Br. J. Pharmac. (1972), **44**, 203–209.

Effect of sympathomimetic drugs in eliciting hypertensive responses to reserpine in the rat, after pretreatment with monoamineoxidase inhibitors

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Summary

1. The effects of some rapidly metabolized sympathomimetic amines, such as β-phenylethylamine and *p*-tyramine, in eliciting hypertensive responses to reserpine in the anaesthetized rat, have been studied.

2. Retardation of metabolism, by pretreatment with the monoamineoxidase inhibitors iproniazid or phenelzine, causes β-phenylethylamine (which in untreated rats has no effect) to induce hypertensive responses to reserpine. Tyramine and other hydroxy substituted phenylethylamines are much less active in this respect, probably because of relatively poor lipid solubility.

3. Hypertensive responses to reserpine are due to catecholamine release, which is believed to be from stores made accessible to indirectly acting sympathomimetic amines with high lipid solubility by an action of reserpine on cell membranes.

Introduction

Reserpine, when injected intravenously into animals pretreated with monoamineoxidase inhibitors or certain indirectly acting sympathomimetic amines induces a sustained hypertensive response or so called ' reserpine reversal '. This phenomenon has recently been reviewed by Schmitt & Schmitt (1970) who consider that the effect is mediated by release of catecholamines as it is blocked by α-adrenoceptor blocking agents and tricyclic antidepressant drugs. Bonaccorsi (1968) concluded that in the anaesthetized rat, blockade of monoamineoxidase was not important for induction of reserpine reversal. The monoamineoxidase inhibitors with the greatest potency were those with the closest structural similarity to the active sympathomimetic amines and were probably acting as such. Surprisingly tyramine and β-phenylethylamine showed little ' reversal ' although their effect in releasing catecholamines is well documented. In the case of tyramine, Yelnosky, McGill & Mastrangelo (1966) attributed the lack of interaction with reserpine to rapid metabolism, presumably by monoamineoxidase, since reserpine induced a hypertensive response in dogs if given during infusion with tyramine and this response faded rapidly after cessation of the infusion. (Similarly, Schmitt & Schmitt (1970) attribute the lack of effect of phenylethylamine and tyramine to the rapid disappearance of these amines from the bloodstream.)

Since reserpine is extensively used in the search for centrally acting drugs, and in the treatment of hypertension it seemed worthwhile to investigate the interaction

of tyramine and phenylethylamine with reserpine in rats pretreated with monoamineoxidase inhibitors. Twenty-four to forty-eight hours after treatment, the monoamineoxidase levels were severely depressed by inhibitors iproniazid and phenelzine, but reserpine did not give a maintained hypertensive response at this time. We expected that tyramine or phenylethylamine would persist in the tissues under these conditions for sufficient time after acute treatment for interaction with reserpine to occur. It was also thought desirable to include in the study short-acting compounds containing β- and *p*-hydroxyl groups, such as phenylephrine and synephrine, as Schmitt & Schmitt (1970) have reported that this type of chemical substitution reduces the ability of indirectly acting sympathomimetic amines to induce ' reserpine reversal '.

Methods

Experiments were carried out in Wistar rats (200–300 g) bred in our laboratories. When urethane (1·5 g/kg, i.p.) was used as an anaesthetic we were unable to obtain pressor responses to reserpine after dexamphetamine, but the response was regularly observed under barbiturate anaesthesia (phenobarbitone sodium, 80 mg/kg, i.p., plus pentobarbitone sodium, 50 mg/kg, i.p. given 15 min later) which was used in all subsequent experiments.

In most experiments, the rats were artificially ventilated at a pressure of 100 mm H_2O (9·8 mbar) to maintain a steady blood pressure. Blood pressure was monitored from the cannulated carotid artery with a Bell & Howell transducer connected to a Devices M4 recorder. Drugs were injected through a polythene cannula into an external jugular vein. Heparin was not administered systemically, but the carotid cannula contained 50 units/ml in 0·9% NaCl solution.

In experiments in which monoamineoxidase inhibitors were used, iproniazid (50 mg/kg, i.p.) was given approximately 48 h before or phenelzine (10 mg/kg, i.p.) 24 h before the anaesthetic. With this dosing schedule histochemical examination of 15 μm sections of brain and liver for monoamineoxidase showed that the levels were severely depressed compared to normal controls. The sections were stained by the tetrazolium method (Glenner, Burtner & Brown, 1957).

The drugs used were dexamphetamine sulphate, ephedrine hydrochloride, phenylephrine hydrochloride, β-phenylethylamine hydrochloride, *p*-tyramine hydrochloride (Sigma) ; noradrenaline hydrogen tartrate (Bayer) ; mephentermine sulphate (Wyeth) ; N-methylamphetamine hydrochloride (May & Baker) ; *p*-chloromethylamphetamine hydrochloride (Roche) and *p*-hydroxyephredine hydrochloride (Emanuel). Iproniazid phosphate (Roche), tranylcypromine sulphate (S.K.F.) and phenelzine hydrogen sulphate (Warner) were prepared from commercially available tablets. Reserpine (B.D.H.) was prepared as a stable solution (2·5 mg/ml) by the method of Leyden, Pomerantz & Bouchard (1956). Doses refer to the base unless otherwise stated. Stock solutions were prepared in distilled water and diluted with 0·9% NaCl solution before use, except for the reserpine which precipitates in 0·9% NaCl solution and was given in aqueous solution throughout. The dose volumes used were 1 ml/kg.

All rats were initially tested for their sensitivity to noradrenaline. Only those rats with a significant but not excessive sensitivity to an intravenous injection of 0·2 μg/kg were used.

Results

The responses obtained to the intravenous injection of reserpine (0·25 mg/kg) in control rats and in rats treated with monoamineoxidase inhibitors or sympathomimetic amines are shown in Table 1. Reserpine 'reversal' was judged to have occurred if the induced pressor responses were well maintained and this is indicated in the table.

Pressor response to intravenous reserpine in control rats

Small acute pressor responses usually occurred when reserpine was injected into control rats given 0·9% NaCl solution intravenously. Schmitt & Schmitt (1970) consider this effect involves a peripheral mechanism, as it is more constant in pithed rats, and that reserpine 'reversal' after amphetamine and other active compounds is a potentiation of this pressor effect.

Pressor response to intravenous reserpine administered after sympathomimetic amines

The results for dexamphetamine, *p*-chloromethylamphetamine, ephedrine, tyramine and β-phenylethylamine are in agreement with those reported by Bonaccorsi. All except tyramine and β-phenylethylamine were effective in inducing a well maintained pressor response to reserpine. Mephentermine was also effective and like the other sympathomimetics, has an α methyl substituent and is thus a poor substrate for monoamineoxidase. *p*-Hydroxyephedrine was less active than ephedrine, whereas its close analogues synephrine and phenylephrine were without effect.

TABLE 1. *Effect of monoamineoxidase inhibitors and sympathomimetic amines on responses to reserpine in anaesthetized rats*

Pretreatment	Dose mg/kg	Route	Time given before reserpine	Peak pressor response (mmHg*)	Type of response†	P‡
Control (0·9% NaCl solution)		i.v.	5 & 10 min	11·7± 4·0	A	—
Dexamphetamine sulphate	5	i.p.	15 min	89·0± 9·0	M	<0·001
Methylamphetamine hydrochloride	5	i.p.	15 min	91·7± 1·7	M	<0·001
p-Chloromethylamphetamine hydrochloride	5	i.p.	15 min	48·3±15·9	M	<0·01
Ephedrine hydrochloride	5	i.p.	15 min	55·0±12·6	M	<0·01
p-Hydroxyephedrine hydrochloride	5	i.p.	15 min	22·5 (2)	M	—
Mephentermine hydrochloride	0·5	i.v.	5 min	78·0±15·9	M	<0·001
p-Tyramine hydrochloride	0·1×2	i.v.	5 & 10 min	15·0± 7·6	A	NS
β-Phenylethylamine hydrochloride	0·1×2	i.v.	5 & 10 min	23·1± 1·9	A	<0·05
Phenylephrine hydrochloride	0·005×2	i.v.	5 & 10 min	22·5± 2·5	A	NS
Synephrine hydrochloride	0·1×2	i.v.	5 & 10 min	10·0 (1)	A	—
Tranylcypromine	5	i.p.	15 min	75·0 (2)	M	<0·001
Phenelzine	10	i.p.	24 h	18·3± 2·1	A (or M)	NS
Iproniazid	50	i.p.	24 h	61·7±24·2	A (or M)	<0·01
	50	i.p.	48 h	22·5± 3·2	A	NS

*Pressor response to reserpine (0·25 mg/kg i.v.). Mean ± standard error where appropriate. Number of experiments in parentheses. †Type of response. A=acute, poorly maintained response; M= maintained pressor response. Parentheses indicate the less frequent response. ‡P determined in Student's t test, difference from 0·9% NaCl controls.

Pressor responses to intravenous reserpine in rats pretreated with monoamineoxidase inhibitors

Treatment with phenelzine 24 h before reserpine induced a slight pressor response which was generally short-lived but occasionally persisted. Iproniazid, given 24 h before reserpine, induced a larger pressor response than phenelzine but when administered 48 h before reserpine only small, brief pressor responses were observed. Tranylcypromine, which is a closer analogue of the sympathomimetics, given 15 min beforehand, induced a large well maintained pressor response to reserpine, confirming the finding of Bonaccorsi, who also observed that tranylcypromine was effective when given 18 h before reserpine.

Responses to intravenous reserpine administered after sympathomimetic amines in rats pretreated with monoamineoxidase inhibitors

The absence of maintained pressor responses to reserpine after pretreatment with iproniazid, given 48 h before and phenelzine, given 24 h before, justified the use of these procedures to reduce severely tissue monoamineoxidase concentrations whilst studying the effect of other drugs on the reserpine response. Tranylcypromine is not suitable as a monoamineoxidase inhibitor in this respect.

Table 2 shows the results obtained with indirectly acting sympathomimetics lacking α methyl substitution (thus being highly susceptible to attack by monoamineoxidase). β-Phenylethylamine, which in normal rats failed to induce 'reserpine reversal' (Fig. 1a), induced pressor responses to reserpine after iproniazid (Fig. 1b), or phenelzine. Tyramine was much less effective (Fig. 1c) and only occasionally produced an effect after either monoamineoxidase inhibitor. Synephrine and phenylephrine were inactive. In these experiments the sympathomimetics were administered intravenously as their cardiovascular effects by this route are well documented. For comparison, we tested dexamphetamine using the same dosage

TABLE 2. *Effect of sympathomimetic amines lacking α methyl substitution on responses to reserpine in anaesthetized rats pretreated with monoamineoxidase inhibitors*

Monoamineoxidase inhibitor		Sympathomimetic amines				
Drug*	Dose mg/kg (i.p.)	Drug	Dose mg/kg i.v.†	Peak pressor response mmHg‡	Type of response§	*P*¶
Iproniazid	50	Phenylethylamine hydrochloride	0·1 × 2	75·0±10·1	M	<0·001
Iproniazid	50	Tyramine hydrochloride	0·1 × 2	30·0±10·0	A (or M)	NS
Iproniazid	50	Synephrine hydrochloride	0·1 × 2	5·0 (1)	A	—
Iproniazid	50	Phenylephrine hydrochloride	0·005 × 2	25·0± 5·0	A	NS
Phenelzine	10	Phenylethylamine hydrochloride	0·1 × 2	65·0± 7·2	M	<0·001
Phenelzine	10	Tyramine hydrochloride	0·1 × 2	15·0± 7·6	A	NS

* Iproniazid given 48 h before and phenelzine given 24 h before reserpine. † Doses given 5 and 10 min before reserpine. ‡ Pressor response to reserpine 0·25 mg/kg intravenously. Mean ± standard error where appropriate. Number of experiments in parentheses. § Type of response. A=acute, poorly maintained response; M=maintained pressor response. Parentheses indicate the less frequent response. ¶ *P* determined in Student's *t* test, difference from 0·9% NaCl controls (Table 1).

regimen. Figures 1d and 1b show that 2×200 μg/kg dexamphetamine were required to induce a pressor response to reserpine in untreated rats comparable with that produced by 2×100 μg/kg phenylethylamine in rats pretreated with iproniazid.

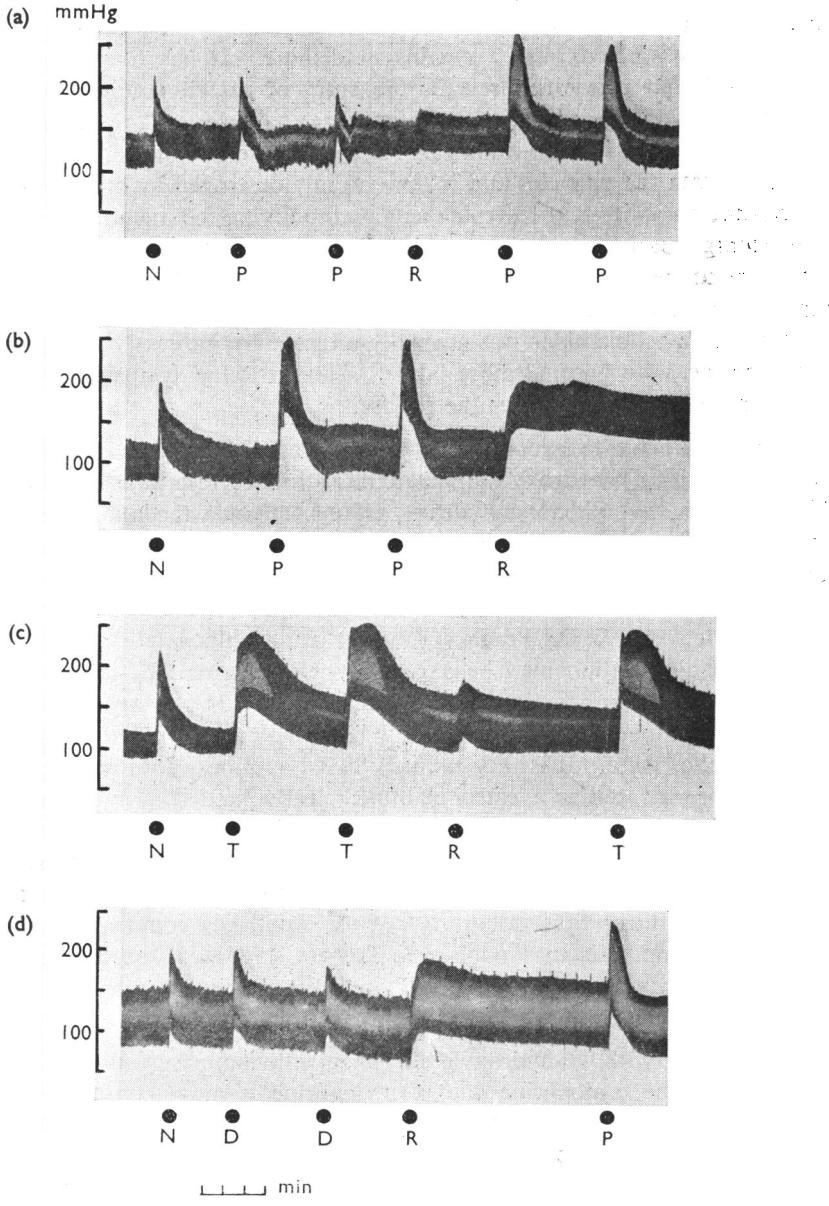

FIG. 1. Pressor responses elicited by reserpine (R), 250 μg/kg, given intravenously, after treatment with (a) β-phenylethylamine (P), 2×100 μg/kg; (b) β-phenylethylamine, 2×100 μg/kg, after iproniazid, 50 mg/kg, given intraperitoneally 48 h previously; (c) tyramine (T), 2×100 μg/kg, after iproniazid given intraperitoneally 48 h previously; (d) dexamphetamine (D), 2×200 μg/kg. Rats were tested for their sensitivity to the pressor action of noradrenaline (N), 0·2 μg/kg, and drugs were given intravenously at about 5 min intervals thereafter.

Discussion

When given shortly after β-phenylethylamine, reserpine induces a maintained pressor response in rats with low monoamineoxidase concentrations. We therefore must conclude that agents capable of inducing this type of response are present in the tissues at the time the reserpine is administered and assume that the reported absence of this effect in normal rats was due to rapid inactivation of phenylethylamine. The mode of action of reserpine in depleting tissue catecholamines has been extensively studied but it remains in dispute. It has been postulated (see Iversen, 1967) that reserpine prevents the entry of catecholamines into a storage compartment within adrenergic nerve storage particles. Noradrenaline, newly synthesized in these particles, cannot be retained in the storage compartment, is lost by diffusion from the particles and is then rapidly destroyed by monoamineoxidase. This hypothesis implies that reserpine acts by modifying the membrane permeability of the storage particles. A similar action is probably involved when reserpine elicits a pressor response in animals pretreated with dexamphetamine or other active sympathomimetic drugs. In this case the reserpine seems to alter permeability so that stores of catecholamines not normally affected by indirectly acting sympathomimetic amines, now become more labile. Thus it is the sympathomimetic amine and not the reserpine that causes the release.

It is more difficult to account for the reduced ability of tyramine and other ethylamines such as *p*-hydroxy amphetamine to induce reserpine reversal. Iversen (1967) considers that phenolic hydroxy groups enhance the affinity for the noradrenaline uptake site in sympathetic nerves, so that tyramine and related compounds would be taken up into the storage granules (and would be unavailable for noradrenaline release). This hypothesis would not be tenable if the *in vitro* findings of Berneis, Pletscher & Da Prada (1969) are applicable *in vivo*. These workers postulate that aggregation may be a general principle for the storage of biogenic amines in the storage organelles of the adrenal medulla and sympathetic nerves, but found that tyramine is weak in forming high molecular weight aggregates with adenosine triphosphate. It is thus unlikely that tyramine would be sufficiently well bound to be inactivated as a catecholamine releaser, and it is more probable that the low activity of tyramine and related compounds is due to poor lipid solubility. High lipid solubility is probably essential for penetration to the storage sites associated with this phenomenon and the introduction of hydroxyl groups into the phenyl moiety reduces lipid solubility. This hypothesis could also apply to the so-called ' tyramine resistant ' pools (see Iversen, 1967) of stored catecholamines, and it may be that ' reserpine reversal ' involves release of catecholamines from these sites.

The situation with β-hydroxylated phenylethylamines such as ephedrine, *p*-hydroxyephedrine, synephrine and phenylephrine is more complicated. In our experiments they were given intravenously, before reserpine, at doses giving approximately equal acute pressor responses. Sympathomimetic amines all have both direct and indirect actions on adrenoceptors and noradrenaline stores, respectively, and the overall effect depends on the relative potency of the two types of action (see Iversen, 1967). Thus in compounds with predominantly direct actions, such as synephrine and phenylephrine, only a small portion of the pressor response would be due to an indirect action (associated with ' reserpine reversal '). Changes in blood pressure will thus not adequately reflect the catecholamine releasing activity

of such compounds and relative potency can only be assessed by direct measurement of noradrenaline efflux. Some separation of direct and indirect effects may be possible by recording pressor responses before and after administration of tricyclic antidepressant drugs, such as imipramine, which block catecholamine release. In estimating the proportion of the pressor response attributable to direct action it would be necessary to take into account the potentiation of directly acting sympathomimetics which occurs after tricyclic antidepressants.

Schmitt & Schmitt (1970) hold the view that only neuronal storage sites are implicated in the hypertensive responses elicited by reserpine. They consider the response to be due to an increased release of noradrenaline from postganglionic sympathetic fibres. A non-neuronal site could well be involved, the noradrenaline originally having accumulated by the 'Uptake-2' process demonstrated by Iversen (1965) in rat heart muscle and later shown to occur in other muscle tissues such as spleen, cat nictitating membrane and vas deferens (see Iversen, 1971).

A similar type of interaction between amphetamine and reserpine has been demonstrated *in vitro* with the rat isolated vas deferens by Laporte, Cuenca, Radriguez & Valdecasas (1969), showing that the phenomenon is not restricted to cardiovascular responses. Reserpine alone at up to 3×10^{-4} g/ml has no effect on this preparation but when added after recovery from a spasmogenic dose of amphetamine, reserpine caused contractions in concentrations of only 5×10^{-6} to 5×10^{-5} g/ml. In common with the effect of reserpine in eliciting hypertensive responses in anaesthetized rats after amphetamine, marked tachyphylaxis was evident (Bonaccorsi, 1968).

There is little doubt that the hypertensive responses to reserpine are due to the release of catecholamines. These experiments indicate that reserpine makes additional stores available to indirectly acting sympathomimetic drugs with comparatively high lipid solubility.

The substantial technical assistance of Mr. D. Burleigh is gratefully acknowledged. I should also like to thank Dr. W. E. Brocklehurst and Dr. B. A. Callingham for help in preparing the manuscript, and Mr. J. Denne for histochemistry.

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(*Received September* 27, 1971)