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Effects of amphetamine and its hydroxylated derivatives on newly synthesized hypothalamic norepinephrine; study *in vitro*

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Sympathetic stimulations release norepinephrine from a newly synthesized pool [1, 2] as do amphetamine and its hydroxylated derivatives in peripheral tissues [3–5]. In this report we present evidence that newly synthesized norepinephrine is preferentially released in brain tissue by both amphetamine and its metabolites. The latter have a longer biological half-life in the brain and this may explain some aspects of the pharmacological action of amphetamine [6, 7].

Male rats (C. Rivers) received intraventricular (i.v.) injections of 10 μCi/kg of ^{3}H-dopamine (specific radioactivity: 17·5 Ci/m-mole). The animals were killed after 15 min by decapitation and the brain was rapidly removed. The hypothalamus was isolated and sliced into sections. These sections were incubated for 45 min in Mac Ilwain medium with one of the following drugs: *d*-amphetamine (De Laire), *dl*-hydroxyamphetamine (SKF), or *dl*-α-methyloctopamine (Aldrich) at a concentration of 2×10^{-6} moles/ml. Control slices were incubated under the same conditions, but without sympathomimetic amines. At the end of incubation (45 min) the hypothalamic sections were homogenized in 5 ml of $HClO_4$ 0·4 N at 0°. The supernatant was acidified in the same way.

The ^{3}H-norepinephrine was isolated from ^{3}H-dopamine by filtering through a Dowex 50 WX 4 (H+) column [8]. The norepinephrine fraction was purified by alumin adsorption [9]. The endogenous norepinephrine was determined by fluorometric method. There is a 90 per cent recovery using this method [5].

The endogenous norepinephrine levels (μg/g) in hypothalamic slices and the supernatant are shown in Fig. 1. In addition, the newly formed ^{3}H-norepinephrine is expressed as a per cent of total radioactivity.

Amphetamine does not change the level of endogenous norepinephrine in the tissues, even though it does increase the concentration of the transmitter in the supernatant. On the other hand, hydroxyamphetamine and α-methyloctopamine, a false neurotransmitter [10], deplete the tissue of norepinephrine while increasing the level of transmitter in the supernatant. This well established tissue depletion [11, 12] is the result of granular penetration by the hydroxylated derivatives [13]. These findings for endogenous norepinephrine are qualitatively similar to those for newly formed ^{3}H-norepinephrine. There is a relationship between the increase of norepinephrine in the supernatant under the influence of the three amines, and its decrease in the tissue when the slices are incubated with either hydroxyamphetamine or α-methyloctopamine. In the case of amphetamine, the loss of tissue norepinephrine is not significant. In all cases (Table 1) the ratio of the specific radioactivity of the hypothalamic slices and that of the supernatant shows that there is a preferential release of newly synthesized norepinephrine. Despite these similarities, there are large differences in the mechanism of the three amines' activity. With both amphetamine and α-methyloctopamine there is a decrease in the specific radioactivity of tissue norepinephrine and an increase in the specific radioactivity of supernatant norepinephrine. This increase is not very important. It is partially masked by the more important release of endogenous transmitter, resulting from the granular penetration of the false transmitter. In the case of hydroxyamphetamine, there is an

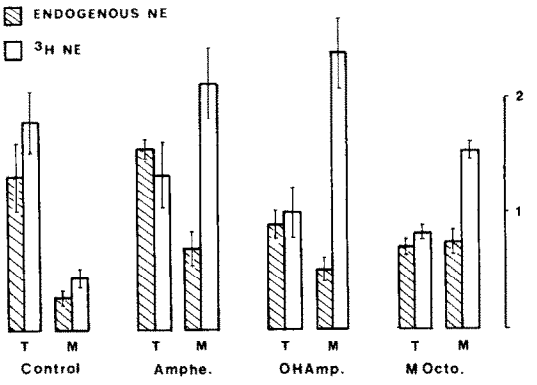

Fig. 1. Endogenous and tritiated norepinephrine (NE) released by amphetamine (Amphe), hydroxyamphetamine (OH-Amp) and α-methyloctopamine (M.Octo.) from hypothalamic slices. In tissues (T) and medium (M), endogenous NE was expressed in μg/g; ^{3}H-NE was expressed as per cent of the whole radioactivity (n = 6).

Table 1. Effects of amphetamine and its hydroxylated derivatives on the specific radioactivity (SR: mCi/m-mole) of norepinephrine (in tissue and medium)

	Hypothalamic SR	Medium SR	R = Hypothalamic SR / Medium SR
Controls	10·3 ± 1·4	11·0 ± 2·2	0·93 ± 0·14
Amphetamine	6·3 ± 1·0*	22·5 ± 3·8*	0·28 ± 0·04*
Hydroxyamphetamine	9·1 ± 1·7	34·3 ± 3·6*	0·26 ± 0·03*
α-Methyloctopamine	6·4 ± 0·3*	15·5 ± 0·7	0·41 ± 0·02*

* $P \leqslant 0{\cdot}01$.

increase in the specific radioactivity of the norepinephrine in the supernatant, but there is no effect in the tissue. These apparently contradictory results can be explained by an accelerated synthesis of ^{3}H-norepinephrine in the tissue.

In conclusion, the three amines studied, having different mechanisms of activity, produce the same results, preferential release of newly synthesized norepinephrine.

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Inhibition of beef plasma amine oxidase by clorgyline

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The antidepressant drug Clorgyline® [*N*-methyl-*N*-propargyl-3(2,4 dichlorophenoxy) propylamine HCl] was first described by Johnston [1] who showed it to be capable of selectively and irreversibly inhibiting the activity of the FAD^+-linked mitochondrial monoamine oxidase toward certain substrates at lower concentrations than were required to inhibit the oxidation of others. Graphs of percentage inhibition vs log. Clorgyline® concentration gave biphasic curves under certain conditions, and several workers now use this as a criterion of multiplicity [2–4]. Recently we have proposed a model for multiplicity of FAD^+-linked monoamine oxidase in rat liver mitochondria [5]. However in a large number of studies in which clorgyline has been used, either crude homogenates of tissue [see e.g. refs 6–10] or a "high speed" (approx. 375,000 *g* min) pellet derived from such an homogenate [see e.g. refs 1–3, 11–13] have been used as the enzyme source. It is possible that certain of these preparations may well be contaminated with the soluble pyridoxal phosphate-Cu^{2+}-dependant monoamine oxidase present in plasma, as it has been shown that this enzyme is capable of adhering to membranes [14] (platelet plasma membranes). Thus it was decided to investigate the effect of Clorgyline® on a purified preparation of beef plasma monoamine oxidase.

Purification of the enzyme was basically by a published procedure [15], up until the calcium phosphate gel step whence ammonium sulphate fractionation was carried out and the activity in the 45–50 per cent (saturation) precipitate was used for assay after dialysis against 3mM potassium phosphate buffer, pH 7·2. The resultant preparation showed a single activity band on continuous polyacrylamide gel (5 per cent) electrophoresis [16] with benzylamine as substrate. The gel being stained for activity using a novel method that has previously been used for staining other