the peripheral nervous system and was used in this study to examine central dopaminergic neurons.

Our data show that the response of the nigro-neostriatal and mesolimbic dopaminergic neurons to increased impulse flow produced by electrical stimulation is similar to the response of peripheral noradrenergic neurons to increased impulse flow. Increased impulse flow in these dopaminergic neurons leads to increased synthesis of the neurotransmitter dopamine, much as increased impulse flow produces increased norepinephrine synthesis in peripheral noradrenergic neurons (4–8).

Electrical stimulation caused a significant increase in the specific activity of dopamine in the neostriatum (Table 1) when [^{3}H]tyrosine was used as precursor. Since stimulation did not alter endogenous dopamine or tyrosine levels and did not increase tyrosine specific activity in stimulated neostriata, the increase in dopamine specific activity is not likely due to alterations in endogenous dopamine levels, to increased transport of tyrosine into the stimulated neostriata, or to preferential uptake of labeled tyrosine. The increase in dopamine specific activity appears to be

TABLE 7

Effect of stimulation on dopa accumulation in neostriatum following Ro 4-4602

Rats were injected with Ro 4-4602 (800 mg/kg intraperitoneally) at various times before or immediately after stimulation. Stimulations were applied as described in METHODS for various lengths of time and were 400-μamp biphasic pulses, 15 Hz, 3.0-msec duration. Controls represent nonstimulated contralateral neostriata. Values are means and standard errors of the number of samples shown.

Treatment	Dopa	*n*	Change[a]
	μg/g, wet wt		%
Ro 4-4602, 30 min			
Control	1.20 ± 0.05	12	
Stimulation, 20 min	2.27 ± 0.23[b]	4	+106 ± 22
Stimulation, 30 min	4.40 ± 0.28[b]	4	+298 ± 37
Ro 4-4602, 20 min			
Control	0.67 ± 0.07	8	
Stimulation, 20 min	1.76 ± 0.21[b]	8	+169 ± 23
Ro-4-4602, 15 min			
Control	0.44 ± 0.02	5	
Stimulation, 20 min, before Ro 4-4602	1.22 ± 0.09[b]	3	+175 ± 15

[a] Change of stimulated side compared with the contralateral unstimulated side.

[b] Significantly different from control; $p < 0.0005$.

TABLE 8

Effect of stimulation frequency on accumulation of dopa in neostriatum

Rats were injected intraperitoneally with Ro 4-4602 (800 mg/kg), and stimulation as described in METHODS was immediately begun (400 μamp biphasic, 3.0-msec duration, 15 Hz, 20 min). At the end of the stimulation period the rats were decapitated and the neostriata were assayed for dopa as described in METHODS. Controls represent contralateral nonstimulated neostriata. Values are the means and standard errors of the number of samples shown.

Frequency	Dopa		*n*	Change[a]
	Control	Stimulated		
Hz	*μg/g, wet wt*			%
5	0.81 ± 0.10	1.20 ± 0.17[b]	4	+50 ± 10
10	0.67 ± 0.17	2.00 ± 0.45[b]	4	+221 ± 38
15	0.66 ± 0.07	1.76 ± 0.21[b]	8	+169 ± 23
30	0.66 ± 0.02	5.01 ± 0.64[b]	4	+671 ± 125

[a] Change of stimulated sides compared with the contralateral unstimulated sides.

[b] Significantly different from control; paired *t*-test, $p < 0.025$.

due to increased synthesis of dopamine from tyrosine.

The most effective frequency for increasing dopamine synthesis with 20 min of stimulation was 15 Hz (Table 1), the same frequency found optimal for increasing 3,4-dihydroxyphenylacetic acid accumulation (37, 47) and one close to those found optimal for increasing serotonin catabolism (11) and synthesis (14) and central norepinephrine catabolism (15). This frequency, which is close to the physiological firing rate of these neurons (36), was also found to be effective in producing persistent behavioral changes, particularly turning behavior, and increased dopamine synthesis in awake animals with chronically implanted electrodes (48). Since the nigro-neostriatal fibers are thin, unmyelinated fibers (49) and probably do not carry impulses much beyond a frequency of 50 Hz (19), the high frequency employed and differences in length of stimulation may explain the discrepancies between our results and those of Schlehuber *et al.* (50).

Lesion of the dopaminergic neurons has been shown to produce a short-term increase in dopamine synthesis (51, 52). Sham stimulations were carried out to determine whether part or all of the increases in dopamine specific activity were due to nonspecific damage of the dopaminergic neurons by the electrodes. Our results (Tables 1 and 2) indicate that this was not the case and that the increase in dopamine specific activity was due to increased impulse flow as a result of electrical stimulation. The data on increased dopamine specific activity produced by electrical stimulation were paralleled by increases in total radioactivity in the dopamine fractions from the neostriatum and olfactory tubercles (Table 2). Thus it appears that the mesolimbic system is quite similar to the nigro-neostriatal system in its response to increased impulse flow, as suggested by earlier studies in which the accumulation of 3,4-dihydroxyphenylacetic acid was examined (47).

The similar effects on dopamine synthesis from [^{3}H]tyrosine in the neostriatum seen with stimulation of the nigro-neostriatal pathway and of the zona compacta of the substantia nigra indicate that the observed effects are due to direct stimulation of the dopaminergic neurons and not to stimulation of some other pathway which exerts an influence on the dopaminergic neurons.

The possibility that phenylalanine might be a substrate for dopamine synthesis in the neostriatum was of interest, since phenylalanine had been reported to be a precursor for dopamine and norepinephrine in whole mouse brain (53). Our experiments suggest that phenylalanine can serve as a precursor for dopamine *in vivo* in rat neostriatum (Table 5), although some conversion of phenylalanine to tyrosine in the periphery prior to its entry into the brain cannot be ruled out.

In these experiments the specific activities of the tyrosine and phenylalanine used were approximately the same. Since the circulating levels of endogenous phenylalanine are lower (about 35% less) than those of tyrosine (54), the specific activity of the phenylalanine reaching the central nervous system should be at least as great as that of tyrosine. Phenylalanine appears to be much less efficient as a dopamine substrate, since much greater levels of [^{3}H]phenylalanine were used (350 μCi vs. 200 μCi of [^{3}H]tyrosine) to produce much less accumulation of label in the neostriatal dopamine fraction.

Other differences between phenylalanine and tyrosine as precursors were seen. When phenylalanine was used as a precursor, a much greater percentage of the radioactivity was found in the norepinephrine fractions from Amberlite chromatography than when tyrosine was used, even if no allowances were made for loss of label in converting [2,3-^{3}H]phenylalanine to [^{3}H]norepinephrine. The maximum percentage increase in radioactivity in the dopamine fraction due to stimulation was greater with phenylalanine (+138%) than with tyrosine as precursor (+94%).

These data suggest, as did the work of Nybäck *et al.* (53), that phenylalanine is normally of minor importance as a precursor for dopamine but that during periods of increased demand on dopamine synthesis relatively more phenylalanine may be used as precursor. These experiments do not rule out the possibility that the

[^{3}H]phenylalanine was converted to [^{3}H]-tyrosine outside the neostriatum and that this [^{3}H]tyrosine then served as substrate for dopamine synthesis. However, the quantitative differences seen between experiments using phenylalanine and tyrosine as labeled precursors suggest that phenylalanine is used directly as a precursor for dopamine, an idea supported by the findings that phenylalanine can serve as a substrate for dopamine synthesis in synaptosomes prepared from neostriatum (55) and that it is also a substrate for tyrosine hydroxylase (56).

Amberlite CG-120 column chromatography was used to demonstrate that the catecholamine fraction being studied in these experiments as the dopamine fraction was not seriously contaminated with norepinephrine. Our results show that in both control and stimulated preparations dopamine accounted for 99% of the radioactivity in the neostriatal catecholamine fraction when [^{3}H]tyrosine was used as precursor. Thus changes in the catecholamine fraction due to electrical stimulation could be properly attributed to changes in dopamine. Similar results were reported by Jonason (57), using rabbit caudate slices.

The time course of the increase in dopamine specific activity with electrical stimulation (Fig. 3) indicates that there is a stimulation-induced increase in the specific activity of dopamine in the neostriatum lasting for 20 min. Beyond 20 min there does not appear to be any further significant increase in dopamine specific activity. The most likely explanation for this effect is that after 20 min of stimulation a temporary steady-state level of dopamine specific activity is reached. This steady-state level is probably governed by several factors, such as the specific activity of the precursor, tyrosine, in the neuron and the release of newly synthesized dopamine, possibly a preferential release (58).

The demonstration of a post-stimulation increase in dopamine specific activity (Table 8) indicates that the increased synthesis of dopamine continues for some time following the cessation of electrical stimulation. Thus the rate-limiting step in the synthesis of dopamine, presumably tyrosine hydroxylation (59), which is accelerated by stimulation, is not under direct control by impulse flow and remains in an activated state for some time after impulse flow has returned to control levels.

The response of the nigro-neostriatal and mesolimbic dopaminergic neurons to increased impulse flow due to electrical stimulation appeared quite similar to the response of peripheral sympathetic neurons to increased impulse flow. Since it had been shown that peripheral sympathetic neurons increase norepinephrine synthesis by increasing the activity of the rate-limiting enzyme, tyrosine hydroxylase (5, 8), it was of interest to see whether dopaminergic neurons would respond in a similar manner. Accumulation of dopa following decarboxylase inhibition was used as an index of tyrosine hydroxylase activity *in vivo* (60), and with this technique it was found that electrical stimulation of the nigro-neostriatal pathway did increase tyrosine hydroxylase activity in the neostriatum (Tables 7 and 8).

Unlike the increase in dopamine specific activity (Table 1), the increased accumulation of dopa produced by electrical stimulation following decarboxylase inhibition continued for at least 30 min in the neostriatum (Table 7). The reasons for this difference are unclear and may be due to any of several possibilities. As tyrosine hydroxylase becomes more active, dopa decarboxylase may become the rate-limiting enzyme in dopamine biosynthesis, thus limiting the amount of dopamine synthesized but having no significant effect on dopa synthesis. The increase in dopamine specific activity is limited by the specific activity of the tyrosine pool in the neostriatum. After 20 min of stimulation the specific activity of dopamine may be as high as is possible following a single injection of labeled tyrosine. Moreover, labeled dopamine is being released as the stimulation continues, and possibly is preferentially released over older, unlabeled dopamine (58). This would also tend to limit the amount and specific activity of radioactive dopamine accumulating in the neostriatum. Dopa, on the other hand, is not readily taken up by the synaptic vesicles and so apparently is not released from the nerve

ending upon stimulation. Moreover, following dopa accumulation, synthesis of dopamine is blocked by Ro 4-4602. Dopamine levels would thus be expected to fall, removing a potent feedback inhibitor of tyrosine hydroxylase. Dopa, on the other hand, appears to be much weaker as a feedback inhibitor of neostriatal tyrosine hydroxylase (44). These factors would also tend to extend the time necessary for dopa levels to reach a steady state.

Accumulation of dopa also differed from the increase in dopamine specific activity in the stimulation frequency necessary to produce an optimal effect. Dopa accumulation was found to be optimal at a frequency of 30 Hz among those frequencies tested (Table 8).

The post-stimulation accumulation of dopa (Table 7) paralleled the results for post-stimulation increase in dopamine specific activity (Table 6). Thus dopa accumulation for 15 min immediately following electrical stimulation was much greater in stimulated neostriata than in contralateral unstimulated neostriata. This indicates that the post-stimulation increase in dopamine specific activity is due, at least in part, to an increased activity of tyrosine hydroxylase, which is maintained for some time after stimulation has ceased.

The dopa accumulation experiments clearly demonstrate that an increase in impulse flow results in an increase in tyrosine hydroxylase activity. These experiments indicate that the nigro-neostriatal and mesolimbic dopaminergic neurons are similar to peripheral noradrenergic neurons in their response to increased impulse flow; i.e., increased impulse flow leads to an increase in synthesis of the neurotransmitter from the precursor, tyrosine. The fact that an increase in impulse flow results in an apparent activation of tyrosine hydroxylase which persists following the termination of the stimulation period makes it feasible to investigate further the mechanisms underlying the stimulus-induced activation. Such experiments are currently in progress.

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