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Regulation of Dopamine Synthesis in Rat Brain Striatal Synaptosomes

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SUMMARY

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The properties of dopamine synthesis in rat brain striatal synaptosomes were examined. Addition of catecholamines inhibited the conversion of tyrosine to dopamine, with 50% inhibition occurring at 0.5 μM dopamine. Cocaine antagonized the dopamine-induced inhibition of synthesis to an extent similar to its blockade of dopamine uptake. Stimulation with depolarizing concentrations of veratridine produced a calcium-dependent increase in the conversion of tyrosine to dopamine. Strontium, although less effective, could substitute for calcium in the veratridine-induced stimulation. Veratridine treatment was associated with a significant decline in the specific activity of tyrosine in the tissue, a finding consistent with the concept that the increased rate of dopamine formation is caused by an activation of synthesis rather than by preferential labeling of the precursor. The decrease in tyrosine specific activity was due to a decreased accumulation of tyrosine produced by veratridine. The veratridine-induced inhibition of tyrosine accumulation appears to be related to the ability of the alkaloid to alter sodium permeability, since it was blocked by tetrodotoxin. The tyrosine apparent K_m for synthesis was much lower than the apparent K_m for tyrosine uptake and, along with the apparent V_{max} for synthesis, was significantly increased by veratridine. In contrast to dopamine synthesis from tyrosine, synthesis from dopa was not inhibited by the addition of dopamine, nor was it activated by veratridine. These studies indicate that isolated synaptosomal preparations respond to depolarizing agents with an activation of tyrosine hydroxylation, a property which suggests their suitability as a useful model for studying synthesis regulation in the central nervous system.

INTRODUCTION

Stimulation of adrenergic neurons is often accompanied by an increase in the rate of catecholamine formation (1-4). It has been suggested that this increase is caused by a reduction of catecholamine feedback inhibition of tyrosine hydroxylase (EC 1.14.3a) by means of the stimulus-induced

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release of catecholamines (1–5). This hypothesis is supported by observations that catecholamines can inhibit tyrosine hydroxylase both in intact systems *in vitro* (3, 6, 7) and in purified enzyme preparations (8–12), and also by the finding that the stimulus-induced increase in synthesis is prevented by omitting calcium from the incubation medium (3), a condition which inhibits transmitter release (3, 13). A direct role of calcium in regulating synthesis has also been proposed (14), possibly in conjunction with a stimulus-induced change in the kinetic properties of tyrosine hydroxylase (12).

Isolated synaptosomal preparations offer an opportunity to study regulation of catecholamine synthesis in the central nervous system at the nerve-ending site where synaptic release occurs. They contain all the enzymes necessary for catecholamine synthesis (7, 15, 16) and maintain the ability to release catecholamines in response to depolarizing agents such as potassium (17, 18) and veratridine (18), an alkaloid capable of producing depolarization by increasing sodium permeability (19, 20). We have previously shown that striatal synaptosomes can also respond to depolarizing concentrations of potassium or veratridine with an increase in the rate of dopamine formation (21, 22).

The present studies were undertaken to gain further insight into the interactions between depolarizing agents and synaptosomal dopamine synthesis by studying the veratridine-induced increase in dopamine synthesis with regard to the time course of synthesis activation, the divalent cation requirements, the effect of veratridine on tyrosine flux and specific activity, the effects of veratridine on the kinetic constants for dopamine synthesis, and determination of the step(s) in the biosynthetic pathway that is activated by veratridine.

METHODS AND MATERIALS

Preparation of crude synaptosomal fraction. Male Sprague-Dawley rats (200–250 g) were decapitated and the striata were dissected out and placed in polyethylene tubes in ice. The P_2 fraction (containing synaptosomes, mitochondria, and myelin) was prepared according to Gray and Whittaker (23). The tissue was homogenized in 20 volumes of 0.32 M sucrose, pH 7.4, in a 0.25-mm-clearance Teflon-glass homogenizer and centrifuged at 1000 × *g* for 15 min to remove nuclei and unbroken cells, and the supernatant was centrifuged at 20,000 × *g* for 20 min to sediment the P_2 fraction. Most of the tyrosine hydroxylase activity in the P_2 fraction has been shown to be associated with the synaptosomal component of this fraction following sucrose density gradient centrifugation (15).

Assay for dopamine synthesis. The P_2 pellet was resuspended in a Tris-buffered medium with the following composition (normal control medium): NaCl, 125 mM; KCl, 5 mM; $CaCl_2$, 1 mM; $MgCl_2$, 1 mM; glucose, 10 mM; ascorbic acid, 1 mM (made fresh daily); and Tris-HCl, 50 mM, at pH 7.4. The addition of ascorbic acid and labeled tyrosine lowered the pH to approximately 7.0. None of the agents tested in this study had any further significant effect on pH. This buffer was chosen to provide the tissue with an environment similar both to physiological conditions and to conditions under which calcium-dependent synaptosomal catecholamine release has been demonstrated to occur (18).

For each incubation, 15–20% of the P_2 fraction from one rat was used. Dopamine synthesis was measured by incubating the tissue at 37° with either L-[1-^{14}C]tyrosine or DL-[1-^{14}C]dopa and monitoring the production of $^{14}CO_2$, as previously described (21). Essentially all of the dopa newly formed from tyrosine is converted to dopamine under these conditions (21). Concentrations greater than 5 μM tyrosine and 10 μM dopa were produced by combining the isotope with unlabeled amino acid. Isotope added to the incubation medium served as the reagent blank, which averaged approximately 0.02% of the total disintegrations per minute for tyrosine and 0.04% for dopa. Synthesis was proportional to tissue concentration up to at least 25% of the P_2 from one rat, and to time for at least 15 min. For a 5-min synthesis period, the tissue to blank ratios were approximately 15:1 for the lower, and 5:1 for the higher, tyrosine concentrations. At low dopa con-

centrations this ratio was approximately 16:1, and at higher concentrations, 5:1. The presence of ascorbic acid was necessary to prevent nonenzymatic dopa decarboxylation. All final reaction volumes were 1 ml. The apparent synthesis rate was calculated by dividing the disintegrations per minute of product formed per hour per gram of original tissue by the specific activity of the tyrosine added to the medium. Hypotonic lysis of the P_2 fraction with distilled water abolished dopamine formation from L-[1-^{14}C]tyrosine, indicating that synthesis could occur under these assay conditions only in intact synaptosomes.

Tyrosine assay. Tyrosine was assayed according to the procedure of Ambrose *et al*. (24), which is a modification of the fluorometric procedure of Waalkes and Udenfriend (25). Following incubation in the presence of labeled tyrosine, eight tubes were placed in ice, pooled, and centrifuged at 20,000 × *g* for 20 min. The supernatant was discarded, and the pellet was washed twice with 5 ml of ice-cold 0.32 M sucrose. The pellet was then lysed by the addition of 1.5 ml of 0.06 N trichloracetic acid and mixed with an orbital electric mixer for 30 sec. Protein was precipitated by centrifugation at 20,000 × *g* for 10 min, and aliquots were taken from the lysate for the determination of radioactivity (0.05 ml) and tyrosine (0.5 ml). Dopamine, up to at least 4 μg/assay tube, did not produce a measurable reading in the tyrosine assay, nor did it interfere with the tyrosine determinations. Application of the trichloracetic acid supernatant to a Bio-Rad AG 50-X8 (100–200 mesh, H^+) ion-exchange resin removed 97% of the labeled tyrosine, and decreased the tissue sample fluorescent readings to the same extent.

Uptake studies. The uptake of L-[3,5-^{3}H]tyrosine, DL-[1-^{14}C]dopa, and [*G*-^{3}H] dopamine was measured by separating the tissue from the medium on a 0.65-μm Millipore filter. The uptake of labeled amino acids was linear with time for 1 min. By the use of Bio-Rad AG 50 (H^+) ion-exchange resin following incubation with [*G*-^{3}H]dopamine, it was possible to show that fewer than 15% of the counts in the tissue were due to dopamine oxidation products. Previous work had demonstrated less than 2% metabolism of labeled tyrosine under these conditions (21). Although no differences were observed between washing the [^{3}H]tyrosine-treated tissue with 37°, 0.32 M sucrose compared to 0°, 0.32 M sucrose, the Millipore wash was performed with one 10-ml volume of 37°, 0.32 M sucrose to avoid possible temperature-induced artifacts (26). When samples were washed with distilled water to produce hypotonic synaptosomal lysis, only 10% of the labeled tyrosine or dopa remained on the Millipore filter, suggesting their intrasynaptosomal localization.

Efflux studies. The P_2 fraction was incubated for 7 min at 37° in the incubation buffer, followed by the addition of 5 μCi of L-[3,5-^{3}H]tyrosine per P_2 (10 μM), and the incubation was continued for an additional 8 min. The tubes were then placed in ice and centrifuged at 20,000 × *g* for 20 min. The pellets were washed with 5 ml of ice-cold 0.32 M sucrose, and the labeled P_2 was resuspended in the incubation buffer.

Statistical methods. Student's *t*-test was used to determine statistical significance (27). A value of p less than 0.05 was considered significant. To obtain the best estimation of Michaelis constants, maximal velocities, and their standard errors, a computer program described by Cleland (28) and adapted to a Wang Model 700 computer was used.

Materials. L-[1-^{14}C]Tyrosine (specific activity, 55.8 mCi/mmole), DL-[1-^{14}C]dopa (specific activity, 13.2 mCi/mmole), [*G*-^{3}H] dopamine (specific activity, 9.3 Ci/mmole), and L-[3,5-^{3}H]tyrosine (specific activity, 57.5 Ci/mmole) were purchased from New England Nuclear Corporation. Veratridine, obtained from K & K Laboratories, was freshly prepared by dissolving it in 0.1 N HCl heated to approximately 60°, followed by partial neutralization with an equal volume of 0.08 N NaOH. L-Tyrosine, L-norepinephrine HCl, and dopamine HCl were obtained from Regis; tetrodotoxin, from Calbiochem; and DL-dopa, catechol, and tyramine HCl, from Sigma. Apomorphine HCl and cocaine HCl were obtained from Merck. Millipore filters (DAWP

02500) of size 0.65 μm, 25 mm in diameter, were purchased from Millipore Corporation.

RESULTS

Effects of various dopamine-related compounds on dopamine synthesis. Dopamine, apomorphine (a putative dopamine receptor activator), and norepinephrine markedly inhibited dopamine synthesis from tyrosine at 1 μM (Table 1). All three catecholamines could inhibit synthesis over 90% at 100 μM. Catechol was a relatively ineffective inhibitor at 1 μM, although it could markedly inhibit synthesis at 100 μM, a concentration at which tyramine (a non-catechol sympathomimetic agent) inhibited synthesis by 30%. Uptake of 10 μM tyrosine was not inhibited by any of the following compounds: dopamine (1 or 100 μM), apomorphine (1 μM), tyramine (10 μM), or catechol (100 μM). In contrast to dopamine synthesis from tyrosine, synthesis from dopa (1 or 10 μM) was not inhibited by dopamine or apomorphine at any of the concentrations employed in Table 1.

Effect of cocaine on dopamine-induced inhibition of synthesis. In agreement with the findings for synaptosomes isolated from the cortex plus striatum (21), cocaine, an inhibitor of catecholamine uptake, markedly antagonized the dopamine-induced synthesis inhibition in the striatal synaptosomal preparation (Table 2). Cocaine prevented 64% of the synthesis inhibition produced by 1 μM dopamine, and inhibited the uptake of dopamine to a similar extent (Table 2).

Time course of dopamine synthesis stimulation by veratridine. Exposure of the synaptosomes to veratridine produced an increase in dopamine synthesis (Fig. 1). This increase was markedly time-dependent, as evidenced by the finding that the veratridine-treated samples were significantly elevated over controls 2.5, 5, and 10 min after veratridine exposure, but not at 15 or 20 min.

Effect of various veratridine concentrations on synaptosomal dopamine synthesis. Significant, and similar, increases in dopamine synthesis were obtained with 25, 75, and 150 μM veratridine (Fig. 2). However, 7.5 μM veratridine had no effect on synthesis.

Effect of veratridine on synaptosomal dopamine synthesis at various calcium or strontium concentrations. As previously reported (22), the veratridine-induced increase in dopamine synthesis was prevented by omitting calcium from the normal incubation medium (Fig. 3). Addition of 0.1 mM calcium to the medium was not sufficient to restore the veratridine response, while in the presence of calcium concentrations of 0.3, 1, 3, and 10 mM, veratridine did produce a significant in-

TABLE 1

Effects of various dopamine-related compounds on dopamine synthesis

Aliquots of the striatal P_2 fraction were incubated for 10 min at 37°, followed by the addition of L-[1-^{14}C]tyrosine, 10 μM, and the samples were incubated for an additional 10 min. In the tyramine-treated samples, the tissue was first incubated for 10 min in the presence of tyramine. All other agents were added together with the tyrosine. All drugs were initially prepared in 1 mM HCl. Values are means ± standard errors of the percentage of control activity. The number of observations is shown in parentheses. The control activity was 10.4 ± 0.44 nmoles/hr/g (N = 29).

Compound	Dopamine synthesis			
	0.1 μM	1 μM	10 μM	100 μM
	% control			
Dopamine	85 ± 3(11)	40 ± 1 (17)	20 ± 3 (6)	7 ± 1 (6)
Apomorphine	82 ± 4 (6)	52 ± 2 (6)	14 ± 1 (6)	2 ± 0.2 (6)
L-Norepinephrine	92 ± 2 (6)	59 ± 2 (13)	22 ± 2 (9)	5 ± 0.4 (6)
Tyramine	96 ± 3 (3)	87 ± 3 (3)	80 ± 1 (4)	67 ± 3 (4)
Catechol	97 ± 5 (6)	96 ± 2 (6)	87 ± 3 (6)	59 ± 2 (5)

TABLE 2

Effect of cocaine on dopamine-induced inhibition of synthesis and on dopamine uptake

For the measurement of dopamine synthesis, aliquots of the striatal P_2 fraction were incubated for 10 min at 37°, either with or without cocaine (80 μM), followed by the addition of L-[1-^{14}C]tyrosine (10 μM) together with dopamine, and the samples were incubated for an additional 10 min. For the measurement of dopamine uptake, the samples were treated as above, except that [*G*-^{3}H]dopamine was added following the initial 10-min incubation period. Values are the means ± standard errors of the numbers of observations shown in parentheses.

Conditions	Dopamine synthesis	Dopamine uptake
	nmoles/hr/g	*nmoles/g*
Controls	9.75 ± 0.62 (9)	
Cocaine	9.71 ± 0.28 (9)	
Dopamine (1 μM)	3.68 ± 0.19[a] (9)	9.51 ± 0.16 (5)
Dopamine (1 μM) + cocaine	7.60 ± 0.28[b] (9)	1.73 ± 0.060[c] (5)
Dopamine (0.5 μM)	4.68 ± 0.16[a] (5)	
Dopamine (0.5 μM) + cocaine	8.00 ± 0.29[d] (5)	

[a] $p < 0.001$ vs. controls.
[b] $p < 0.001$ vs. corresponding amine alone; $p < 0.001$ vs. cocaine alone.
[c] $p < 0.001$ vs. dopamine without cocaine.
[d] $p < 0.001$ vs. corresponding amine alone; $p < 0.01$ vs. cocaine alone.

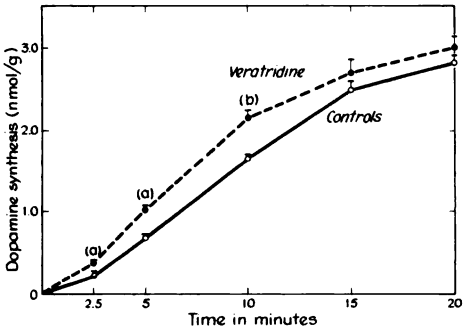

FIG. 1. *Time course of dopamine synthesis stimulation by veratridine*

Aliquots of the striatal P_2 fraction were incubated for 5 min at 37°, followed either by the simultaneous addition of veratridine (75 μM) plus L-[1-^{14}C]tyrosine (10 μM) or else by the addition of tyrosine alone, and incubated again for the times indicated. Each point represents the mean ± standard error of four observations. Significant values of veratridine-treated samples compared to controls: *a*, $p < 0.001$; *b*, $p < 0.02$.

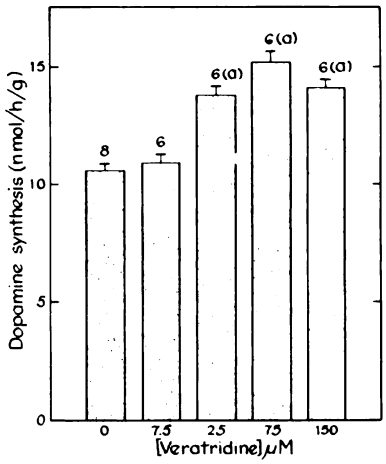

FIG. 2. *Effect of various veratridine concentrations on synaptosomal dopamine synthesis*

Aliquots of the striatal P_2 fraction were incubated for 5 min at 37°, followed either by the simultaneous addition of L-[1-^{14}C]tyrosine (10 μM) and veratridine at the concentrations indicated or by the addition of tyrosine alone, and incubated for an additional 5 min. Values are the means ± standard errors of the numbers of observations indicated. *a*, $p < 0.001$ vs. controls.

crease in dopamine synthesis. No significant effect on the basal synthesis rate was observed by varying the calcium concentration in the medium. Strontium could substitute for calcium in the veratridine-induced synthesis stimulation, although the percentage increase was not as great as with calcium (Fig. 3). Varying the strontium concentration also had no significant effect on the basal synthesis rate.

Effect of veratridine on tyrosine specific activity. To determine whether veratridine was producing an increase in apparent syn-

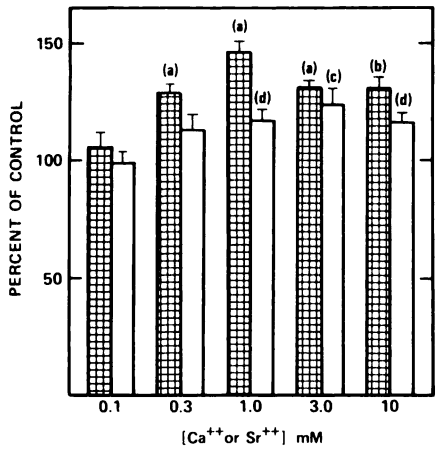

FIG. 3. *Effect of veratridine on synaptosomal dopamine synthesis at various calcium* (▦) *or strontium* (□) *concentrations*

Aliquots of the striatal P_2 fraction were incubated for 5 min at 37° at the calcium or strontium concentration indicated, followed by the simultaneous addition of veratridine (75 μM) and L-[1-^{14}C]tyrosine (10 μM), and incubated for an additional 5 min. Each value represents the mean ± standard error of five or six observations, expressed as the percentage of the corresponding control tissue incubated at the same calcium or strontium concentration without veratridine. The means ± standard errors, in nanomoles of dopa formed per hour per gram of original tissue, are 8.82 ± 0.38 for calcium-free tissue incubated in the absence of veratridine and 9.18 ± 0.19 in the presence of veratridine (N = 12). Significant values of veratridine-treated samples compared to controls: *a*, $p < 0.001$; *b*, $p < 0.01$; *c*, $p < 0.02$; *d*, $p < 0.05$.

thesis rate by increasing the specific activity of tyrosine in the tissue, specific activity measurements of tyrosine were made 2.5 and 5 min after veratridine exposure. As can be seen in Table 3, veratridine actually produced a significant decline in tyrosine specific activity; although the total tyrosine content was not significantly altered by veratridine, there was a significant reduction in the accumulation of tyrosine.

Effect of veratridine on tyrosine accumulation. In order to study the effect of veratridine on the accumulation of tyrosine in more detail, samples were incubated for various periods of time with veratridine and the amount of labeled tyrosine in the tissue at each time point was measured. In addition, the effect of tetrodotoxin, an agent which can antagonize veratridine-induced depolarization by blocking sodium channels (29), was studied. The data in Table 4 show that the inhibition of tyrosine accumulation by veratridine increased with time of incubation, from 25% inhibition at 30 sec to 64% inhibition at 20 min. In addition, the observation that tetrodotoxin could completely prevent the veratridine-induced inhibition of tyrosine accumulation (Table 4) suggests that this action of veratridine is related to its depolarizing properties.

Effect of veratridine on tyrosine efflux. Since the ability of veratridine to inhibit tyrosine accumulation may have been due to an increase in tyrosine efflux, the effect of veratridine on tyrosine efflux from the P_2 fraction that had been labeled with tyrosine was examined. Table 5 shows that the tyrosine efflux from labeled tissue was in fact markedly stimulated by veratridine. Also, as with the tyrosine accumulation studies, this action of veratridine was blocked by prior incubation with tetrodotoxin. These results indicate that at least part of the ability of veratridine to inhibit the accumulation of tyrosine was due to stimulated efflux of newly taken up tyrosine.

Effect of veratridine on synaptosomal dopamine synthesis at various tyrosine concentrations. Figure 4 demonstrates the complex nature of the effect of veratridine on dopamine synthesis from tyrosine at various tyrosine concentrations. The apparent K_m for synthesis was increased from 1.17 ± 0.18 μM to 2.19 ± 0.32 μM ($p < 0.01$), and the apparent V_{max} was increased from 12.4 ± 0.62 to 17.2 ± 1.0 nmoles/hr/g ($p < 0.001$).

Effect of veratridine on synaptosomal dopa decarboxylation at various dopa concentrations. Synthesis of dopamine from dopa was not activated by veratridine, even at saturating dopa concentrations (Fig. 5). At low dopa concentrations a significant inhibition of synthesis was observed. The apparent K_m values for synthesis were 52.7 ± 7.5 μM and 84.5 ± 15.6 μM, and the apparent V_{max} values were 393 ± 16 and 360 ± 21 nmoles/hr/g for controls

TABLE 3

Effect of veratridine on tyrosine specific activity

The striatal P_2 fraction was prepared for the determination of labeled and total tyrosine content as described in METHODS AND MATERIALS. The tissue was incubated for 5 min at 37°, followed either by the simultaneous addition of veratridine (75 μM) plus L-[3,5-^{3}H]tyrosine (1 μCi/tube, 10 μM) or else by the addition of tyrosine alone, and incubated again for the times indicated. Each value represents the mean ± standard error of four observations (each observation resulting from eight pooled samples).

Time	Labeled tyrosine		Total tissue tyrosine		Tyrosine specific activity	
	Controls	Veratridine	Controls	Veratridine	Controls	Veratridine
min	*dpm/g* (× 10^{-6})		*μg/g*		*dpm/μg* (× 10^{-5})	
2.5	6.51 ± 0.32	3.46 ± 0.14[a]	36.3 ± 1.2	32.1 ± 1.7	1.79 ± 0.057	1.08 ± 0.066[a]
5	6.33 ± 0.30	3.04 ± 0.17[a]	32.8 ± 2.5	29.9 ± 1.9	1.95 ± 0.106	1.02 ± 0.070[a]

[a] $p < 0.001$ vs. controls.

TABLE 4

Effect of veratridine on tyrosine accumulation

Aliquots of the striatal P_2 fraction were incubated for 5 min in control medium or in 0.2 μM tetrodotoxin-containing medium, followed either by the simultaneous addition of veratridine (75 μM) plus L-[3,5-^{3}H]tyrosine (10 μM) or else by the addition of tyrosine alone, and incubated again for the times indicated. Each value represents the mean ± standard error of the number of observations shown in parentheses.

	Tyrosine uptake				
Time	Controls	Veratridine		Tetrodotoxin controls	Tetrodotoxin + veratridine
min	*nmoles/g*	*nmoles/g*	*% control*	*nmoles/g*	*nmoles/g*
0.5	7.49 ± 0.62 (7)	5.59 ± 0.41[a] (7)	75		
1	13.5 ± 0.75 (10)	9.43 ± 0.68[b] (11)	70	18.2 ± 1.1 (4)	16.7 ± 0.50[c] (4)
2	21.4 ± 1.3 (5)	13.5 ± 0.81[d] (5)	63		
5	29.2 ± 1.1 (8)	14.6 ± 0.68[b] (9)	50	33.6 ± 0.92 (4)	32.6 ± 1.1[c] (4)
10	31.3 ± 2.0 (4)	13.4 ± 1.0[d] (4)	43		
15	32.3 ± 1.6 (4)	13.1 ± 1.7[b] (4)	41		
20	30.8 ± 1.3 (4)	11.2 ± 0.73[b] (4)	36		

[a] $p < 0.05$ vs. controls.
[b] $p < 0.001$ vs. controls.
[c] Not significantly different from controls.
[d] $p < 0.01$ vs. controls.

and veratridine-treated tissues, respectively. Neither of these quantities was significantly altered by veratridine.

Tyrosine uptake at various tyrosine concentrations. Since at least part of the inhibition of tyrosine accumulation by veratridine was due to stimulated efflux (see above), the kinetic constants for tyrosine uptake were examined only in control tissue. The apparent K_m for tyrosine uptake was 88.8 ± 8.9 μM and the apparent V_{max} was 10,280 nmoles/hr/g. These data indicate that dopamine synthesis did not saturate at approximately 1 μM (Fig. 4) because of saturation of precursor uptake.

Dopa uptake at various dopa concentrations. The apparent K_m for DL-dopa uptake was somewhat higher than that for L-tyrosine uptake, with an apparent K_m of 190 ± 22 μM, while the V_{max} for dopa uptake (9364 ± 564 nmoles/hr/g) was similar to that for tyrosine.

DISCUSSION

The ability of isolated synaptosomal preparations to respond to depolarizing agents such as potassium and veratridine with a release of putative neurotransmit-

TABLE 5

Effect of veratridine on tyrosine efflux

The striatal tyrosine-labeled P_2 fraction was prepared as described in METHODS AND MATERIALS. It was incubated for 5 min at 37° in control medium or in 0.2 μM tetrodotoxin-containing medium, followed either by the simultaneous addition of veratridine (75 μM) plus tyrosine (10 μM) or else by the addition of tyrosine alone, and incubated again for the times indicated. The tissue was separated from the medium by Millipore filtration, as described in METHODS AND MATERIALS. Under these conditions, in controls, only about 6% of labeled tyrosine in the medium was taken up into the tissue, which should therefore have minimized any possible effect of veratridine on tyrosine reuptake. The amount of tyrosine remaining in the tissue is expressed as a percentage of the amount of tyrosine in the tissue following the initial 5-min incubation, which was 2.53 ± 0.23 × 10^6 dpm/g of original tissue for controls and 2.78 ± 0.36 × 10^6 dpm/g of original tissue for tetrodotoxin-treated samples. Each value represents the mean ± standard error of six observations for controls and four observations for tetrodotoxin-treated tissue.

Time	Tyrosine remaining in tissue			
	Controls	Veratridine	Tetrodotoxin controls	Tetrodotoxin + veratridine
min	%	%	%	%
1	74 ± 3.6	65 ± 1.2	73 ± 1.0	69 ± 3.0
2	65 ± 1.8	49 ± 2.1[a]	61 ± 2.9	59 ± 2.9
5	47 ± 1.5	30 ± 1.2[a]	46 ± 1.5	44 ± 2.7

[a] $p < 0.001$ vs. controls.

ters such as norepinephrine (18), glutamate and aspartate (30), γ-aminobutyric acid (30, 31), and acetylcholine (32) suggests their potential as a valuable system for studying mechanisms of transmitter release in the central nervous system. The present studies, along with previous work (21, 22), suggest further that synaptosomal preparations can also serve as a model system for studying regulation of neurotransmitter synthesis in the central nervous system. Synaptosomes respond to the addition of catecholamines to the medium with a decline in catecholamine synthesis rate (Table 1) (7, 21, 33), a response similar to that seen in intact neurons (3, 6, 34). The finding that dopamine synthesis from DL-[1-^{14}C]dopa was not inhibited even at the highest concentrations of dopamine studied indicates that the catecholamine-induced inhibition of dopamine synthesis from L-[1-^{14}C]tyrosine was due to inhibition of the tyrosine hydroxylation step, not to inhibition of the decarboxylase step. However, since not all of the dopa decarboxylase (EC 4.1.1.26) activity is located in

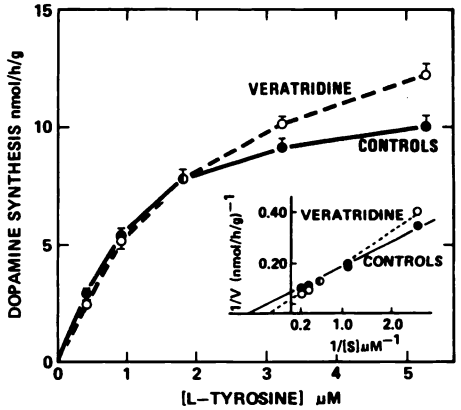

FIG. 4. *Effect of veratridine on synaptosomal dopamine synthesis at various tyrosine concentrations*

Aliquots of the striatal P_2 fraction were incubated for 5 min at 37°, followed either by the simultaneous addition of veratridine (75 μM) and L-[1-^{14}C] tyrosine at the concentrations indicated or by the addition of tyrosine alone, and incubated for an additional 5 min. Each point represents the mean ± standard error of 6–10 observations.

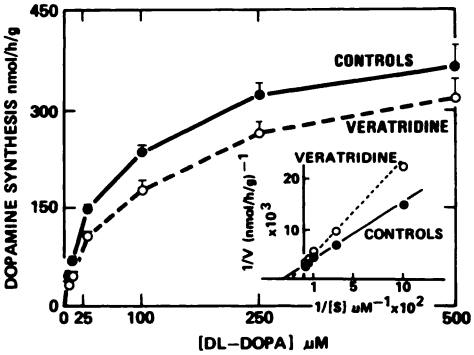

FIG. 5. *Effects of veratridine on synaptosomal dopa decarboxylation at various dopa concentrations*

Aliquots of the striatal P_2 fraction were incubated for 5 min at 37°, followed either by the simultaneous addition of veratridine (75 μM) and DL-[1-^{14}C]dopa at the concentrations indicated or by the addition of dopa alone, and incubated for an additional 5 min. Each point represents the mean ± standard error of four to six observations.

the adrenergic component of the striatal P_2 fraction (35), the possibility that catecholamine-induced inhibition of adrenergic decarboxylation was masked by nonadrenergic decarboxylation cannot be ruled out.

The catecholamine-induced inhibition of synthesis in both striatal synaptosomes (Table 2) and cortex slices (3) is antagonized by prior incubation with cocaine. The observation that cocaine blocked dopamine uptake and the dopamine-induced synthesis inhibition to a similar extent (Table 2) suggests that in isolated striatal synaptosomes a major part, but not necessarily all, of the dopamine-induced inhibition is a result of dopamine action within the synaptosome. The tyramine-induced inhibition (Table 1) may be the result of intrasynaptosomal displacement of endogenous dopamine, which would then be free to interact directly with tyrosine hydroxylase (36).

The time course of the veratridine-induced increase in dopamine synthesis (Fig. 1) illustrates the necessity of using relatively short (i.e., 10 min or less) periods of exposure in order to obtain significant elevations in synthesis rate. Whether or not this time dependency is related to a similar time course of transmitter release is currently being investigated. The finding that the veratridine-induced inhibition of the accumulation of tyrosine actually increased with time may also contribute to a diminished apparent synthesis rate over the longer time periods (Table 4). Veratridine concentrations from 25 to 150 μM produced significant increases in dopamine synthesis (Fig. 2), indicating that veratridine concentrations which can produce catecholamine release are also able to increase synthesis (18, 37). Omitting calcium from the incubation medium prevented the veratridine-induced increase in dopamine synthesis (Fig. 3) (22). A similar finding has been reported for the potassium-induced increase in catecholamine synthesis in cortex slices (3). The synaptosomal system did not demonstrate an absolute requirement for calcium, however, since strontium could substitute for calcium, although not as effectively (Fig. 3). The addition of various calcium concentrations to the medium did not significantly affect the basal synthesis rate. This is in contrast to the findings of Goldstein *et al.* (38), who reported that the addition of calcium to striatal slices incubated in a calcium-free medium inhibited the basal synthesis rate. This difference may be due to the different types of preparations used (synaptosome vs. slice), or to a different time course of treatment.

The finding that the specific activity of tyrosine in the tissue was actually decreased by veratridine (Table 3) is consistent with the concept that the increased rate of dopamine formation is caused by an activation of synthesis, rather than by a preferential labeling of precursor. However, the observation that veratridine preferentially stimulated the efflux of newly taken up tyrosine (Table 3) suggests that this tyrosine does not rapidly equilibrate with the total tyrosine pool. This indication of multiple tyrosine pools, plus the fact that this preparation consists of a mixture of different types of nerve endings, means that measurements of changes in total tyrosine specific activity may not necessarily reflect changes in the adrenergic catecholamine tyrosine precursor pool. The observation that the veratridine-induced inhibition of tyrosine accumulation and stimulated efflux was prevented by tetrodotoxin (Tables 4 and 5) suggests that these two phenomena are related to the depolarizing capabilities of the alkaloid. Veratridine also significantly inhibited the accumulation of dopa (data not shown).

Veratridine produced an increase in both the apparent K_m for tyrosine and the apparent V_{max} (Fig. 4). A similar result has been found for the potassium-induced increase in dopamine synthesis in striatal slices (39). In contrast to the effect of veratridine on dopamine formation from L-[1-^{14}C]tyrosine, dopamine formation from DL-[1-^{14}C]dopa was not stimulated by veratridine (Fig. 5). Dopamine synthesis was, in fact, significantly inhibited by veratridine at low dopa concentrations. The ability of veratridine to inhibit amino acid accumulation (Table 4) may explain why veratri-

dine inhibits dopamine synthesis from dopa to a greater extent at low dopa concentrations compared to higher dopa concentrations (Fig. 5) and also why dopamine synthesis from tyrosine is increased by veratridine only at high tyrosine concentrations (Fig. 4), since only at saturating substrate concentrations would the amount of dopamine formed not be significantly affected by a reduction in substrate concentration. An alternative explanation, that veratridine might inhibit amino acid accumulation to a greater extent at lower amino acid concentrations, is ruled out, since veratridine inhibited the accumulation of tyrosine and dopa uniformly over the entire range of amino acid concentrations used in this study (data not shown).

While the present studies indicate that both adrenergic synaptosomal preparations and intact adrenergic neurons (1–4) respond to stimulation with an increase in neurotransmitter synthesis, the exact mechanism(s) for this response remains to be elucidated. If stimulated transmitter release leading to a reduction in feedback inhibition of tyrosine hydroxylase is the mechanism, then studying transmitter release at various calcium, veratridine, and strontium concentrations should give results that parallel the synthesis stimulation results (Figs. 2 and 3). It is also possible that cyclic nucleotides may play a role in synthesis stimulation (40, 41). Veratridine produces a calcium-sensitive increase in cyclic AMP formation in cortex slices (42). Although a depolarization-induced increase in cyclic AMP has been reported not to occur in cortex synaptosomes (43), attempts to determine whether veratridine increases cyclic AMP formation in striatal synaptosomes should be of interest.

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