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The role of *p*-hydroxylation of amphetamine in its peripheral mode of action

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Desipramine completely abolished the blood pressure response to (+)-amphetamine in the rat, whereas the tricyclic antidepressant iprindole did not reduce this response. Since both tricyclic drugs inhibit the aromatic hydroxylation of amphetamine, these studies do not support the hypothesis that hydroxylated metabolites of amphetamine, *p*-hydroxyamphetamine and *p*-hydroxynorephedrine are essential for the peripheral sympathomimetic effects of amphetamine.

Peripheral effects of amphetamine e.g. cardiovascular responses are reduced or blocked by tricyclic antidepressants (Sigg, 1959; Bonaccorsi & Hrdina, 1967; Schmidt & Schmidt, 1970). Since imipramine-like drugs inhibit the *p*-hydroxylation of amphetamine, it has been suggested that the hydroxylated metabolites, *p*-hydroxyamphetamine and *p*-hydroxynorephedrine, major metabolites in some species,may mediate the peripheral cardiovascular effects of the parent drug (Sulser & Sanders-Bush, 1970; Clay, Cho & Roberfroid, 1971; Ross & Renyi, 1971). We have tested this hypothesis further, using the antidepressant drug iprindole as a tool, since it has been shown to share, with imipramine-like antidepressants, the ability to inhibit the aromatic hydroxylation of amphtamine in the rat without interfering with neuronal uptake mechanisms (Freeman & Sulser, 1972).

METHODS

Analysis of cardiovascular responses

Male Sprague-Dawley rats (290–310 g) were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}, i.p.) and the trachea, carotid artery and external jugular vein exposed, and cannulated. Increasing doses of (+)-amphetamine were injected intravenously in a volume of 0·1 ml at 10 min intervals through the PE 20 polyethylene catheter in the external jugular vein. The catheter was flushed with 0·1 ml saline after each injection. Heparin sodium (10 mg kg^{-1}, i.v.) was used as an anticoagulant.

Determination of endogenous noradrenaline and of [^3H]noradrenaline and its metabolites

Endogenous noradrenaline was analysed fluorometrically following its elution from alumina (Whitby, Axelrod & Weil-Malherbe, 1961), according to the procedure of Chang (1964). [^{3}H]noradrenaline and its metabolites were analysed by alumina adsorption and ion exchange chromatography according to Freeman & Sulser (1972).

Drugs and radioactive materials

Iprindole and desipramine were donated by Wyeth Laboratories and Geigy

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Pharmaceuticals respectively, and were injected intraperitoneally as the hydrochloride salts. (+)-Amphetamine sulphate was purchased from Sigma Chemical Corp. and was injected as the base. (±)-[^{3}H]Noradrenaline (5–13 Ci mmol^{-1}) and Aquasol were purchased from New England Nuclear Corp.

RESULTS

Comparative effects of desipramine and iprindole on the blood pressure response elicited by (+)-amphetamine

Dose-response curves of blood pressure were obtained after the intravenous administration of (+)-amphetamine (16–660 μg kg^{-1}) with and without pretreatment with iprindole or desipramine (10 mg kg^{-1}, i.p.). (+)-Amphetamine elicited an increase in pressure with the maximum response occurring at 330 μg kg^{-1} (Fig. 1). Pretreatment with iprindole did not appreciably change the dose-response curve of amphetamine, whereas pretreatment with desipramine completely abolished the response to amphetamine. Since both iprindole and desipramine are potent *in vivo* inhibitors of the aromatic hydroxylation of amphetamine (Freeman & Sulser, 1972), the cardiovascular effects of amphetamine clearly are not mediated by *p*-hydroxyamphetamine or *p*-hydroxynorephedrine.

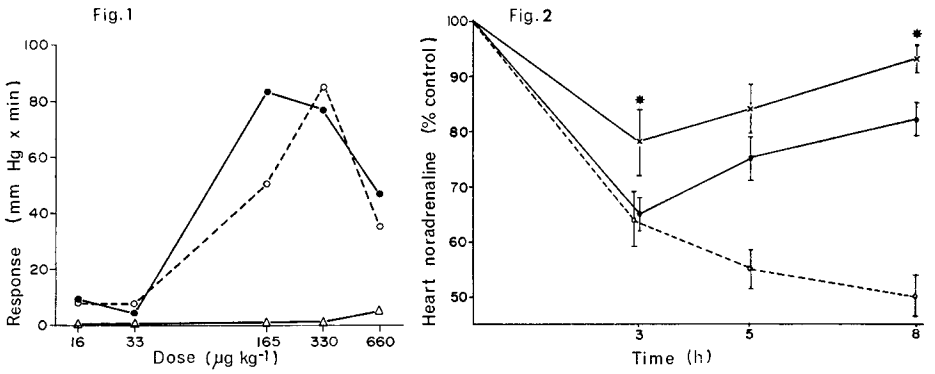

FIG. 1. Comparative effects of pretreatment with desipramine or iprindole on the blood pressure response to amphetamine. Iprindole and desipramine (10 mg kg^{-1}, i.p.) were administered 30 min before the intravenous injection of the lowest dose of amphetamine. Blood pressure response is expressed as the product (maximum height in mm Hg × duration in minutes). Control systolic blood pressure: 134 ± 4 mm Hg. N=6. △——△, Desipramine + amphetamine; ●——● iprindole + amphetamine; ○ - - ○, amphetamine alone.

FIG. 2. Comparative effects of pretreatment with desipramine or iprindole on the amphetamine induced reduction of noradrenaline in the rat heart. Iprindole or desipramine (10 mg kg^{-1} i.p.) were given 30 min before the administration of (+)-amphetamine (5 mg kg^{-1} i.p.). The animals were killed at various times after the administration of amphetamine. Noradrenaline is expressed as % of the control value ±s.e. Control mean values of noradrenaline: 0·93 ± 0·02 μg g^{-1}. N=6. *P <0·05. (Significance of difference from the corresponding iprindole group). ×—× Desipramine + amphetamine; ●——● iprindole + amphetamine; ○ - - - ○ Amphetamine alone.

Comparative effects of desipramine or iprindole on the amphetamine-induced reduction of noradrenaline in the rat heart

To ascertain whether desipramine, in contrast to iprindole, could prevent the release of noradrenaline elicited by amphetamine, iprindole or desipramine (10 mg kg^{-1}) were administered intraperitoneally 30 min before (+)-amphetamine (5 mg kg^{-1}). The

animals were killed at various times after (+)-amphetamine and the hearts analysed for noradrenaline. Pretreatment with desipramine slightly reduced the rate of reduction in heart noradrenaline caused by amphetamine, whereas pretreatment with iprindole did not alter the initial reduction in heart noradrenaline (Fig. 2). Moreover, the level of noradrenaline in the hearts of rats pretreated with desipramine returned to control values sooner than that of rats pretreated with iprindole. In animals given amphetamine alone, the concentration of noradrenaline in heart was still significantly depleted at 8 h. Iprindole or desipramine alone did not cause a significant change in the levels of noradrenaline in the heart.

Effect of desipramine or iprindole on the initial accumulation and metabolism of [^3H]-noradrenaline in the rat heart

[^{3}H]Noradrenaline (100 μCi kg^{-1}) was administered intravenously via the tail vein to unanaesthetized rats, injected 90 min previously with desipramine or iprindole (10 mg kg^{-1}, i.p.). The animals were killed 10 min following the administration of the labelled amine and the hearts were analysed for [^{3}H]noradrenaline and its metabolites.

Pretreatment with desipramine lowered significantly the amount of total radioactivity and [^{3}H]noradrenaline accumulated in the heart. When expressed as a percentage of the total radioactivity, [^{3}H]normetanephrine, [^{3}H]deaminated catechols and [^{3}H]deaminated-*O*-methylated metabolites were increased significantly above control values (Table 1). In confirmation of earlier studies, iprindole did not alter either the initial accumulation or the metabolism of [^{3}H]noradrenaline.

Effect of desipramine or iprindole on the metabolism of [^3H]noradrenaline in the rat heart

As in previous experiments, [^{3}H]noradrenaline (100 μCi kg^{-1}) was administered intravenously 90 min after pretreatment with iprindole or desipramine (10 mg kg^{-1}). The animals were killed 90 min after the administration of the tritiated amine. Again,

Table 1. *Effect of desipramine or iprindole on initial accumulation and metabolism of [^3H]noradrenaline in rat heart.* Desipramine or iprindole were administered 90 min before the intravenous injection of 100μCi kg^{-1} of [^{3}H]noradrenaline. Animals were killed either 10 or 90 min following administration of the labelled amine. Results are expressed as a percent of the total radioactivity ±s.e., n=6.

	Total radioactivity (%)					
	10 min			90 min		
	Control	Desipramine	Iprindole	Control	Desipramine	Iprindole
[^{3}H]Noradrenaline	84 ± 1	24 ± 4[a]	83 ± 1	90 ± 1	56 ± 4[a]	91 ± 0·4
[^{3}H]Normetanephrine	7 ± 1	46 ± 4[a]	7 ± 1	3 ± 1	6 ± 0·3[a]	4 ± 0·3
[^{3}H]Deaminated catechol metabolites	3 ± 0·3	6 ± 1[a]	3 ± 0·2	2 ± 0·2	5 ± 1[b]	2 ± 0·1
[^{3}H]Deaminated-*O*-methylated metabolites	6 ± 2	24 ± 3[a]	7 ± 0·4	4 ± 1	32 ± 3[a]	3 ± 0·1
Total radioactivity (nCi ± s.e.)	430 ± 18	147 ± 9[a]	423 ± 20	395 ± 40	40 ± 5[a]	413 ± 18

[a] $P < 0{\cdot}001$ [b] $P < 0{\cdot}005$

while iprindole did not alter either the amount of total radioactivity or the metabolic pattern of [^{3}H]noradrenaline, desipramine caused highly significant changes in the metabolism of the tritiated amine (Table 1). The total radioactivity was reduced from a control value of 395 to 40 nCi. The radioactivity residing in the noradrenaline fraction was also reduced, while that of normetanephrine was increased, and, unexpectedly, the deaminated and the deaminated-*O*-methylated metabolites of [^{3}H]noradrenaline, expressed as a percentage of the total radioactivity, were still increased significantly if compared to control values.

DISCUSSION

Tricyclic antidepressants such as desipramine block the rise in blood pressure elicited by intravenously administered amphetamine (Sigg, 1959; Bonaccorsi & Hrdina, 1967; Schmidt & Schmidt, 1970). Since desipramine, in addition to its blocking effect on neuronal uptake mechanisms, is a potent inhibitor of the metabolism of amphetamine, it has been suggested that the cardiovascular effects of amphetamine may be elicited by the hydroxylated products of amphetamine, *p*-hydroxyamphetamine or *p*-hydroxynorephedrine. The present results put this hypothesis in doubt because iprindole, a potent inhibitor of the aromatic hydroxylation of amphetamine (Freeman & Sulser, 1972) does not block the blood pressure response to amphetamine in the rat. Gluckman & Baum (1969) reported earlier that iprindole, which does not block the blood pressure response to amphetamine in the dog is only a moderately effective hydroxylator of amphetamine (Dring, Smith & Williams, 1970). Furthermore, Lewander (1971) found that amphetamine depleted noradrenaline in the hearts of guinea-pigs, a species which does not hydroxylate amphetamine (Axelrod, 1954). These results provide additional evidence that the hydroxylated metabolites of amphetamine are not essential for peripheral effects elicited by the parent drug.

Since amphetamine appears to be an indirectly acting sympathomimetic amine in the peripheral autonomic nervous system (Burn & Rand, 1958; Trendelenburg, Muskus & others, 1962), the possibility has to be considered that desipramine may inhibit the uptake of amphetamine into the neurons, thus preventing the release of noradrenaline. A number of investigators have indeed provided evidence that low concentrations of amphetamine are transported into the adrenergic neuron via the amine transport mechanism of the cell membrane and that tricyclic antidepressants interfere with this mechanism (Obianwu, Stitzel & Lundborg, 1968; Lundborg & Waldeck, 1971; Azzaro, Ziance & Rutledge, 1974). Our biochemical data are compatible with such a proposal as the amphetamine-induced initial reduction of heart noradrenaline is less pronounced in animals pretreated with desipramine than in those pretreated with iprindole.

Considering that desipramine completely blocks the blood pressure response to amphetamine, it is, at first glance, difficult to visualize why noradrenaline is depleted to any extent in those animals given desipramine plus amphetamine. However, the effect of amphetamine on blood pressure is an acute phenomenon whereas the curve of noradrenaline depletion in the heart represents effects occurring over a long period of time. It is conceivable that desipramine has completely blocked the initial uptake of the small amounts of amphetamine and thus abolished the blood pressure response whereas in the experiments on noradrenaline depletion, the high concentrations of amphetamine may have partially overcome the blockade by desipramine of the amine

transport. The sustained depletion of noradrenaline in the heart following amphetamine alone is related to the accumulation of PHN in tissue and is prevented by both desipramine (Lewander, 1968; Groppetti & Costa, 1969) and iprindole (Freeman & Sulser, 1972).

It is of interest that in the cns, where the action of amphetamine is potentiated by desipramine, the tricyclic antidepressant causes a shift in the metabolic pattern of noradrenaline consistent with a blockade of uptake of noradrenaline through the neuronal membrane; that is, an increase in the concentration of normetanephrine and a decrease in that of the deaminated catechols (Glowinski, Axelrod & Iversen, 1966; Schildkraut, Dodge & Logue, 1969; Freeman & Sulser, 1972). It remains to be seen whether the shift by desipramine of the metabolic pattern of tritiated noradrenaline in the heart will contribute towards an understanding of the intriguing difference in the DMI-amphetamine interaction in the peripheral (blockade) and central (enhancement) nervous system.

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