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SHORT COMMUNICATION

The inhibition *in vivo* of norepinephrine synthesis by adrenalone*

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THE BIOGENESIS of norepinephrine involves the oxidation of phenylalanine to tyrosine followed by hydroxylation to 3,4-dihydroxyphenylalanine, decarboxylation to dopamine (3,4-dihydroxyphenylethylamine) and β-hydroxylation of dopamine to norepinephrine. The rate-limiting step in this reaction sequence may be the conversion of dopamine to norepinephrine, and the inhibition of dopamine-β-hydroxylation may produce lower levels of norepinephrine and consequently higher levels of dopamine.

The enzyme that catalyses the conversion of dopamine to norepinephrine was isolated from bovine adrenal medulla[1] and it was shown not to be specific for dopamine. In view of this finding the enzyme was named phenylamine-β-hydroxylase.[2] Many compounds were tested as possible inhibitors of the conversion of dopamine to norepinephrine by the hydroxylating enzyme.[2] Among the compounds thus far tested, adrenalone was the most effective *in vitro*.[3] At a concentration of the substrate, dopamine, of 2 μmoles/ml, the formation of norpinephrine in a standard incubation mixture at pH 6·4 is inhibited approximately 50 per cent by adrenalone in a concentration of 0·2 μmoles/ml. At lower pHs, the inhibition by adrenalone decreases sharply. A Lineweaver-Burk plot revealed that the inhibition by adrenalone is not of a competitive nature. The effective inhibition *in vitro* of norepinephrine synthesis by adrenalone at physiological pHs suggests that this compound may also be active *in vivo*. The inhibition *in vivo* was investigated by a comparison of the content of norepinephrine-H^3 formed from dopamine-H^3 in several organs of rabbits treated and untreated with adrenalone.

Each rabbit was treated with 100 mg iproniazid/kg 16 hr before the infusion of dopamine-H^3. The adrenalone-treated rabbits received 4 mg adrenalone/kg i.p. both 12 hr and 1 hr before the infusion and, in addition, 2 mg/kg i.v. during the infusion of dopamine-H^3; 0·2 mg of dopamine-α-H^3, with the specific activity 10^8 cpm/mg, in a 20-ml solution was infused during a period of 20 min into the rabbit's ear vein. The rabbits were killed one hr after the infusion. The liver, heart, and spleen were removed and homogenized, and the homogenate was deproteinized with 5% trichloracetic acid. After centrifugation the acid metabolites of dopamine and the excess of trichloracetic acid were removed by extraction three times with an equal volume of ethylacetate. The aqueous phase was adjusted to pH4 and the amines were acetylated as previously described.[4] The acetylated amines were chromatographed in the "C" solvent system of Bush, and the dry chromatograms were scanned for radioactive zones.

The radiochromatograms obtained from the spleen of the rabbits treated and untreated with adrenalone are presented in Fig. 1. It is evident that in the spleen of the adrenalone-treated rabbit the content of norpinephrine-H^3 is decreased and the content of dopamine-H^3 is increased, as compared with the control. The radiochromatograms obtained from the hearts and livers of adrenalone-treated rabbits also show a decrease in the norepinephrine-H^3 and an increase in the dopamine-H^3 content, as compared with the controls. Upon elution of the corresponding radioactive peaks, the amounts of norepinephrine-H^3 and dopamine-H^3 in each organ were determined in a Packard liquid scintillation spectrometer. Table 1 shows that the content of norepinephrine-H^3 is decreased approximately 50 per cent in the heart and spleen of adrenalone-treated animals. The content of dopamine-H^3 is increased almost to the same extent in these organs. The inhibition of norepinephrine synthesis by adrenalone in the liver is less effective. The results given in Table 1 are averages of three experiments.

It may also be noted from Table 1 that the total radioactivity of the amines in the tissues, and especially in the heart of the adrenalone-treated rabbits, is somewhat lower than that in tissues of the

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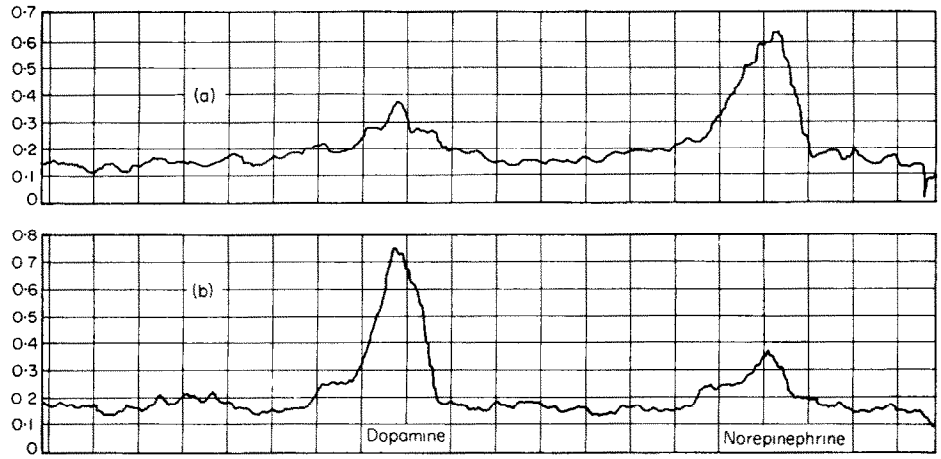

FIG. 1. Radiochromatograms of acetylated catecholamines from rabbit spleens 1 hr after infusion with dopamine-H^3.

A: The rabbit was treated with 100 mg/kg iproniazid 16 hr before infusion of dopamine-H^3.

B: The rabbit was treated as above, and adrenalone was administered as described in the text before the infusion of dopamine-H^3.

TABLE 1. NOREPINEPHRINE-H^3 AND DOPAMINE-H^3 ISOLATED FROM VARIOUS TISSUES OF ADRENALONE-TREATED AND CONTROL RABBITS*

Tissue	Total radioactivity of the amines		Dopamine-H^3		Norepinephrine-H^3	
	Control	Treated	Control	Treated	Control	Treated
Heart	6·9 ± 0·7†	5·0 ± 0·5	0·9 ± 0·09	1·3 ± 0·1	4·2 ± 0·4	2·3 ± 0·2
Spleen	5·1 ± 0·5	4·8 ± 0·5	1·3 ± 0·11	2·6 ± 0·25	3·1 ± 0·3	1·5 ± 0·15
Liver	0·53 ± 0·05	0·44 ± 0·04	0·15 ± 0·01	0·19 ± 0·02	0·28 ± 0·03	0·2 ± 0·02

* In counts per min per 1g tissue × 10^4.

† Standard deviation.

control animals. This result may indicate that adrenalone causes a release of the catecholamines from the tissues. However, the observed decrease in the norepinephrine content of the organs is primarily due to the inhibition of synthesis and not to release of norepinephrine. This is evident from the following findings: (1) the much greater decrease of norepinephrine-H^3 content as compared with the decrease of the total radioactivity; (2) the decrease in the norepinephrine content is associated with a comparable increase of the dopamine content in all organs.

This study has demonstrated that adrenalone is an effective inhibitor *in vivo*. Further studies concerned with the time interval for which the inhibition by adrenalone is effective are now in progress. Since the physiological significance of dopamine and norepinephrine in tissues is still in doubt, the inhibition of phenylamine-β-hydroxylase by adrenalone or other potent inhibitors may be useful in the evaluation of the physiological and pharmacological roles of these two catecholamines separately.

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