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PHENYLETHYLAMINE EFFECTS ON DOPAMINE SYNTHESIS

STRUCTURE–ACTIVITY RELATIONSHIPS*

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Abstract—The effects of various substituents on phenylethylamine- and amphetamine-induced alterations in dopamine synthesis have been studied in rat brain synaptosomal preparations isolated from the corpus striatum. The stimulation of synthesis normally observed with phenylethylamine and amphetamine was converted into inhibition by the addition of a *p*-hydroxy substituent on the ring. Thus, whereas 20 μM phenylethylamine produced a 30 per cent stimulation of synthesis, 20 μM *p*-hydroxyphenylethylamine (tyramine) produced a 30 per cent inhibition, and whereas 20 μM *d*-amphetamine increased synthesis by 40 per cent, 20 μM *dl*-*p*-hydroxyamphetamine inhibited synthesis by 20 per cent. β-Hydroxy substitution on the side chain somewhat diminished, but did not prevent, synthesis stimulation, whereas the presence of both the β-hydroxy and *p*-hydroxy substituents appeared to increase the inhibitory effects, with 45 per cent inhibition produced by 20 μM *dl*-octopamine and 50 per cent inhibition produced by 20 μM *dl*-*p*-hydroxynorephedrine. The synthesis stimulation produced by *dl*-norephedrine was dependent upon the presence of calcium in the incubation medium. The addition of an *N*-methyl substituent on *d*-amphetamine to form *d*-methamphetamine did not alter significantly the stimulatory potency of amphetamine. Tyramine-induced synthesis inhibition was prevented by preincubation with cocaine, suggesting an intrasynaptosomal site of action. Amphetamine antagonized the synthesis stimulation produced by the depolarizing agent veratridine, but tyramine did not have this effect. These data suggest that the parent phenylethylamine compounds interact with an intracellular regulatory site, possibly related to calcium flux and/or dopamine release, that is not affected by the *p*-hydroxy derivatives.

Interest in the interactions between phenylethylamine compounds and neurotransmitter functioning in the central nervous system stems from: (1) the behavioral stimulation produced by sympathomimetics such as phenylethylamine and amphetamine [1, 2], (2) the widespread use of amphetamine (alpha-methylphenylethylamine) and related stimulants for both medical and non-medical purposes [3], and (3) the presence of phenylethylamine and related compounds in the central nervous system [4–6]. The importance of catecholamines in mediating behavioral stimulations produced by these compounds can be seen in: (1) the antagonism of amphetamine-induced stereotyped behavior in rats by neuroleptics known to block dopamine receptors [2], (2) prevention of phenylethylamine-induced sterotypy by adrenergic blocking agents [1], and (3) antagonism of both amphetamine- and phenylethylamine-induced stereotypy by alpha-methyltyrosine, an inhibitor of catecholamine synthesis [1, 2]. Studies on catecholamine release, both *in vitro* [7–10] and *in vivo* [11], are also consistent with catecholamine involvement in the action of phenylethylamines. In recent studies employing synaptosomal preparations [12], we have found that both amphetamine and phenylethylamine produce an increase in dopamine formation that is: (1) calcium-dependent, (2) tyrosine-dependent, and (3) not additive to the stimulation produced by the depolarizing agent veratridine. Since stimulation by veratridine is also calcium- and tyrosine-dependent [13], this suggests a common site of action for depolarization- and phenylethylamine-induced synthesis stimulations.

Although various laboratories have reported that amphetamine increases dopamine synthesis in synaptosomes [12, 14–16], reports on the effects of phenylethylamine derivatives have not been consistent. Thus, *p*-hydroxyamphetamine has been reported to have either inhibitory [15] or excitatory [14] effects on synaptosomal dopamine synthesis, and *p*-hydroxyphenylethylamine (tyramine) has also been reported to be either inhibitory [10] or excitatory [14]. One problem with comparing the results from different laboratories is that different assay conditions (buffer, pH, time–course of treatment) have inevitably been used by different investigators. The purpose of the present investigation was to examine systematically the effects of phenylethylamine and amphetamine derivatives on dopamine formation under identical incubation conditions so that direct comparisons between the effects of different substituent groups could be made. To further the comparisons among the modes of action of phenylethylamine compounds, the effect of tyramine on depolarization-induced synthesis stimulation has

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been analyzed and compared to that of amphetamine.

METHODS

Preparation of the crude synaptosomal fraction. Male Sprague–Dawley rats (200–250 g) were decapitated and the striatal tissue was rapidly dissected, essentially according to the procedure of Glowinski and Iversen [17], and placed in polyethylene tubes in ice. The striatal tissue averaged 100 mg/rat. The P_2 fraction containing synaptosomes, myelin, and mitochondria was prepared as described previously [18, 19]. Most of the tyrosine hydroxylase activity in the P_2 fraction has been found to be associated with the synaptosomal fraction after sucrose density gradient centrifugation [20].

Tissue incubations. Unless otherwise indicated, the P_2 pellet was resuspended in a Tris-buffered medium with the following composition: 125 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, 1 mM ascorbic acid (freshly prepared), and 50 mM Tris–HCl at pH 7.4. The addition of ascorbic acid to this medium lowered the pH to 7.2. None of the agents tested in this study had any further significant effect on pH. In some experiments, where indicated, the P_2 pellet was resuspended in a phosphate-buffered medium identical to the Tris-buffered medium described above with two exceptions: (1) a mixture of mono- and dibasic sodium phosphate (30 mM, pH 7.2) replaced the Tris, and (2) the medium also contained 50 mM sucrose. For each incubation, 15–20 per cent of the P_2 from one rat was used (equivalent to 15–20 mg of original weight) per ml of incubation volume. Unless otherwise indicated, all incubations were at 37°, with a final reaction volume of either 0.5 or 1 ml.

Dopamine synthesis assay. Dopamine synthesis was measured by incubating the tissue with L-[1-^{14}C]tyrosine and monitoring the production of $^{14}CO_2$ as described previously [18, 21]. Essentially all of the DOPA* newly formed from tyrosine is converted to dopamine under these conditions [18]. Unless otherwise indicated, all incubations were carried out in the presence of 10 μM tyrosine, a concentration that was produced by combining the isotope with unlabeled amino acid. This concentration of tyrosine is approximately eight times the apparent K_m for tyrosine [13]. Isotope added to the incubation medium served as the reagent blank, which averaged approximately 0.02 per cent of the total tyrosine disintegrations per minute. For a 5-min synthesis period, the tissue/blank ratio was approximately 6:1. 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.

Uptake studies. The uptake of L-[3,5-^{3}H]tyrosine and [^{3}H-G] tyramine was determined by separating the tissue from the medium on a 0.65 μm Millipore filter as described previously [13]. At the end of the incubation, a 0.2-ml aliquot was placed on the Millipore filter, followed by one 10-ml washing with 0.32 M sucrose at 37°. The Millipore filter was then counted in 10 ml of Bray's solution.

Statistical methods. Student's t-test was used to determine statistical significance [22]. A P value of less than 0.05 was considered significant.

Materials. L-[1-^{14}C]Tyrosine (sp. act. 51.1 mCi/mmole), L-[3,5-^{3}H]tyrosine (sp. act. 52.1 Ci/mmole), and [^{3}H-G]tyramine (sp. act. 4.6 Ci/mmole) were purchased from the New England Nuclear Corp. (Boston, MA). Veratridine and *dl*-*p*-hydroxynorephedrine HCl were obtained from the Aldrich Chemical Co. (Milwaukee, WI); tyramine HCl, β-phenylethylamine HCl, *d*-amphetamine sulfate, *dl*-norephedrine HCl, *dl*-β-hydroxyphenylethylamine, *l*-ephedrine HCl and *d*-methamphetamine HCl from the Sigma Chemical Co. (St. Louis, MO); L-tyrosine from CalBiochem (San Diego, CA); cocaine HCl from Merck, Sharp & Dohme (West Point, PA); *dl*-*p*-hydroxyamphetamine HBr and *l*-amphetamine sulfate from Smith Kline and French Laboratories (Philadelphia, PA); pargyline HCl from Abbott Laboratories (North Chicago, IL); and Millipore filters (DAWP 02500) from the Millipore Filter Corp. (Bedford, MA). Control experiments with sodium sulfate and sodium bromide indicated no significant effects of either sulfate or bromide salts on dopamine synthesis at the concentrations used in this study. All drug concentrations are expressed in terms of the free drug.

RESULTS

Effects of phenylethylamine derivatives on dopamine synthesis. Both phenylethylamine and *dl*-β-hydroxyphenylethylamine signficantly increased dopamine formation, with the phenylethylamine effects statistically significant at 2 and 20 μM, and the β-hydroxyphenylethylamine effect significant at 20 μM (Fig. 1). The effects of *p*-hydroxy derivatives, however, are quite different from the parent compounds, since both *p*-hydroxyphenylethylamine (tyramine) and *dl*-octopamine produced a marked inhibition of synthesis at both 2 and 20 μM (Fig. 1). Additional studies with tyramine showed synthesis inhibition from 0.2 to 600 μM, with no inhibition of tyrosine uptake observed up to 100 μM tyramine (the highest concentration tested for uptake effects).

Effects of amphetamine derivatives on dopamine synthesis. Stimulation of synthesis can be seen with *d*-amphetamine (2 and 20 μM), *dl*-β-hydroxyamphetamine (norephedrine) at 20 μM and *l*-β-hydroxymethamphetamine (ephedrine) at 20 μM (Fig. 2). Again, however, *p*-hydroxy substitution dramatically altered the effects of the parent compounds, with significant inhibition (19–31 per cent) observed with *dl*-p-hydroxyamphetamine from 0.2 to 200 μM and an even greater inhibition (51 per cent) produced by 20 μM *dl*-*p*-hydroxynorephedrine (Fig. 2).

Calcium dependency of norephedrine-induced stimulation. Although the magnitude of the norephedrine-induced synthesis stimulation was appreciably less than that produced by *d*-amphetamine (Fig. 2), the data presented in Fig. 3 suggest that

* Abbreviations: DOPA, dihydroxyphenylalanine; Amph, amphetamine; PEA, phenylethylamine; and MAO, monoamine oxidase.

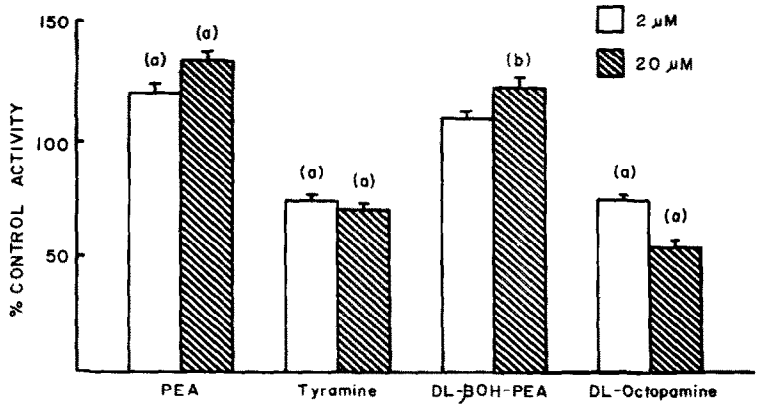

Fig. 1. Effects of phenylethylamine derivatives on dopamine synthesis. Aliquots of the striatal P_2 fraction were incubated for 5 min at 37° either in control medium or in the presence of the drug indicated, followed by the addition of 10 μM L-[1-^{14}C]tyrosine and incubation for an additional 5 min. Each value is the mean ± S.E. of six to twelve observations, except for 2 μM βOH-PEA (N = 3). Key: (a) $P < 0.001$ vs controls; (b) $P < 0.01$ vs controls.

the mechanism of action of these two compounds may be similar, since norephedrine-induced stimulation was completely calcium-dependent, which is also true for amphetamine-induced stimulation [12].

Comparison of d-*amphetamine to* l-*amphetamine and* d-*methamphetamine.* In agreement with the work of Kuczenski and Segal [23], we found that the *d*-isomer of amphetamine stimulated synthesis at a lower concentration (0.2 μM) than the *l*-isomer, but that a similar maximal stimulation was obtained for both isomers (Fig. 4A). In contrast, stimulation by *d*-methamphetamine at 0.2 μM was similar to that observed for *d*-amphetamine (Fig. 4B). Synthesis effects at 2 and 20 μM were also not significantly different between *d*-methamphetamine and *d*-amphetamine.

Effect of cocaine on tyramine-induced synthesis inhibition. Since cocaine is known to inhibit the uptake of phenylethylamine-related compounds such as amphetamine [24], we tested the effect of cocaine on tyramine-induced synthesis inhibition. Table 1 shows that the marked synthesis inhibition produced by 0.2 μM tyramine was indeed completely reversed by preincubation with 80 μM cocaine, which by itself had no significant effect on synthesis.

Effect of cocaine on tyramine uptake. To determine if the antagonism of tyramine-induced inhibition by cocaine was, in fact, associated with an inhibition of tyramine uptake, the effect of cocaine on tyramine uptake was studied. Incubation conditions were the same as for the synthesis experiments, except that 100 μM pargyline was included to inhibit MAO-induced tyramine metabolism. We have shown previously that this concentration of pargyline essen-

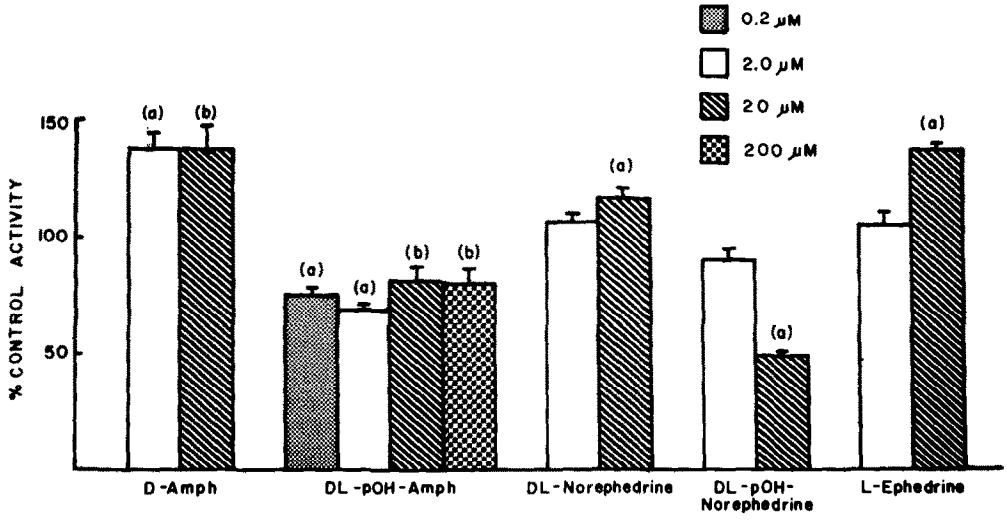

Fig. 2. Effects of amphetamine derivatives on dopamine synthesis. Aliquots of the striatal P_2 fraction were incubated for 5 min at 37° either in control medium or in the presence of the drug indicated, followed by the addition of 10 μM L-[1-^{14}C]tyrosine and incubation for an additional 5 min. Each value is the mean ± S.E. of four to ten observations, except for 2 μM pOH-norephedrine (N = 3). Key: (a) $P < 0.001$ vs controls; (b) $P < 0.01$ vs controls.

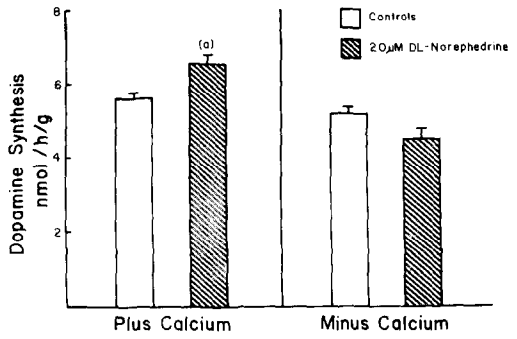

Fig. 3. Effect of calcium on norephedrine-induced stimulation. Aliquots of the striatal P_2 fraction were incubated for 5 min at 37° either in control medium or calcium-free medium in the presence or absence of norephedrine, followed by the addition of 10 μM L-[l-^{14}C]tyrosine and incubation for an additional 5 min. Each value is the mean ± S.E. of seven observations. Key: (a) $P < 0.01$ vs controls.

tially completely inhibits MAO-induced dopamine metabolism in this preparation [25]. Cocaine inhibited by 64 per cent the amount of tyramine associated with the tissue following incubation at 37° (Table 2). A similar inhibition was observed by incubating the tissue at 0°. Thus, cocaine appeared to completely inhibit the temperature-dependent tyramine uptake, with the remaining tyramine associated with the tissue presumably representing material bound to the tissue but not taken up and/or taken up by a temperature- and cocaine-independent mechanism, such as diffusion.

Comparison of amphetamine and tyramine effects on veratridine-induced synthesis stimulation. In the presence of 200 μM amphetamine, veratridine-induced synthesis stimulation decreased from 54 to 10 per cent (Table 3, Expt. I). In contrast, preincubation in the presence of tyramine did not antagonize veratridine-induced synthesis stimulation.

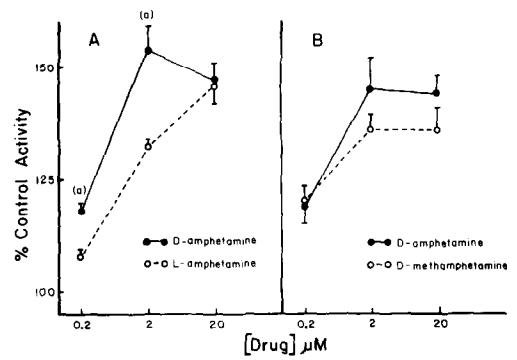

Fig. 4. Comparison of effects of *d*-amphetamine to *l*-amphetamine and *d*-methamphetamine. Aliquots of the striatal P_2 fraction were incubated for 6 min at 37° either in control phosphate-buffered medium or in the presence of the drug indicated, followed by the addition of 10 μM L-[l-^{14}C]tyrosine and incubation for an additional 6 min. Each value is the mean ± S.E. of seven to eleven observations. All drug-treated samples are significantly different from controls at a P value of less than 0.001, except for 0.2 μM *l*-amphetamine ($P < 0.01$). Key: (a) $P < 0.01$ vs corresponding concentration of *l*-amphetamine.

Table 1. Effect of cocaine on tyramine-induced synthesis inhibition*

	Dopamine synthesis (nmoles·hr^{-1}·g^{-1})
Controls	6.04 ± 1.18
Tyramine	4.19 ± 0.18†
Cocaine	6.37 ± 0.19
Cocaine plus tyramine	6.27 ± 0.08

* Aliquots of the striatal P_2 fraction were incubated for 10 min at 37° in control buffer or in the presence of 80 μM cocaine, followed by the addition of 0.2 μM tyramine and 10 μM L-[1-^{14}C]tyrosine and incubation for an additional 10 min. Each value is the mean ± S.E. of seven to eight observations.

† $P < 0.001$ vs controls; $P < 0.001$ vs cocaine plus tyramine samples.

Thus, although 20 μM tyramine significantly inhibited synthesis, treatment with veratridine not only increased synthesis to a rate higher than that observed with tyramine alone, but also to a rate higher than the control value (Table 3, Expt. II).

DISCUSSION

The major structure–activity conclusions to be drawn from the present studies are: (1) addition of a *p*-hydroxy substituent onto the parent phenylethylamine compound can change the effects on dopamine synthesis from excitatory to inhibitory, (2) addition of a β-hydroxy group may somewhat attenuate, but does not prevent or reverse, synthesis stimulation, (3) addition of an *N*-methyl substituent does not lower synthesis stimulation, and (4) the parent phenylethylamine compounds interact with an intracellular regulatory site, possibly related to calcium flux and/or dopamine release, that is not affected by the *p*-hydroxy derivatives.

The last point is made in light of the findings that, although amphetamine markedly antagonized synthesis stimulation produced by veratridine, tyramine did not interfere with veratridine-induced synthesis stimulation (Table 3). We have shown previously

Table 2. Effect of cocaine on tyramine uptake*

	Tyramine uptake (nmoles/g)
37° Controls	0.294 ± 0.028 (8)
37° Cocaine	0.107 ± 0.012† (8)
0° Controls	0.101 ± 0.008† (4)

* Aliquots of the striatal P_2 fraction were incubated for 10 min at the temperature indicated in control buffer or in the presence of 80 μM cocaine, followed by the addition of 100 μM pargyline, 0.2 μM tyramine and 20 μM L-tyrosine, and incubation for an additional 10 min. The Millipore filters were washed with 0.32 M sucrose at the same temperature as the sample incubations. Each value is the mean ± S.E. of the number of observations indicated in parentheses.

† $P < 0.001$ vs controls.

Table 3. Comparison of amphetamine and tyramine effects on veratridine-induced synthesis stimulation*

Treatment	Dopamine synthesis (nmoles·hr^{-1}·g^{-1})	% Respective control
Experiment I		
Controls	5.90 ± 0.15	100 ± 2.6
Veratridine	9.09 ± 0.66†	154 ± 11.2‡
Amphetamine (200 μm)	6.14 ± 0.13	100 ± 2.1
Veratridine plus amphetamine	6.73 ± 0.31	110 ± 5.0
Experiment II		
Controls	6.11 ± 0.15	100 ± 2.5
Veratridine	10.1 ± 0.55†	166 ± 9.0
Tyramine (20 μM)	4.34 ± 0.13†	100 ± 3.0
Veratridine plus tyramine	8.43 ± 0.54§	194 ± 12.4

* Aliquots of the striatal P_2 fraction were incubated for 5 min at 37° in control buffer or in the presence of the indicated concentration of either amphetamine or tyramine, followed by the addition of 10 μM L-[1-^{14}C]tyrosine plus or minus 75 μM veratridine and incubation for an additional 5 min. Each value is the mean ± S.E. of five to eight observations for Experiment I and six to twelve observations for Experiment II.

† $P < 0.001$ vs respective controls.

‡ $P < 0.01$ vs veratridine plus amphetamine.

§ $P < 0.001$ vs tyramine alone; $P < 0.001$ vs controls.

that the synthesis stimulations produced by both phenylethylamine and amphetamine share several properties in common with depolarization-induced stimulation, namely (1) calcium dependency, (2) tyrosine dependency (i.e. synthesis stimulation observed only with saturating concentrations of tyrosine), and (3) non-additivity with combinations of veratridine and either phenylethylamine or amphetamine [12]. All of the data available to date suggest that some sort of calcium flux is necessary for veratridine- [25] and amphetamine-induced stimulations [12, 26], either as a necessary first step for stimulating dopamine release and relieving tyrosine hydroxylase from feedback inhibition, or as a means of producing some other calcium-related event that could serve as a signal for catecholamine synthesis stimulation. If we, for the sake of simplicity, consider the dopamine release concept, then it would appear that the parent phenylethylamine compounds can release dopamine from the same pool as does depolarization. This could explain why their stimulations are not additive. At higher phenylethylamine concentrations (200 μM), release of dopamine from an inhibitory pool would be counterbalanced by a direct vesicular release of dopamine into cytoplasm, where its inhibitory effects on tyrosine hydroxylase would negate the stimulatory effects observed at lower concentrations. Also, at this higher phenylethylamine concentration, veratridine could not stimulate synthesis, since the pool of dopamine that is normally released to activate synthesis would no longer be present. On the other hand, the *p*-hydroxy derivatives, although they can release catecholamines in synaptosomal preparations [7, 10], apparently do not cause release from this postulated inhibitory dopamine pool since: (1) they do not stimulate synthesis over a wide range of concentrations, and (2) they do not block veratridine-induced synthesis stimulation. Although this explanation is based on a dopamine release model of stimulation, a very similar argument could be constructed based on a possible action of calcium not directly related to dopamine release. The inhibition of synthesis by the *p*-hydroxylated compounds could thus be viewed as a result of their displacement of vesicular dopamine without the stimulatory component possessed by the parent phenylethylamine compounds.

Effects on tyrosine uptake do not seem to be involved in phenylethylamine effects on synthesis, since neither amphetamine [12] nor tyramine (present studies) has any significant effect on synaptosomal tyrosine uptake. Direct effects on tyrosine hydroxylase also appear unlikely, since neither amphetamine [14] nor tyramine [27] has any pronounced effects on soluble or particulate tyrosine hydroxylase activity.

The cocaine experiments presented here (Tables 1 and 2) and elsewhere [12, 24] suggest that all of the major phenylethylamine-induced effects that we have observed are dependent upon the uptake of the phenylethylamine compounds into the tissue. Although inhibition of dopamine reuptake may make some contribution to the synthesis stimulation produced by phenylethylamine compounds, the fact that cocaine, at concentrations that markedly inhibit dopamine reuptake, does not activate synthesis during a similar time course of incubation [28] suggests that dopamine reuptake inhibition *per se* is not a sufficient stimulus for synthesis activation.

At the present time, we cannot explain the differences between our results and those of Kuczenski [14] who did not find inhibition by *p*-hydroxyamphetamine or tyramine at pH 6.6. In testing various incubation conditions, we have found tyramine-induced inhibition in a Tris-buffered incubation medium at pH 7.2 (present studies) and also in a phosphate-buffered medium at pH 7.2 and pH 6.6, although inhibition was markedly antagonized at pH 6.2 [21]. Our findings are in agreement with those of Harris *et al.* [15], who found inhibition by *p*-

hydroxyamphetamine at pH 6.7. It should be emphasized that all of the *p*-hydroxylated phenylethylamine compounds that we have tested (tyramine, octopamine, *p*-hydroxyamphetamine and *p*-hydroxynorephedrine) have proved to be inhibitory over a wide concentration range, whereas all of the non-*p*-hydroxylated compounds have been stimulatory, although they can become inhibitory at high concentrations.

The exact role of the naturally occurring phenylethylamine compounds in mediating endogenous or drug-induced behaviors is, of course, still to be ascertained, as is the question of the contribution of the amphetamine and phenylethylamine metabolites to the biochemical and behavioral alterations produced by these agents. It is hoped that the present studies will help to clarify the effects of phenylethylamine substituents on one basic aspect of dopamine biochemistry, namely regulation at the level of transmitter formation within the nerve terminal.

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