Increased Synthesis of Catecholamines in the Intact Rat following Administration of α-Adrenergic Blocking Agents

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SUMMARY

The present study was undertaken to evaluate the effects of α-blocking agents on the rate of catecholamine synthesis *in vivo*. It was found that phentolamine and phenoxybenzamine increase the formation of radioactive norepinephrine and epinephrine in rat tissues when L-tyrosine-^{14}C is used as a precursor but not when L-dopa-^{3}H is the precursor. These findings indicate that α-blocking agents increase catecholamine synthesis by stimulating tyrosine hydroxylase activity.

INTRODUCTION

The rate of synthesis of norepinephrine in sympathetically innervated tissues varies with the degree of nerve stimulation. The latter conclusion, which is based on studies in intact animals (1–4) and in isolated innervated organs (5–10), indicates that a mechanism exists in the nerves for regulating norepinephrine synthesis. Studies *in vivo* with radioactive tyrosine and 3,4-dihydroxyphenylalanine (dopa), two precursors of norepinephrine, suggest that regulation occurs at the tyrosine hydroxylase step (11) apparently through a mechanism involving end-product inhibition (11, 12). In support of this type of regulatory mechanism it has been shown that, in rats, elevation of tissue norepinephrine levels resulting from monoamine oxidase inhibition is accompanied by decreased conversion of tyrosine-^{14}C to norepinephrine. In an attempt to elevate tissue norepinephrine levels even higher, α-adrenergic blockers were administered to permit the rate to tolerate large amounts of injected norepinephrine (5 mg/kg). Surprisingly, it was found that the α-adrenergic blockers phentolamine and phenoxybenzamine themselves brought about a marked increase in norepinephrine synthesis in peripheral tissues and in brain. Details of these studies and a discussion as to their significance are presented in this report.[2]

METHODS

Female Sprague-Dawley rats, 160–200 g, were used in these studies. Phentolamine (5 mg/kg), phenoxybenzamine (25 mg/kg), or diluent was injected intraperitoneally.

Tyrosine-^{14}C (uniformly labeled, 376 μC/μmole) was obtained from New England Nuclear Corporation. 3,4-Dihydroxy-L-phenylalanine (ring-2,5,6-^{3}H, 34.7 mC/μmole) was obtained from Nuclear-Chicago Corporation.

Rates of incorporation of isotope into catecholamines starting from labeled tyrosine or dopa were measured as described previously (13). Tracer doses of either tyrosine-^{14}C (25 μC) or dopa-^{3}H (75–100 μC) were administered intravenously, and animals were killed by decapitation 1 hr

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later. Heart, brain, and adrenal glands were removed and immediately frozen. Blood samples were also taken at this time. Tyrosine, dopa, dopamine, and norepinephrine were isolated from tissues by procedures which have been described previously (14). Norepinephrine was assayed by a modification of the trihydroxyindole procedure (15), and dopamine as described by Drujan *et al.* (16). Tyrosine was determined according to the method of Waalkes and Udenfriend (17). For radioassay all samples were dissolved in Bray's solution (18) and radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer. Counts were corrected to an efficiency of 15% for tritium and 75% for ^{14}C. Beef adrenal tyrosine hydroxylase was purified to the ammonium sulfate stage (19), and enzymatic activity was assayed according to Nagatsu *et al.* (20). To test the effects of α-adrenergic blocking agents on the enzyme *in vivo*, rats were treated with phentolamine (5 mg/kg) and killed 2 hr later or with phenoxybenzamine (25 mg/kg) and killed 22 hr later; their adrenals were removed and homogenized, and tyrosine hydroxylase activity was assayed.

Blood pressure was recorded directly on anesthetized rats through a femoral artery catheter connected to a Statham transducer on a Grass polygraph. The α-adrenergic blocking actions of the drugs were monitored by their prevention of the norepinephrine pressor response.

Statistical analyses were performed using the Student's *t*-test. Those values found to be significantly different at the 0.05 probability level or lower are indicated in the tables.

RESULTS

Effect of phentolamine treatment on the incorporation of label from tyrosine-^{14}C into catecholamines. Phentolamine has been shown to be a short acting α-adrenergic blocking agent (21). Table 1 shows that 1 hr after administration of phentolamine (5 mg/kg) the incorporation of label from tyrosine-^{14}C into norepinephrine in brain and brainstem was increased appreciably above control values. Incorporation of radioactivity into dopamine, although slightly elevated in one experiment, was not statistically different from control values. In the heart and the adrenals the effects of phentolamine on norepinephrine and epinephrine were striking. As shown in Table 2, the incorporation of label from tyrosine-^{14}C into heart norepinephrine and adrenal epinephrine 1 hr after drug administration was increased 3- to 4-fold above control values.

Correlation between the α-adrenergic blocking activity of phentolamine and its effects on the incorporation of label from

TABLE 1

Effect of phentolamine on the incorporation of label from tyrosine-^{14}C into catecholamines of rat brain

Rats were given phentolamine (5 mg/kg) intraperitoneally. One hour later, tyrosine-^{14}C (25 μC) was injected intravenously. One hour following the administration of radioactive tyrosine, the animals were killed, and brain or brainstem was removed and examined for catecholamines. In this and subsequent tables, each number represents the value obtained on a single animal. The different experiments refer to studies carried out on different days.

Expt.	Tissue	Catecholamine assayed	Newly formed catecholamine	
			Control	Phentolamine
			cpm/g tissue	
1	Brain	Norepinephrine	136; 169; 177	289; 237; 262[a]
2	Brainstem	Norepinephrine	388; 472; 404	703; 558; 625[a]
1	Brain	Dopamine	518; 528; 603	750; 716; 617
3	Brain	Dopamine	603; 732; 515	703; 558; 625

[a] Significantly different from control ($p < .05$).

TABLE 2

Effect of phentolamine on the incorporation of label from tyrosine-^{14}C into catecholamines of rat heart and adrenals

Experimental conditions were identical with those described for Table 1.

Expt.	Tissue	Catecholamine assayed	Newly formed catecholamine[a]	
			Control	Phentolamine
			cpm/g tissue	
1	Heart	Norepinephrine	268; 340; 327	1240; 1250; 915[b]
2	Heart	Norepinephrine	340; 239; 276	794; 790; 740[b]
1	Adrenals	Epinephrine	929; 793	2939; 2764[b]

[a] Adrenal data are expressed as counts per minute per adrenal pair.

[b] Significantly different from control ($p < .05$).

tyrosine-^{14}C into catecholamine was demonstrated in another experiment. As shown in Table 3, 1 hr after phentolamine administration, when α-adrenergic blockade was maximal as indicated by a 32% reduction in blood pressure and the prevention of the norepinephrine pressor response, incorporation of label from tyrosine-^{14}C into norepinephrine and epinephrine was increased markedly. After 7 hr, norepinephrine again elicited its normal pressor response; however, the blood pressure was 80% of the control value. At this time, when the blocking effects had largely worn off, incorporation of label from tyrosine-^{14}C into catecholamines had fallen considerably.

Effect of phenoxybenzamine on the incorporation of label from tyrosine-^{14}C into catecholamines. To determine whether similar stimulatory results could be obtained by a long acting α-adrenergic blocking agent, phenoxybenzamine was administered 16 or 22 hr prior to the administration of tyrosine-^{14}C. Table 4 shows that phenoxybenzamine caused a 4- to 5-fold increase in the incorporation of label from tyrosine into norepinephrine in the heart and a 2-fold increase in the brainstem. There was a 3-fold increase in the incorporation of label into adrenal epinephrine.

Effect of α-blockers on the incorporation of label from dopa-^{3}H into catecholamines.

TABLE 3

Incorporation of label from tyrosine-^{14}C into catecholamines 1 or 7 hr following phentolamine administration

Rats were given phentolamine (5 mg/kg) intraperitoneally, and 1 or 7 hr later 25 μC of tyrosine-^{14}C were injected intravenously. One hour following the administration of radioactive tyrosine, the rats were killed and tissues were examined for catecholamines.

Expt.	Tissue	Catecholamine assayed	Newly formed catecholamine[a]		
			Control	1 hr after phentolamine	7 hr after phentolamine
				cpm/g tissue	
1	Heart	Norepinephrine	268; 340; 327	1240; 1250; 915[b]	607; 542[b,c]
2	Heart	Norepinephrine	501; 370		279; 609; 416
1	Adrenals	Epinephrine	929; 793	2939; 2764[b]	1330; 1180[c]
1	Brain	Norepinephrine	136; 169; 177	289; 237; 262[b]	174; 172[c]
1	Brain	Dopamine	518; 528; 603	750; 716; 617	851; 676

[a] Adrenal data are expressed as counts per minute per adrenal pair. For clarity, some data have been repeated from other tables.

[b] Significantly different from control ($p < .05$).

[c] Significantly different from 1-hr values ($p < .05$).

TABLE 4

Incorporation of label from tyrosine-^{14}C into catecholamines 16 hr following phenoxybenzamine administration

Rats were given phenoxybenzamine (25 mg/kg) intraperitoneally. Either 16 (Expt. 1) or 22 hr (Expt. 2) later they were given tyrosine-^{14}C (25 μC) intravenously and killed 1 hr later. Appropriate tissues were removed and analyzed for endogenous and labeled catecholamines.

Expt.	Tissue	Catecholamine assayed	Newly formed catecholamine[a]	
			Control	Phenoxybenzamine
			cpm/g tissue	
1	Heart	Norepinephrine	190; 295; 261	1440; 1210; 993[b]
1	Brainstem	Norepinephrine	402; 479; 461	801; 783; 722[b]
2	Adrenals	Epinephrine	1067; 1248; 1144	2777; 3388[b]

[a] Adrenal data are expressed as counts per minute per adrenal pair.

[b] Significantly different from controls ($p < .05$).

Studies were carried out with dopa-^{3}H as the precursor to bypass the tyrosine hydroxylase step. Table 5 shows the incorporation of label from dopa-^{3}H into catecholamines in heart, brain, and adrenals following treatment with either phentolamine or phenoxybenzamine. In contrast to the effects observed with tyrosine-^{14}C as precursor, the α-blockers did not increase the incorporation of label into catecholamines when dopa-^{3}H was used as the precursor.

Endogenous levels of catecholamines and specific activities of radioactive precursors in the tissues following treatment with phentolamine or phenoxybenzamine. Endogenous levels of catecholamines were determined in all experiments (Table 6). Except for the hearts of phenoxybenzamine-treated animals, tissue levels of norepinephrine and epinephrine were essentially the same as those in controls. Tyrosine and dopa were also isolated from plasma and tissues at the time the animals were killed, and their radioactivity was assayed. As shown in Table 6, neither drug changed the levels of radioactive precursors in the tissues.

Effects of α-adrenergic blocking agents on tyrosine hydroxylase activity. To determine whether α-adrenergic blocking agents have a direct effect on tyrosine hydroxylase,

TABLE 5

Effect of α-blockers on incorporation of label from dopa-^{3}H into catecholamines

Rats were given injections of 5 mg/kg of phentolamine or 25 mg/kg of phenoxybenzamine. One hour following phentolamine administration, the rats received intravenous injections of dopa-^{3}H (Expt. 1, 75μC; Expt. 2, 100 μC). Sixteen hours following phenoxybenzamine, the rats were given 100 μC of tritiated dopa. All animals were killed 1 hour after administration of tritiated dopa. Appropriate tissues were removed for catecholamine assay. Neither drug produced changes which were statistically significant.

Expt.	Tissue	Catecholamine assayed	Newly formed catecholamine[a]		
			Control	Phentolamine	Phenoxybenzamine
				cpm/g tissue	
1	Heart	Norepinephrine	6525; 6897; 6013	4066; 4390; 6515	
2	Heart	Norepinephrine	6350; 7160; 11,500	7840; 8480; 7450	8220; 6520; 5300
1	Brain	Norepinephrine	299; 464; 296	173; 378; 359	
2	Brainstem	Norepinephrine	647; 701; 812	635; 702; 849	742; 680; 631
1	Adrenals	Epinephrine	1240; 1330; 1220	957; 830; 1340	
1	Brain	Dopamine	407; 612; 306	247; 464; 281	

[a] Adrenal data are expressed as counts per minute per adrenal pair.

TABLE 6

Tissue levels of endogenous catecholamines and radioactive precursors following administration of α-blocking agents to rats

Data represent means ± the standard error in parentheses. Each value represents a minimum of three animals except for the 7-hr brain norepinephrine, dopamine, and tyrosine-^{14}C data, which represent two animals.

Tissue	Catecholamine assayed[a]	Endogenous levels[b]				Radioactive precursor administered[c]	Radioactive precursors in tissue			
		Control	Phentolamine 1 hr	Phentolamine 7 hr	Phenoxybenzamine		Control	Phentolamine 1 hr	Phentolamine 7 hr	Phenoxybenzamine, 16 hr
		μg/g tissue								
Heart	N	0.75 (0.04)	0.75 (0.0004)	0.75 (0.06)	0.45 (0.07)	T	1727 (75)	1845 (65)		1768 (67)
						D	2175 (378)	1689 (358)		
Adrenal	E	16.5 (0.64)	18.2 (1.66)	18.1 (2.1)	14.4 (0.72)[d]	D	4531 (953)	4268 (1160)		
Brain	N	0.40 (0.01)	0.41 (0.02)	0.37 (0.007)		T	2325 (31)	2650 (182)	2835 (415)	
	DA	0.78 (0.04)	0.77 (0.06)	1.01 (0.001)		D	699 (172)	543 (145)		
Brainstem	N	0.45 (0.02)	0.45 (0.02)		0.47 (0.01)	T	1830 (33)	1888 (43)		1902 (45)
						D	334 (71)	370 (76)		361 (59)
Plasma						T	2310 (270)	2491 (112)	2716 (121)	
							3460 (254)			3272 (153)[d]

[a] N = norepinephrine; E = epinephrine; DA = dopamine.

[b] Adrenal data are expressed as micrograms per adrenal pair.

[c] Tyrosine-^{14}C radioactivity (T) is expressed as counts per minute per microgram of tyrosine; dopa-^{3}H radioactivity (D) is expressed as counts per minute per gram of tissue.

[d] These values were obtained 23 hr after phenoxybenzamine administration.

the drugs, in concentrations from 1×10^{-6} M to 5×10^{-4} M, were incubated at 21° for periods up to 30 min with a partially purified preparation of the beef adrenal enzyme. Saturating concentrations of tyrosine (1×10^{-4} M) and cofactor, 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine (1×10^{-3} M), were used in the enzyme assay. Neither drug affected tyrosine hydroxylase activity. In addition, neither drug overcame the inhibition of the purified tyrosine hydroxylase by added norepinephrine (1×10^{-3} M), which elicited a 60% inhibition of the enzyme activity.

The possibility remained that α-adrenergic blocking agents, when administered, increase the amounts of tyrosine hydroxylase in tissues by some indirect mechanism. As shown in Table 7, however, 2 hr after phentolamine (5 mg/kg) or 22 hr after phenoxybenzamine (25 mg/kg), when α-adrenergic blockade was still maximal and incorporation of tyrosine-^{14}C into adrenal epinephrine was increased several fold, tyrosine hydroxylase activity in adrenal homogenates was not increased over that of the controls when measured in the presence of saturating concentrations of both substrate and cofactor.

TABLE 7

Activity of adrenal tyrosine hydroxylase following administration of phentolamine or phenoxybenzamine to rats

Rats were given phentolamine (5 mg/kg) and killed 2 hr later, or phenoxybenzamine and killed 22 hr later. The adrenals were removed from each animal, homogenized, and assayed for tyrosine hydroxylase activity.

Treatment	Tyrosine converted to dopa
	mμmoles/adrenal pair/10 min
Control	5.3; 9.0; 8.4; 8.1
Phentolamine	8.3; 7.5; 6.9; 9.3
Control	11.2; 11.1; 11.0; 13.0; 13.3
Phenoxybenzamine	11.2; 10.2; 14.1; 12.9; 13.1

DISCUSSION

The procedures for estimating relative rates of norepinephrine synthesis used here have been validated in many previous studies. Thus, inhibitors of tyrosine hydroxylase diminish conversion of radioactive tyrosine to norepinephrine. When labeled dopa is used to bypass tyrosine hydroxylase, these inhibitors actually bring about an increased incorporation of label into the newly formed norepinephrine by decreasing dilution of the injected dopa-^{3}H. Conversely, when the sympathetic nervous system is stimulated, conversion of labeled tyrosine to norepinephrine is increased while labeling from dopa is usually unchanged or decreased. In the present studies, both phentolamine and phenoxybenzamine produced effects which are consistent with increased synthesis of norepinephrine and epinephrine. The fact that increased incorporation of radioactivity was observed only with tyrosine signifies that the effect of the α-blockers is at the tyrosine hydroxylase step.

The finding that α-adrenergic blocking agents increase the rate of catecholamine synthesis is consistent with previously reported effects of these drugs. Millar *et al.* (22) observed increased plasma levels of norepinephrine and epinephrine following the administration of phenoxybenzamine to dogs. The increased plasma levels paralleled the hypotensive effects of the drug. Benfey *et al.* (23) demonstrated an increase in urinary excretion of norepinephrine and epinephrine in dogs treated with phenoxybenzamine. A prolonged elevation in circulating levels of catecholamines is indicative of an increased rate of synthesis or diminished degradation. Hexamethonium, a ganglionic blocking agent, prevents the phenoxybenzamine-induced increase in urinary catecholamines (23). This would seem to indicate that this effect of phenoxybenzamine is mediated by postganglionic nerve activity.

The decreased receptor response and the consequent hypotension brought about by α-blocking agents appear to result in a stimulation of adrenergic nerve activity to maintain homeostasis. An analogous situation may be the increased synthesis of catecholamines following electrical stimulation of the stellate ganglia (7). An explanation for this is that during increased

nerve activity norepinephrine is released from the nerve, resulting in a lower concentration of catecholamines associated with the inhibitory binding site on tyrosine hydroxylase (24) and consequently a stimulation of tyrosine hydroxylase through the release from end-product inhibition. Such a mechanism for α-blockers is consistent with the observations of Dontas and Nickerson (25), who demonstrated increased splenic nerve activity in the cat following treatment with hypotensive doses of phenoxybenzamine. They suggested that this resulted from an attempted reflex compensation mediated through the baroreceptors and observed that the increase in splenic nerve activity could be eliminated by treatment with a ganglionic blocking agent.

The ability of α-blockers to stimulate catecholamine synthesis in the brain may appear surprising, in that these agents are generally thought to act at peripheral receptors. However, if one postulates that the hypotensive effects of these drugs can induce a reflex compensation involving the baroreceptors and medullary components, then it is not surprising that catecholamine synthesis is stimulated centrally. The area of the central nervous system where this increased synthesis occurs may represent the central component of the reflex arc. It should be noted that the catecholamine synthesis observed centrally was not as great as that seen in the peripheral tissues. This might result from the diluting effect of those central nervous system nerve fibers which are not involved in the maintenance of blood pressure.

It appears that α-adrenergic blocking agents may have even more direct effects on the central nervous system. The α-blocker yohimbine is known to cause marked central effects (26). High doses of other α-blockers have been reported to stimulate the central nervous system, while therapeutic doses have in some instances been associated with sedation and lethargy (27). Chlorpromazine, a compound which may exert central adrenergic blocking activity, has been reported by Burkard *et al.* (28) to increase catecholamine synthesis in brain. Nyback *et al.* (29) later observed that only the synthesis of brain dopamine was increased by treatment with chlorpromazine. In the present experiments, phenoxybenzamine and phentolamine had little, if any, effect on dopamine synthesis, but increased the synthesis of brain norepinephrine.

Phentolamine and phenoxybenzamine have been reported to have actions, other than those associated with α-adrenergic blockade, which could account for the observed increases in catecholamine synthesis (30). Both compounds block norepinephrine uptake by the perfused heart (31–33) and could conceivably release tyrosine hydroxylase from end-product inhibition by a lowering of endogenous catecholamine levels. However, in the intact cat, only phenoxybenzamine has been reported to prevent uptake of norepinephrine by the heart (34), while phentolamine (5 mg/kg) has no effect on norepinephrine uptake. Our results are consistent with these findings, in that in the tissues examined, phentolamine did not deplete catecholamines whereas phenoxybenzamine lowered only heart norepinephrine levels. It would appear, therefore, that effects on catecholamine uptake or release are not requisite factors in the observed increase in catecholamine synthesis produced by the α-adrenergic blockers. It should be noted that the ability of phenoxybenzamine to lower endogenous levels of norepinephrine in the heart might also result in a decreased retention of the newly formed radioactive norepinephrine and thus tend to diminish the observed increase in incorporation of label.

One might conceive of some interaction between α-blockers and the cofactor site of tyrosine hydroxylase, since norepinephrine is a competitive inhibitor of the pteridine cofactor site (24) and α-blockers interact at norepinephrine sites. However, a direct effect of the α-blockers on tyrosine hydroxylase is unlikely, since no changes were detected in the activity *in vitro* of adrenal tyrosine hydroxylase preparations or the activity of adrenal homogenates obtained from phentolamine- or phenoxybenzamine-

treated rats. The possibility that the α-adrenergic blockers can increase the synthesis of catecholamines by lowering the K_m for tyrosine is unlikely, since the tissue and plasma concentrations of tyrosine approach 1×10^{-4} M and the K_m for tyrosine may be as low as 1×10^{-5} M (35, 36). Therefore, a lowering of the K_m for tyrosine should not result in a stimulation of tyrosine hydroxylase activity. This is consistent with previous findings, in which the synthesis of catecholamines *in vivo* was increased by exercise and cold stress without any increase in the tissue levels of tyrosine hydroxylase (4).

REFERENCES

1. J. LeDuc, *Acta Physiol. Scand.* **53**, Suppl. 183, 1 (1961).
2. R. Gordon and S. Spector, *Pharmacologist* **1**, 184 (1965).
3. A. Oliverio and L. Stjarne, *Life Sci.* **4**, 2339 (1965).
4. R. Gordon, S. Spector, A. Sjoerdsma and S. Udenfriend, *J. Pharmacol. Exp. Ther.* **153**, 440 (1966).
5. W. C. Holland and H. J. Schümann, *Brit. J. Pharmacol. Chemother.* **11**, 449 (1956).
6. S. Bygdeman and U. S. von Euler, *Acta Physiol. Scand.* **44**, 375 (1958).
7. R. Gordon, J. V. O. Reid, A. Sjoerdsma and S. Udenfriend, *Mol. Pharmacol.* **2**, 610 (1966).
8. N. Weiner and A. Alousi, *Fed. Proc.* **25**, 259 (1966).
9. R. H. Roth, L. Stjarne and U. S. von Euler, *Life Sci.* **5**, 1071 (1966).
10. G. C. Sedvall and I. J. Kopin, *Life Sci.* **6**, 45 (1967).
11. S. Spector, R. Gordon, A. Sjoerdsma and S. Udenfriend, *Mol. Pharmacol.* **3**, 549 (1967).
12. N. H. Neff and E. Costa, *Fed. Proc.* **25**, 259 (1966).
13. S. Udenfriend, P. Zaltzman-Nirenberg, R. Gordon and S. Spector, *Mol. Pharmacol.* **2**, 95 (1966).
14. S. Udenfriend and P. Zaltzman-Nirenberg, *Science* **142**, 394 (1963).
15. R. J. Crout, *Std. Method. Clin. Chem.* **3**, 62 (1961).
16. B. D. Drujan, T. L. Sourkes, D. S. Layne and G. F. Murphy, *Can. J. Biochem. Physiol.* **37**, 1153 (1959).
17. T. P. Waalkes and S. Udenfriend, *J. Lab. Clin. Med.* **50**, 733 (1957).
18. A. G. Bray, *Anal. Biochem.* **1**, 279 (1960).
19. T. Nagatsu, M. Levitt and S. Udenfriend, *J. Biol. Chem.* **239**, 2910 (1964).
20. T. Nagatsu, M. Levitt and S. Udenfriend, *Anal. Biochem.* **9**, 122 (1964).
21. G. Roberts, A. W. Richardson and H. D. Green, *J. Pharmacol. Exp. Ther.* **105**, 466 (1952).
22. R. A. Millar, E. B. Keener and B. G. Benfey, *Brit. J. Pharmacol. Chemother.* **14**, 9 (1959). (1959).
23. B. G. Benfey, G. LeDoux and K. I. Melville, *Brit. J. Pharmacol. Chemother.* **14**, 142
24. S. Udenfriend, P. Zaltzman-Nirenberg and T. Nagatsu, *Biochem. Pharmacol.* **14**, 837 (1965).
25. A. S. Dontas and M. Nickerson, *J. Pharmacol. Exp. Ther.* **120**, 147 (1957).
26. S. L. Garfield, S. Gershon, T. Sletten, D. M. Sundland and S. Ballon, *Int. J. Neuropsychiat.* **3**, 426 (1967).
27. M. Nickerson, *in* "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gilman, eds.), Ed. 3, p. 552. Macmillan, New York, 1965.
28. W. P. Burkard, K. F. Gey and A. Pletscher, *Nature* **213**, 732 (1967).
29. H. Nybäck, G. Sedvall and I. J. Kopin, *Life Sci.* **6**, 2307 (1967).
30. J. Axelrod, G. Hertting and L. Potter, *Nature* **194**, 297 (1962).
31. L. L. Iversen, *J. Pharm. Pharmacol.* **17**, 62 (1965).
32. A. J. Eisenfeld, L. Krakoff, L. L. Iversen and J. Axelrod, *Nature* **213**, 297 (1967).
33. A. J. Eisenfeld, J. Axelrod and L. Krakoff, *J. Pharmacol. Exp. Ther.* **156**, 107 (1967).
34. G. Hertting, J. Axelrod and L. G. Whitby, *J. Pharmacol. Exp. Ther.* **134**, 146 (1961).
35. S. Udenfriend, *Pharmacol. Rev.* **18**, 43 (1966).
36. M. Ikeda, L. A. Fahien and S. Udenfriend, *J. Biol. Chem.* **241**, 4452 (1966).