Effect of Inhibiting both Catechol-O-Methyl Transferase and Monoamine Oxidase on Cardiovascular Responses to Norepinephrine. (26972)

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Norepinephrine is metabolized *in vivo* largely by 2 enzymes—catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO)(1). It should not be assumed, however, that metabolic destruction of norepinephrine by these enzymes is necessarily the major process by which the amine is inactivated pharmacologically. There is some evidence, in fact, that MAO may not participate in terminating the cardio-vascular actions of norepinephrine administered intravenously in the dog; complete inhibition of MAO in this species fails to potentiate the effects of norepinephrine(2). The following study was designed to obtain comparable data on the effect of inhibiting COMT alone and of inhibiting both enzymes simultaneously. This report describes the effect of enzyme inhibition on the cardio-vascular actions of norepinephrine and on the disappearance of norepinephrine from the circulation after its intravenous injection in the dog.

Methods. Studies were performed in open-chest, vagotomized dogs (13-18 kg) anesthetized with a combination of pentobarbital and barbital. Arterial pressure and cardiac contractile force (measured by a strain gauge arch sutured to the right ventricle) were monitored continously as described previously(2). A control dose of *l*-norepinephrine (1-3 μg/kg) was given intravenously into a femoral vein, and blood pressure and heart force responses were recorded. A potent MAO inhibitor, 1-phenyl-3-hydrazinobutane (JB-835), was then administered intravenously in a dose of 5 mg/kg. After 60-90 minutes had elapsed, 2 intravenous doses of pyrogallol (each 37.5 mg/kg) were given 15 minutes apart in order to inhibit COMT. Five to 10 minutes later the pretreatment dose of *l*-norepinephrine was repeated, and cardio-vascular responses were again recorded. After each injection of norepinephrine, blood samples were drawn at 1-5 minute intervals from a femoral artery; plasma norepinephrine concentration was determined in each sample by a fluorometric method(3). In preliminary studies on the effect of COMT inhibition alone, administration of JB-835 and determination of plasma norepinephrine levels were omitted; the procedure was the same in all other respects. At the end of each experiment COMT activity was determined(4) in the myocardium and liver.

Results. Effect of pyrogallol on cardiovascular actions of l-norepinephrine. Preliminary observations were made in 2 dogs treated only with the COMT inhibitor. After injection of pyrogallol the cardiac contractile force increased slowly and progressively; blood pressure also increased, but to a lesser degree. This response to certain polyphenols, among them pyrogallol, has been shown to be due to central nervous system stimulation(5). Since blood pressure and heart force remained near control levels for only about 15 minutes after the second dose of pyrogallol, the study was limited to a comparison of single injections of *l*-norepinephrine before and after COMT inhibition. There was no potentiation of the peak response of the blood pressure or cardiac contractile force to injected norepinephrine (1-2 μg/kg); however, return of these parameters to baseline values was prolonged somewhat after enzyme inhibition. COMT activity was found to be inhibited 100% in the myocardium and 92-100% in the liver by these doses of pyrogallol.

Effect of pyrogallol plus JB-835 on cardiovascular actions of norepinephrine. Since marked potentiation of the action of norepinephrine did not occur after COMT inhibition alone, the experiment was repeated in 3 dogs treated with a MAO inhibitor (JB-835) in addition to pyrogallol. Combined treatment with pyrogallol and JB-835 produced no augmentation of the peak responses to injected norepinephrine (Fig. 1). However,

TABLE I. Prolongation of Cardio-vascular Responses to L-norepinephrine in the Dog by Combined Administration of JB-835 and Pyrogallol.

Dog	Dose of L-norepinephrine (μg/kg)	Time from onset of response to complete return to baseline value: Blood Pressure Before* (sec.)	Blood Pressure After† (sec.)	Blood Pressure % Increase	Cardiac Contractile Force Before (sec.)	Cardiac Contractile Force After (sec.)	Cardiac Contractile Force % Increase
A	3	150	295	97%	180	320	78%
B	2	200	325	63	160	240	50
C	1	140	140	0	140	155	11

* Before = before administration of enzyme inhibitors (control injection).
† After = after administration of enzyme inhibitors (see text for dosages).

he time required for blood pressure and cardiac contractile force to return completely o baseline was moderately prolonged (Table .); degree of prolongation increased as dosage of norepinephrine was increased. In addition, the inhibition of these enzymes delayed the disappearance of norepinephrine rom the arterial plasma (Fig. 2). This delay was present at all times after the injection, but was most pronounced, relative to he control injection, during the latter phases of the disappearance curve (between 2½ and 10 minutes after injection).

Discussion. These results indicate that he cardio-vascular actions of injected norepnephrine can be terminated in the dog in the unctional absence of both enzymes which netabolize the amine. The pharmacological nactivation of circulating norepinephrine unler these conditions occurs more slowly than ıormal. Part (and perhaps all) of this proongation of action can be attributed to a delayed disappearance of norepinephrine from he blood stream, probably due to decreased destruction of circulating amine by the liver and kidney(3). The failure of combined COMT and MAO inhibition to potentiate markedly the cardio-vascular effects of injected norepinephrine suggests that pharmacological inactivation of circulating norepinephrine does not require the intact function of either enzyme at the receptor sites. This conclusion may not necessarily apply to norepinephrine released directly into the tissues by the sympathetic nerve endings.

Dilution and redistribution, as well as metabolic destruction, undoubtedly contribute to the rapid decline in the norepinephrine concentration of the plasma after single intravenous injections of the amine. However, complete equilibration of norepinephrine with total body water would have to occur within a

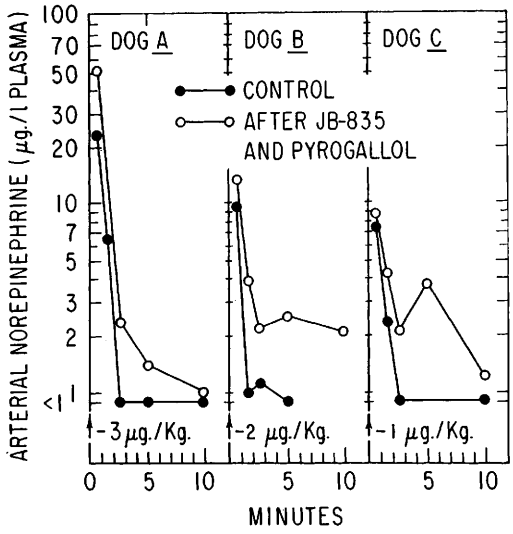

FIG. 2. Concentration of norepinephrine in arterial plasma after single intravenous injections of *l*-norepinephrine in 3 dogs: Before and after simultaneous administration of JB-835 and pyrogallol.

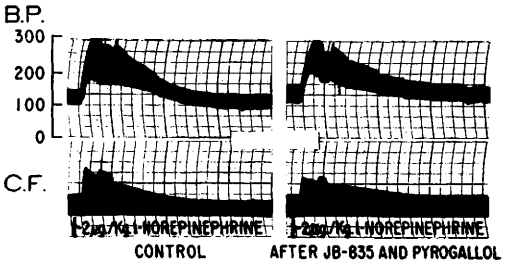

FIG. 1. Effect of JB-835 and pyrogallol on cardio-vascular responses to *l*-norepinephrine (Dog B of Table 1 and Fig. 2). B. P. = blood pressure; C. F. = cardiac contractile force. Each thin vertical line represents 20 sec.

period of 2-2½ minutes to account for the plasma clearance curves of Fig. 2 solely on the basis of redistribution. This rapid rate of clearance, even after the inhibition of amine-metabolizing enzymes, is compatible with the observation that circulating catecholamines are taken up quickly by many tissues of the body(3,6).

Summary. The simultaneous inhibition of catechol-O-methyl transferase and monoamine oxidase (by pyrogallol and JB-835) produced only moderate prolongation of the cardiovascular effects of injected *l*-norepinephrine in anesthetized dogs. This prolongation of pharmacological action in presence of enzyme inhibitors was correlated with a delayed disappearance of injected norepinephrine from the circulation. While these results indicate that metabolism by these enzymes contributes to removal of circulating norepinephrine from the plasma in the dog, they further suggest that the physiological inactivation of circulating norepinephrine at the receptor sites does not require metabolic destruction of the amine by these enzymes.

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1. Axelrod, J., *Physiol. Revs.*, 1959, v39, 751.
2. Goldberg, L. I., Sjoerdsma, A., *J. Pharmacol. Exp. Therap.*, 1959, v127, 212.
3. Crout, J. R., Creveling, C. R., Udenfriend, S., *ibid.*, 1961, v132, 269.
4. Crout, J. R., *Biochem. Pharmacol.*, 1961, v6, 47.
5. Gatgounis, J., Walton, R. P., *J. Pharmacol. Exp. Therap.*, 1960, v128, 363.
6. Axelrod, J., Weil-Malherbe, H., Tomchick, R., *ibid.*, 1959, v127, 251.

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Thyroid: Serum Radioiodide Concentration Ratio During the Estrous Cycle of the Mouse.* (26973)

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The thyroid gland is known to exhibit variations in its activity which are closely correlated with changes in the estrous cycle. The thyroid concentrates more iodide during late estrus in rats(1) and in estrus in rabbits(2). However, in mice it has been reported to be more active during proestrus(3). Because of this apparent species difference it was of interest to reinvestigate thyroid activity in cycling mice. In this investigation thyroid: serum radioiodide concentration ratios (T/S) were determined simultaneously with the identification of the stage of the cycle. This measurement is much less influenced by factors outside the pituitary-thyroid axis than is percentage uptake of I^{131} as has been used by previous investigators. Also, these workers have determined uptakes on the basis of 4 stages of the estrous cycle; it was felt that by subdividing the cycle into 6 stages, more precise information could be obtained concerning the relationship between thyroid function and the reproductive cycle of mice.

Material and methods. Adult Swiss Webster mice weighing between 25 and 30 g were used in this experiment. All were fed Rockland laboratory chow *ad libitum* for at least one week before measurement of the T/S.

To determine T/S, 1 mg of propylthiouracil (PTU) in 0.25 ml of alkaline solution was injected subcutaneously 45 minutes before a subcutaneous injection of 1 μc of carrier-free I^{131} in 0.25 ml of distilled water. One hour later, the animals were anesthetized with ether and blood was obtained from the abdominal aorta. The thyroid was dissected out and weighed on a Roller-Smith torsion balance. One-tenth ml of serum and the thyroid were prepared for radioactivity determination by the method described by VanderLaan and Greer(4).

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