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# THE MECHANISM OF THE AUGMENTATION OF RESPONSES TO INDIRECTLY ACTING SYMPATHOMIMETIC AMINES BY MONOAMINE OXIDASE INHIBITORS

BY

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The potentiation of the actions of tyramine by monoamine oxidase inhibiting drugs has received considerable attention, largely because of the bizarre reactions in patients on monoamine inhibitors after eating cheese and other foodstuffs containing tyramine. It is generally supposed that tyramine, being a substrate for monoamine oxidase, is normally detoxified by that enzyme in the intestinal wall and the liver, so that little or no dietary tyramine acts systemically. Prevention of detoxification of tyramine by monoamine oxidase inhibitors then results in the appearance of reactions to dietary tyramine, and in potentiation of the action of injected tyramine.

The directly acting sympathomimetic amines adrenaline and noradrenaline are substrates for monoamine oxidase, yet their actions are not potentiated by monoamine oxidase inhibitors (Griesemer, Barsky, Dragstdet, Wells & Zeller, 1963; Furchgott, Weinstein, Huebe, Bozorgmehri & Mensendiek, 1955; Elis, Laurence, Mattie & Prichard, 1967; Trinker, Fearn, McCulloch & Rand, 1967). It is recognized, however, that destruction of these amines by monoamine oxidase is not an important factor in the termination of their actions. Rather, their actions are terminated by them being taken up into adrenergic storage sites or being metabolized by catechol-O-methyl transferase (Axelrod, 1959).

Other sympathomimetic amines have their actions potentiated by monoamine oxidase inhibitors: these include amphetamine, mephentermine and ephedrine. These drugs are not substrates for monoamine oxidase; hence, prevention of their detoxification by inhibition of this enzyme does not explain the augmentation of their effects by monoamine oxidase inhibitors.

Tyramine and amphetamine have in common that they are indirectly acting sympathomimetic amines, and their action is due to noradrenaline released from stores in adrenergic neurones (Burn & Rand, 1958; Trendelenburg, Muskus, Fleming & Sierra, 1962; Muscholl, 1966b). Monoamine oxidase inhibitors cause an increase in the content of monoamines in various tissues (Pletscher, Gey & Zeller, 1960; Crout, Creveling & Udenfriend, 1961; Spector, 1963; Pletscher, Gey & Burkard, 1966). The potentiation of the actions of indirectly acting sympathomimetic amines by monoamine oxidase inhibitors could therefore be caused by the release of greater amounts of noradrenaline from the

increased store. Furthermore, it has been suggested that some of the noradrenaline released from the intraneuronal storage sites may be deaminated before it leaves the neurone by monoamine oxidase in the axoplasm (Smith, 1966). Consequently, inhibition of the enzyme would result in the release of increased amounts from the neurone. It has been suggested that the potentiation of indirectly acting sympathomimetic amines by monoamine oxidase inhibitors indicates the functional significance of intraneuronal monoamine oxidase (Goldberg, 1964; Sjöqvist, 1965; Smith, 1966).

The experiments reported in this paper were undertaken in the first instance to explore the various mechanisms that may be involved in the potentiation of amphetamine by monoamine oxidase inhibitors. This work has some practical value because untoward reactions from combinations of these drugs are not uncommon, and elucidation of the mechanisms involved may aid in predicting, avoiding and treating the reaction. It was also anticipated that the findings from the experiments would have theoretical significance in that they may throw light on the role of monoamine oxidase in adrenergic mechanisms. None of the mechanisms mentioned above explained the potentiation, however, and in fact the results indicate that monoamine oxidase is not involved. The main findings and conclusion were communicated to the meeting of the British Pharmacological Society in April, 1967.

## METHODS

### *Experiments with cats and rats*

Albino rats weighing 190–220 g were pithed by the method of Shipley & Tilden (1947). Cats weighing 2–3 kg were made spinal according to the method described by Burn (1952) Arterial blood pressure was recorded from a carotid artery by a Statham pressure transducer coupled to an Offner Dynograph pen-recorder.

Drugs were injected either systemically by way of a femoral or jugular vein or into the portal circulation by way of a splenic or portal vein.

Some cats were eviscerated after they had been made spinal. In this, the entire gastro-intestinal tract was removed by dividing between pairs of ligatures tied as follows (and in this order): the rectum, inferior mesenteric artery, superior mesenteric artery, coeliac axis, portal vein and bile duct, and oesophagus. The renal arteries were then ligated in the hili of the kidneys.

### *Kitten isolated atria*

Kittens weighing approximately 1 kg were anaesthetized with paraldehyde (4–6 ml./kg) injected intraperitoneally. The heart was promptly removed and the atria were dissected from the ventricles and surrounding tissues. They were suspended in a bath of McEwen solution (McEwen, 1956) at 37° C and vigorously bubbled with 5% carbon dioxide in oxygen. One atrial appendage was pierced by two fine platinum wire electrodes and the other was tied to a strain gauge coupled to an Offner Dynograph pen-recorder. The atria were electrically driven with stimuli of 2–4 V and 5 msec pulse width, at a frequency varying from 180 to 240 pulses/sec. This rate was higher than their intrinsic rate which ranged from 100 to 150 beats/min. The reason for driving the atria at a constant rate was to obviate any effect of changes in heart rate on the amplitude of contractions. The amplification used in recording the amplitude was constant in all experiments so that direct comparisons could be made between them.

### *Drugs*

The following drugs were used: L-adrenaline tartrate, DL-amphetamine sulphate, isocarboxazid, mebanazine oxalate, α-methyldopa, L-α-methyl noradrenaline, nialamide monohydrochloride.

L-noradrenaline bitartrate, pargyline hydrochloride, phenelzine sulphate, proadifen hydrochloride-SKF 525-A, tranylcypromine sulphate and tyramine hydrochloride.

Doses of drugs referred to in the text are in terms of these compounds. Animals that were pretreated with monoamine oxidase inhibitors were given the drugs 16 to 22 hr before an experiment, except that in one group of rats pargyline was given daily for 5 days before the experiment. Animals pretreated with α-methyl dopa were given the drug for 5 consecutive days.

## RESULTS

### *Pressor responses in pithed rats*

*Intravenous injections.* Responses to noradrenaline (10 ng), adrenaline (10 ng), tyramine (50 μg) and amphetamine (50 μg) were obtained in control rats, and in rats pretreated with monoamine oxidase inhibitors. Figure 1 illustrates the comparison of the effects of these drugs between control rats and rats treated with pargyline: the responses to noradrenaline and adrenaline were essentially unchanged, whereas the pressor responses to both tyramine and amphetamine were much enhanced in comparison with those obtained in untreated control rats. Similar observations were made with the other five monoamine oxidase inhibitors and all the data are summarized in Table 1. The mean

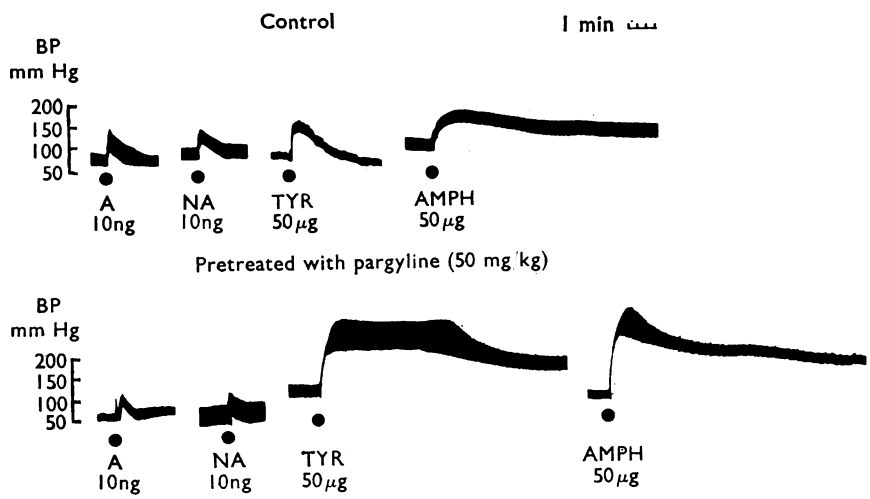


Fig. 1. Records of blood pressure of pithed rats: upper, control rat; lower, rat treated with pargyline (50 mg/kg) injected intraperitoneally on each of 5 days. The pressor responses to adrenaline (A, 10 ng) and noradrenaline (NA, 10 ng) were not significantly modified by pargyline treatment, while responses to tyramine (TYR, 50 μg) and amphetamine (AMPH, 50 μg) were enhanced and prolonged.

values of the pressor responses to noradrenaline and adrenaline in rats pretreated with the monoamine oxidase inhibitors did not differ significantly from the mean values obtained with untreated rats, except for responses to noradrenaline in rats pretreated with tranylcypromine or isocarboxazid which were reduced, being 77% and 63% respectively of those in control rats, the differences being significant at $P<0.02$ by $t$ test. On the other hand, the mean values of the pressor responses to tyramine and amphetamine were

consistently above the mean values in untreated rats, the degree of enhancement of the responses being from 2 times to 2.7 times the control responses: the differences from the control responses were highly significant by $t$ test with values of $P<0.001$ in each case.

TABLE 1

EFFECT OF PRETREATMENT WITH MONOAMINE OXIDASE INHIBITORS ON THE PRESSOR RESPONSES OF PITHED RATS TO SYMPATHOMIMETIC AMINES

* Intraperitoneal injections for 5 days. All other monoamine oxidase inhibitors were administered 18–22 hr before the experiment. † Mean value significantly less than control at $P<0.02$ by $t$ test.

| | | Mean increase in arterial pressure in mm Hg ± S.E.M. | | | |
|---|---|---|---|---|---|
| | No. of animals | Noradrenaline (10 ng) | Adrenaline (10 ng) | Tyramine (50 μg) | Amphetamine (50 μg) |
| Control | 14 | 34·9±1·9 | 28·8±5·4 | 50·0±1·4 | 47·4± 3·9 |
| Nialamide (50 mg/kg) | 6 | 36·3±3·3 | 15·5±0·5 | 120·5±2·6 | 125·0± 9·5 |
| Pargyline* (50 mg/kg) | 6 | 42·1±6·0 | 29·4±2·9 | 115·3±9·3 | 129·7±11·0 |
| Tranylcypromine (25 mg/kg) | 6 | 27·0±1·5† | 22·0±2·7 | 131·0±5·6 | 107·3±11·8 |
| Phenelzine (20 mg/kg) | 6 | 38·6±2·9 | 34·0±1·5 | 129·4±7·9 | 95·5± 5·1 |
| Mebanazine (20 mg/kg) | 5 | 38·2±3·8 | 36·7±2·8 | 129·5±3·7 | 134·7±10·5 |
| Isocarboxazid (50 mg/kg) | 6 | 22·2±1·2† | 18·8±1·4 | 110·0±7·2 | 112·4±11·5 |

The finding that monoamine oxidase inhibitors did not increase the responses to the catecholamines indicates that the enzyme does not play a significant part in terminating the pressor action of injected catecholamines. This indication was further tested by making observations with α-methylnoradrenaline, which is not a substrate for monoamine oxidase. The data are summarized in Table 2. In doses of 10, 20, 30 and 40 ng, noradrenaline and α-methylnoradrenaline were found to be approximately equipotent on the blood pressure of pithed rats; there were no significant differences in responses to these amines at any dose level. Neither catecholamine was affected at any dose level by pretreatment of rats with intraperitoneal injections of nialamide (50 mg/kg) given 18–22 hr beforehand.

TABLE 2

COMPARISON OF PRESSOR RESPONSES TO NORADRENALINE AND α-METHYLNORADRENALINE IN CONTROL AND NIALAMIDE PRETREATED PITHED RATS

| | | Mean increase in blood pressure in mm Hg ± S.E.M. | | | |
|---|---|---|---|---|---|
| | | Control | | Nialamide treated | |
| Dose | No. of animals | Noradrenaline | α-Methyl-noradrenaline | Noradrenaline | α-Methyl-noradrenaline |
| 10 ng | 9 | 29·1±2·7 | 28·1±2·7 | 28·2±1·9 | 30·6±2·4 |
| 20 ng | 10 | 41·7±3·2 | 38·6±3·2 | 40·9±3·3 | 43·6±3·3 |
| 30 ng | 12 | 50·1±5·4 | 53·1±2·8 | 54·6±4·2 | 52·6±3/9 |
| 40 ng | 9 | 64·0±3·5 | 59·0±2·3 | 63·1±7·7 | 65·4±3·6 |

*Intraportal injections.* The liver is an important site for the destruction of sympathomimetic amines because it contains monoamine oxidase and also a microsomal enzyme system (see DISCUSSION). The role of the liver as a site of loss of pharmacological activity

of injected sympathomimetic amines in the pithed rat was determined by comparing the effects of injections into the portal vein and into a systemic vein. The pressor responses obtained with injections into the portal vein are summarized in Table 3. The doses are the same as those used to provide the data on responses to injections into the jugular vein in Table 1 which allows a direct comparison between the responses obtained with the two routes of injection. The mean values of the responses obtained with intraportal injections have been expressed as a percentage of the corresponding values obtained with intrajugular injections and these percentages are given in parentheses in Table 3. The responses were considerably decreased when the drugs were injected intraportally, amphetamine being the most affected.

TABLE 3

PRESSOR RESPONSES TO SYMPATHOMIMETIC AMINES INJECTED INTO THE PORTAL VEIN IN CONTROL AND NIALAMIDE TREATED PITHED RATS

Figures in parentheses express the values of the responses in this table as a percentage of the corresponding response after intrajugular injection as given in Table 1.

| | Mean increase in blood pressure in mm Hg ± S.E.M. | |
|---|---|---|
| | Control rats | Nialamide pretreated rats (50 mg/kg intraperitoneally 18–22 hr before) |
| Noradrenaline (10 ng) | 14·0±1·0 (40%) | 14·3± 1·2 (39·4%) |
| Adrenaline (10 ng) | 8·0±1·0 (27·7%) | 8·0± 1·0 (51·6%) |
| Tyramine (50 μg) | 18·0±1·7 (36%) | 35·0± 6·1 (29%) |
| Amphetamine (50 μg) | 6·0±2·1 (12·7%) | 57·3±16·5 (45·8%) |

In rats pretreated with nialamide, the responses to intraportal injections of noradrenaline and adrenaline did not differ from those in untreated rats, which further confirms the suggestion that monoamine oxidase is not involved in terminating effects of these amines even when they are presented first to the liver which is a rich source of the enzyme. The responses to intraportal injections of tyramine and amphetamine were enhanced in rats pretreated with nialamide, the responses to amphetamine being enhanced more than those to tyramine. Despite these increases in responses to intraportal injections of tyramine or amphetamine, however, their actions were much less marked than those observed when these drugs were given systemically in rats pretreated with nialamide, which indicates that there are sites of loss of activity of these drugs in the rat liver that are not affected by nialamide.

*Rats pretreated with α-methyldopa*

A possible explanation for the enhanced responses to tyramine and amphetamine in rats pretreated with monoamine oxidase inhibitors is that the noradrenaline released from stores in the adrenergic neurones by these indirectly acting sympathomimetic amines is partly metabolized by intraneuronal monoamine oxidase before it leaves the neurone. A test of this explanation was provided by pretreating rats with α-methyldopa so as to replace the noradrenaline in their adrenergic neurones with α-methylnoradrenaline, which is not a substrate for monoamine oxidase. The pressor responses to tyramine and amphetamine of rats pretreated with α-methyldopa did not differ significantly from those in untreated rats. Representative records are shown in Fig. 2A. When treatment with

nialamide was superimposed on rats pretreated with α-methyldopa, however, the responses to tyramine and amphetamine were enhanced (Fig. 2B), the nialamide producing a similar potentiation to that seen in rats without α-methyldopa pretreatment (compare Figs. 1 and 2).

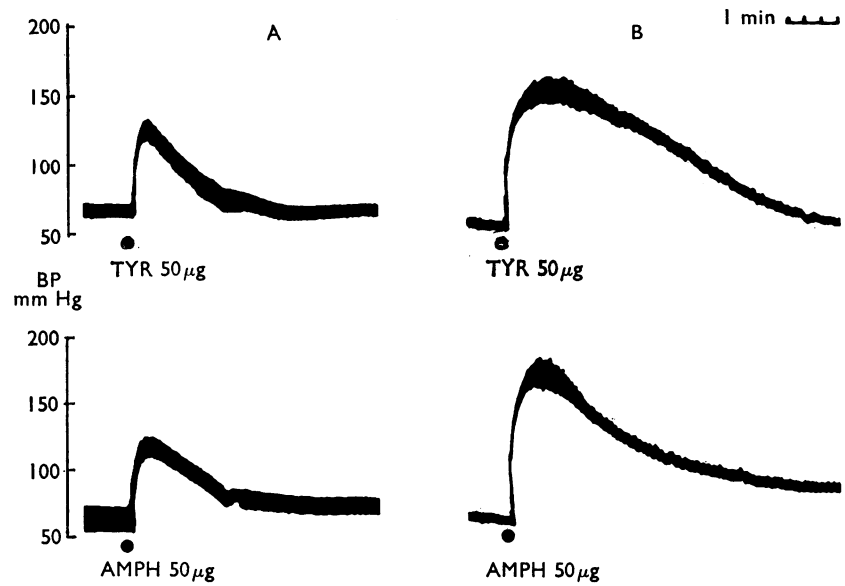


Fig. 2. Blood pressure of pithed rats. A: Pressor responses to tyramine (TYR, 50 μg) and amphetamine (AMPH, 50 μg) in a rat pretreated for 5 days with intraperitoneal injections of α-methyldopa (200 mg/kg/day). B: Observations on a rat pretreated for 5 days with α-methyldopa, and with nialamide (50 mg/kg) treatment superimposed. The nialamide treatment resulted in greatly increased pressor activity of tyramine and amphetamine.

*Experiments with spinal cats*

The pressor effects of injections of tyramine (1 mg) and amphetamine (1 mg) were studied, and the responses obtained by systemic and portal administration of each amine were compared. These doses produced distinct rises of pressure ranging from 50 to 60 mm Hg, but injections into the splenic vein produced almost negligible pressor responses, as illustrated in Fig. 3. Approximately 4 mg of tyramine injected into the splenic vein was necessary to give a pressor response equivalent to 1 mg injected systemically (as shown in Fig. 5). Nialamide (50 mg/kg), injected intraperitoneally 20 hr before the acute experiment, potentiated the actions of tyramine and amphetamine to approximately the same extent, and the magnitudes of the pressor responses were approximately equal irrespective of the route of administration of the drugs (Fig. 3). These findings provide evidence that the liver plays an important part in the termination of the effects of injections of both these sympathomimetic amines in the cat, and that nialamide impairs this role of the liver. Further evidence for this was obtained from experiments carried out on eviscerated cats in which the liver (among other organs) was excluded from the circulation. Figure 4 demonstrates that the pressor actions of amphetamine and tyramine were

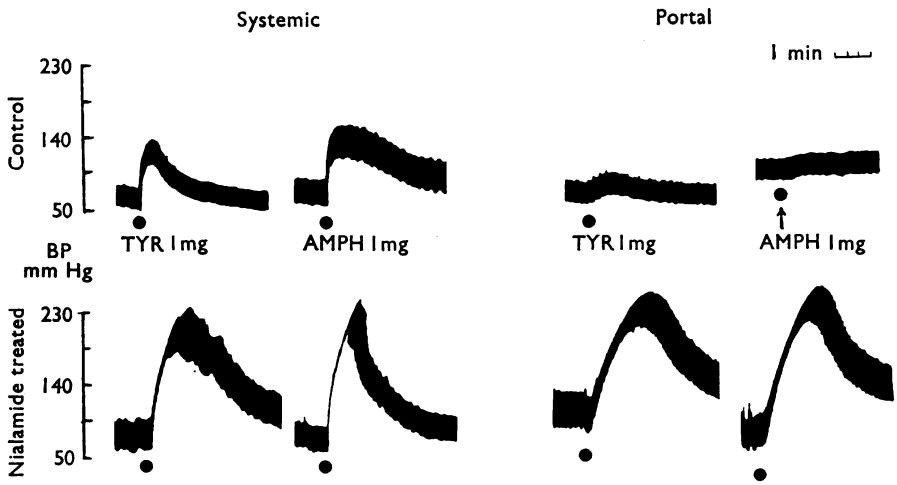


Fig. 3. Pressor responses of spinal cats to tyramine (TYR, 1 mg) and amphetamine (AMPH, 1 mg). Injections into the portal circulation via the splenic vein produced only slight rises in blood pressure which were negligible when compared with the responses produced by systemic administration. In cats pretreated with nialamide (50 mg/kg, injected intraperitoneally) there was a significant enhancement of pressor responses to tyramine and amphetamine and there was little difference in the responses with either the systemic or portal routes of administration.

considerably greater in eviscerated animals than in animals with intact abdominal viscera. Furthermore, in eviscerated spinal cats that were pretreated with nialamide there was no further enhancement of the pressor responses. Table 4 contains a summary of results of a number of similar experiments. It is interesting to note that the mean pressor responses to amphetamine and tyramine in eviscerated cats that were not pretreated with nialamide were almost exactly the same as the mean responses to these drugs in cats with intact viscera that were pretreated with nialamide.

TABLE 4

PRESSOR RESPONSES TO AMPHETAMINE AND TYRAMINE IN SPINAL CATS, COMPARING UNTREATED CATS WITH CATS PRETREATED WITH NIALAMIDE, SYSTEMIC AND INTRA-PORTAL INJECTIONS, AND THE EFFECTS OF EVISCERATION

| | | Mean increase in blood pressure in mm Hg ± S.E.M. | | | |
|---|---|---|---|---|---|
| | | Untreated | | Nialamide treated | |
| | No. of animals | Amphetamine (1 mg) | Tyramine (1 mg) | Amphetamine (1 mg) | Tyramine (1 mg) |
| Systemic injections (femoral vein) | 4 | 54·3±2·2 | 59·1±4·5 | 131·3±0·7 | 107·6±2·8 |
| Portal injections (splenic vein) | 3 | 13·5±1·9 | 18·0±5·2 | 120·6±6·4 | 103·0±2·0 |
| Eviscerated cats | 3 | 127·3±8·9 | 107·3±6·5 | 137·3±6·4 | 113·5±7·8 |

It would seem that monoamine oxidase inhibitors enhance the response to indirectly acting sympathomimetic drugs by inhibiting an enzyme system located in the liver; there are no grounds for assuming that an increase in the amount of noradrenaline released from sympathetic nerve endings contributes to the effect. In the case of

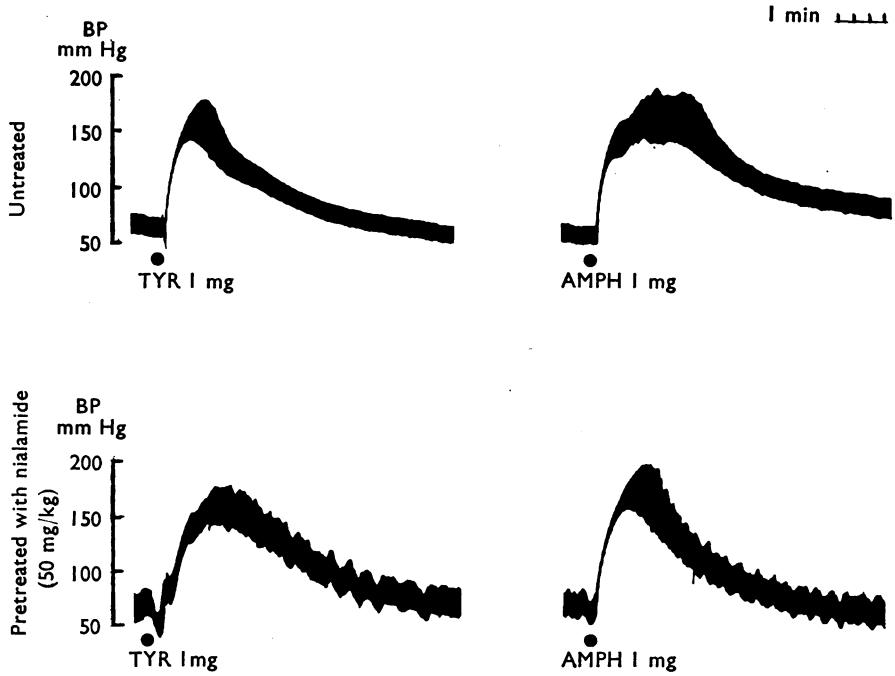


Fig. 4. Pressor responses of eviscerated spinal cats to tyramine (TYR, 1 mg) and amphetamine (AMPH, 1 mg). These injections produced larger increases in the blood pressure than were observed in spinal cats with viscera intact. No greater increase in responses to tyramine or amphetamine was obtained in eviscerated spinal cats pretreated with nialamide (50 mg/kg) injected intraperitoneally 20 hr beforehand.

amphetamine, the enzyme system is probably that located in liver microsomes. In the case of tyramine, it is possible that both monoamine oxidase and the microsomal enzymes are involved. The relative extent to which these two metabolic processes were involved was tested by making observations in cats pretreated with SKF 525-A (20 mg/kg) 1 hr beforehand. The substance SKF 525-A is an inhibitor of liver microsomal enzymes (Brodie, 1956; Kato, Chiesara & Vassanelli, 1964) but does not exhibit any monoamine oxidase inhibiting activity (Dubnick, Morgan & Phillips, 1963). The pressor responses to tyramine were enhanced after treatment of cats with SKF 525-A, and this occurred with injections of tyramine given systemically or into the portal system as illustrated in Fig. 5. In four cats in which observations were made approximately 1 hr after intraperitoneal injections of SKF 525-A (20 mg/kg), the mean pressor responses to intravenous injections of amphetamine (1 mg) and tyramine (1 mg) were $108.8 \pm 8.3$ mm Hg and $136.6 \pm 5.5$ mm Hg, respectively. The degree of enhancement of these responses over control values is approximately the same as that produced by nialamide pretreatment (compare Table 4). These findings indicate that the potentiation of the actions of tyramine and amphetamine is largely the result of inhibition of their metabolism by liver microsomal enzymes. One difference between the effects of SKF 525-A and monoamine oxidase inhibitors on the pressor response to tyramine is that the latter cause

the rapid development of tachyphylaxis, as reported by Day & Rand (1963) and also observed in the present experiments, but there was no tachyphylaxis to tyramine in cats treated with SKF 525-A.

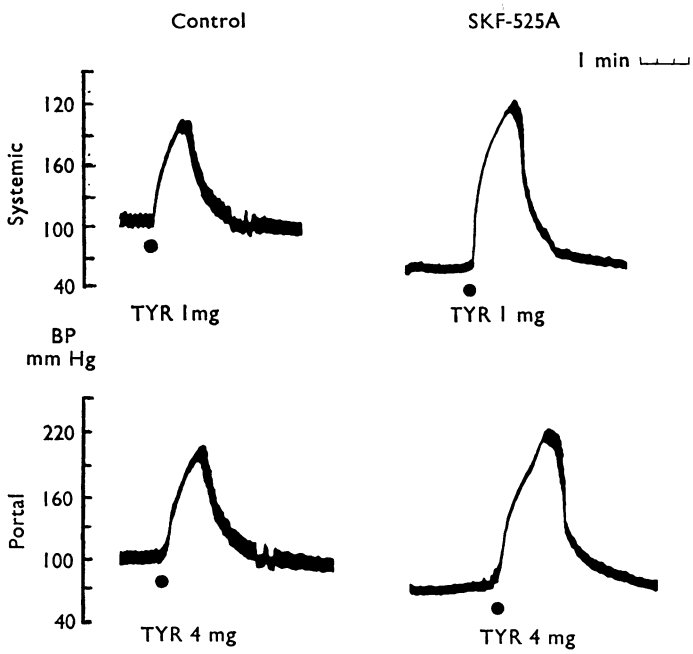


Fig. 5. Pressor responses to tyramine in a spinal cat. Four times the systemic dose of tyramine (TYR, 1 mg) was required to give an aproximately equivalent pressor response when this amine was injected into the portal circulation. SKF 525-A (20 mg/kg), injected intraperitoneally 1 hr previously, potentiated the pressor actions of tyramine injected systemically or into the portal circulation.

*Isolated kitten atria*

In order to determine whether part of the effect was the result of a change in sympathetically innervated effector tissues, experiments were carried out with isolated atria.

Observations were made on the positive inotropic actions of noradrenaline (50 ng/ml.), adrenaline (50 ng/ml.), tyramine (0.5 $\mu$g/ml.) and amphetamine (0.5 $\mu$g/ml.) on atria obtained from normal kittens, and the responses were compared with responses to these drugs in atria taken from kittens pretreated with nialamide (50 mg/kg), injected intraperitoneally 18 to 22 hr beforehand. Figure 6 illustrates that responses to all four sympathomimetic amines were less in atria from a kitten pretreated with nialamide than in atria from an untreated kitten. The mean results from a number of similar experiments are summarized in Table 5.

These findings indicate that inhibition of monoamine oxidase associated with the atria does not lead to potentiation of the actions of sympathomimetic amines.

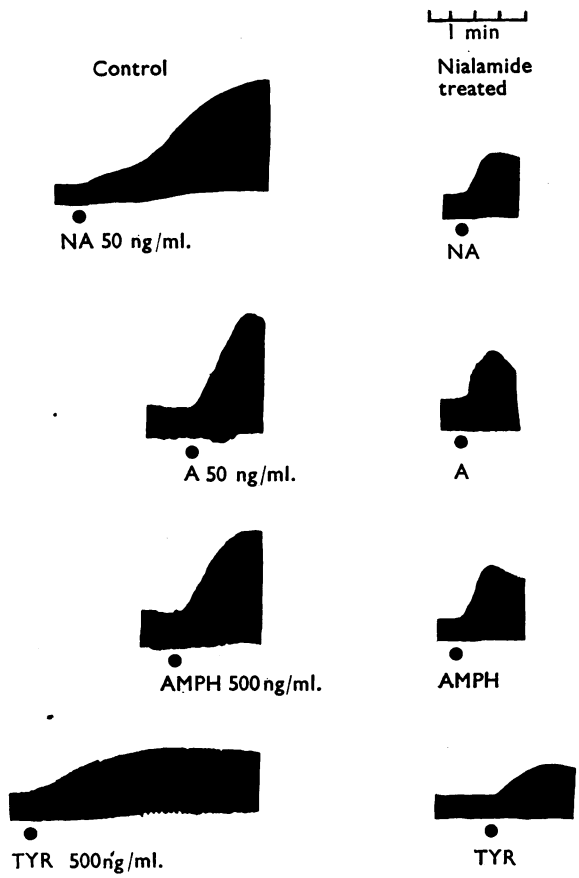


Fig. 6. The positive inotropic effects in isolated kitten atria of noradrenaline (NA, 50 ng/ml.), adrenaline (A, 50 ng/ml.), tyramine (TYR, 500 ng/ml.) and amphetamine (AMPH, 500 ng/ml.). The responses were significantly less in atria taken from a kitten treated with nialamide (50 mg/kg), injected intraperitoneally 18 hr before the experiment.

TABLE 5

POSITIVE INOTROPIC EFFECTS OF SYMPATHOMIMETIC AMINES IN ISOLATED ATRIA FROM UNTREATED AND NIALAMIDE TREATED KITTENS

| | | Increase in amplitude of contraction of atria (mm) | |
|---|---|---|---|
| | No. | Control | Nialamide treated |
| Noradrenaline (50 ng/ml.) | 6 | 28·2±5·3 | 20·0±1·2 |
| Adrenaline (50 ng/ml.) | 5 | 20·0±2·0 | 18·2±1·9 |
| Tyramine (500 ng/ml.) | 4 | 16·2±2·2 | 9·0±2·0 |
| Amphetamine (500 ng/ml.) | 5 | 28·8±4·1 | 17·3±0·8 |

## DISCUSSION

The pressor responses to the indirectly acting amines, tyramine and amphetamine were greatly increased in pithed rats and spinal cats pretreated with the monoamine oxidase inhibitors isocarboxazid, mebanazine, nialamide, pargyline, phenelzine and tranylcypromine. The importance of these observations is reflected in the numerous clinical reports of hypertensive episodes arising from the interaction between indirectly acting amines (whether they be in the form of drugs or constituents of foodstuffs) and the

monoamine oxidase inhibiting drugs available for clinical use (Dally, 1962; Asatoor, Levi & Milne, 1963; Blackwell, 1963; Hodge, Nye & Emerson, 1964; Horwitz, Lovenberg, Engelman & Sjoerdsma, 1964). A possible explanation for the potentiation of the action of tyramine by monoamine oxidase inhibitors is that tyramine reaches its site of action in high concentrations when its enzymatic destruction by monoamine oxidase is inhibited. The action of amphetamine is also potentiated to approximately the same extent, however, yet it is not a substrate for monoamine oxidase.

Tyramine and amphetamine have in common an indirect mode of sympathomimetic action: they both displace noradrenaline from intraneuronal stores. It has been suggested that part of the released noradrenaline is metabolized by monoamine oxidase before it reaches the receptors (Smith, 1966). It is possible, therefore, that monoamine oxidase inhibitors prevent the destruction of noradrenaline after it is displaced from the the binding site, which would result in the release of greater amounts of noradrenaline. In an endeavour to establish the significance of intraneuronal monoamine oxidase in metabolizing the noradrenaline released by tyramine or amphetamine, rats were given large doses of $\alpha$-methyldopa. This results in the replacement of noradrenaline by $\alpha$-methylnoradrenaline in sympathetically innervated tissues (Carlsson & Lindqvist, 1962; Maître & Staehelin, 1963; Day & Rand, 1964; Muscholl, 1966a). The $\alpha$-methylnoradrenaline can be released by the nerve impulse (Muscholl & Maître, 1963) and by indirectly acting sympathomimetic amines (Day & Rand, 1964; Shore, Busfield & Alpers, 1964), but $\alpha$-methylnoradrenaline is not a substrate for monoamine oxidase. The cardiovascular actions of tyramine and amphetamine in rats pretreated with $\alpha$-methyldopa did not differ from those observed in untreated rats. There are contradictory reports in the literature concerning the effect of $\alpha$-methyldopa on the vascular actions of sympathomimetic drugs. Goldberg, DaCosta & Ozaki (1960) reported that, in anaesthetized dogs, pressor responses to noradrenaline and tyramine were not significantly altered by $\alpha$-methyldopa. On the other hand Stone, Ross, Wenger, Ludden, Blessing, Totaro & Porter (1962) claimed that $\alpha$-methyldopa antagonized the cardiovascular actions of amphetamine and phenylethylamine but not those of noradrenaline. Day & Rand (1964) found inconsistent changes in the noradrenaline response (some were increased, others unchanged) and tyramine responses were unchanged after $\alpha$-methyldopa. Tyramine and amphetamine in animals treated with $\alpha$-methyldopa chiefly release $\alpha$-methylnoradrenaline (Shore *et al.*, 1964; Muscholl, 1966a) and the latter was found to be equipotent with noradrenaline in the rat (Maître & Staehelin, 1963; Muscholl & Maître, 1963; Conradi, 1964): these considerations explain our findings of unchanged responses to tyramine and amphetamine in $\alpha$-methyldopa treated rats.

When treatment with the monoamine oxidase inhibitor nialamide was superimposed on the $\alpha$-methyldopa treatment, the cardiovascular responses of amphetamine and tyramine were considerably enhanced. This finding is evidence against the suggestion that the potentiation of the actions of tyramine and amphetamine by monoamine oxidase inhibitors results from the prevention of destruction of the catecholamine released by these indirectly acting sympathomimetic drugs. Furthermore, Kopin, Hertting & Gordon (1962), and Kopin & Axelrod (1963) consider that no deamination occurs when noradrenaline is released by a drug such as tyramine or by nerve stimulation. At this stage in our investigations the possibility of assigning a role to intraneuronal monoamine

oxidase in determining the interaction between monoamine oxidase inhibitors and indirectly acting sympathomimetic amines waned in significance.

It is possible that monoamine oxidase inhibitors could increase the action of indirectly acting sympathomimetic amines by increasing the catecholamine content of tissues. Goldberg (1964), Sjöqvist (1965) and other workers who have reported interactions between monoamine oxidase inhibitors and sympathomimetic amines base their explanations on the following sequence of events: when intraneuronal monoamine oxidase is inhibited the noradrenaline stores increase and hence larger amounts of noradrenaline will be liberated by noradrenaline-releasing drugs. Monoamine oxidase inhibitors have been reported to elevate the content of catecholamines in the brain of rat, rabbit, mouse, monkey and man (Burns & Shore, 1961 ; Crout *et al.*, 1961 ; Wiegand & Perry, 1961 ; Maling, Highman & Spector, 1962 ; Ganrot, Rosengren & Gottfries, 1962 ; Spector, 1963) and in the heart of rat, guinea-pig and dog (Pletscher & Pellmont, 1958 ; Crout *et al.*, 1961 ; Kopin, 1964), but no significant change in the catecholamine level has been found in the brain of cat and dog (Vogt, 1954 ; Spector, Shore & Brodie, 1960 ; Burns & Shore, 1961 ; Maling *et al.*, 1962) or in rabbit and mouse heart (Spector *et al.*, 1960 ; Goldberg & Shideman, 1962). The atria from nialamide treated kittens exhibited diminished inotropic activity in response to direct and indirectly acting sympathomimetics. Monoamine oxidase inhibitors do not change or slightly decrease the catecholamine content of cat cardiac tissue (Bernheim & Bernheim, 1945 ; Goldberg & Shideman, 1962 ; Davey, Farmer & Reinert, 1963 ; Pletscher *et al.*, 1966). A decrease in content is a possible explanation for the diminished responses observed with tyramine and amphetamine. The absolute noradrenaline content of tissues, however, does not necessarily correlate with the activity of indirectly acting sympathomimetic drugs. Thus Smith (1966) found no significant change in the noradrenaline content of atria from guinea-pigs treated with pargyline and yet observed enhanced inotropic effects of tyramine and amphetamine. It may be possible to resolve the discrepancy by arguing that a change in the level of cardiac catecholamines does not allow one to distinguish between different pools of noradrenaline which may be involved in the adrenergic neurone. Furthermore, there is a species difference which is significant, for it has been shown that monoamine oxidase inhibitors do not increase catecholamine levels in cat heart whereas they do in guinea-pig heart (for references, see above). One conclusion which may be drawn from our experiments with kitten atria is that no significant amount of noradrenaline released by either tyramine or amphetamine is normally deaminated before reaching receptor sites. This is in accord with the conclusions reached by other lines of reasoning.

The liver is a rich source of monoamine oxidase and also contains microsomal enzymes that may metabolize amphetamine. Injections of tyramine and amphetamine into the portal vein produced much smaller pressor responses than were obtained with injections into a systemic vein. An interpretation of these results is that tyramine is metabolized in the liver by monoamine oxidase contained in mitochondria (Blaschko, 1952, 1963), and amphetamine by the microsomal enzymes (Axelrod, 1954, 1955). In the rat or guinea-pig, amphetamine predominantly undergoes hydroxylation of the aromatic ring (Axelrod, 1955 ; Dring, Smith & Williams, 1966 ; Ellison, Guzait & Van Loon, 1966). In other species, such as rabbit, monkey and man, the principal route of amphetamine metabolism is oxidative deamination by an enzyme system located in liver microsomes

which is quite distinct from monoamine oxidase (Axelrod, 1954, 1955 ; Asatoor, Galman, Johnson & Milne, 1965 ; Dring *et al.*, 1966 ; Ellison *et al.*, 1966). In dogs, amphetamine undergoes deamination (Dring *et al.*, 1966) or parahydroxylation equally well (Ellison *et al.*, 1966).

It has been shown that monoamine oxidase inhibitors, particularly those with the hydrazine moiety, interfere with enzyme systems other than monoamine oxidase, such as diamine oxidase, choline oxidase (Shore & Cohn, 1960 ; Burkard, Gey & Pletscher, 1960, 1962 ; Gey, Pletscher & Burkard, 1963) and amphetamine oxidase (Fouts & Brodie, 1956). Thus it was not surprising that in rats pretreated with monoamine oxidase inhibitors intraportal injections of tyramine and amphetamine produced increased pressor responses. Nevertheless, the degree of potentiation was much less than that observed when these amines were administered systemically in pretreated rats. An explanation for these differences is that in rats amphetamine undergoes parahydroxylation and monoamine oxidase inhibitors selectively depress the activity of deaminating enzymes in liver microsomes. In cats, there was a more marked potentiation of tyramine given intraportally, and it may be suggested that deamination is the sole route of metabolism in this species.

Evisceration of spinal cats resulted in enhanced pressor responses to tyramine and amphetamine and these responses were quantitatively the same as those in eviscerated animals which had previously received a monoamine oxidase inhibitor. This finding again indicates that the site at which monoamine oxidase inhibitors interact with sympathomimetic amines is solely in the liver.

The microsomal enzyme inhibitor SKF 525-A (Brodie, 1956 ; Kato et al., 1964) does not inhibit monoamine oxidase (Dubnick *et al.*, 1963) and lacks the pharmacological effects of monoamine oxidase inhibitors (Axelrod, Reichenthal & Brodie, 1954 ; Fouts & Brodie, 1955). This drug augmented the cardiovascular actions of tyramine. Thus, although tyramine is a substrate for monoamine oxidase, at least some of the tyramine in passing through the liver may be degraded by non-specific microsomal enzymes.

The experimental evidence indicates that the mechanism by which monoamine oxidase inhibitors enhance the pressor activity of amphetamine and tyramine is by retarding their metabolism in the liver. Other workers (Fouts & Brodie, 1956 ; Brodie, Gillette & La Du, 1958 ; Witt, Brettschneider & Boris, 1961 ; Westermann & Stock, 1962) have suggested that inhibition of the oxidative deaminating enzyme system of liver cell microsomes may be responsible for enhancing the effects of many types of drugs, including barbiturates, and morphine and its congeners as well as amphetamine. Clineschmidt & Horita (1967) observed that phenelzine and pheniprazine potentiated their own inherent sympathomimetic action on the cat nictitating membrane. They found that only phenelzine was a substrate for monoamine oxidase yet both drugs exhibited the phenomenon of auto-potentiation. Even nialamide, which is devoid of any noradrenaline-releasing activity, could enhance the sympathomimetic effect of phenelzine and pheniprazine. It seems reasonable to suggest that both phenelzine and pheniprazine may be substrates for liver microsomal enzymes and hence their auto-potentiating effects could be explained by a common mechanism.

In accordance with the findings of most other investigators (Griesemer *et al.*, 1953 ; Furchgott *et al.*, 1955 ; Elis *et al.*, 1967), we found that the effects of adrenaline and

noradrenaline were unchanged in rats or cats pretreated with any of the six monoamine oxidase inhibitors used. In fact tranylcypromine and isocarboxazid depressed the cardiovascular activity of the two catecholamines, the blood pressure rise and the positive inotropic effect on the heart being less than normal as reported by Crout (1961) and Vanov (1962). Moreover, the effects of $\alpha$-methylnoradrenaline, which is not a substrate for monoamine oxidase, were neither potentiated nor depressed by monoamine oxidase inhibitors. These findings suggest that neither monoamine oxidase nor the liver microsomal enzyme system is concerned in terminating the pharmacological effects of these catecholamines.

The decreased response to the catecholamines after intraportal injection, coupled with the lack of effect of monoamine oxidase inhibitors on these responses, suggests that they may be metabolised by catechol-O-methyl transferase in the liver and not by monoamine oxidase (Axelrod, 1959 ; Kopin, Axelrod & Gordon, 1961 ; Kopin & Gordon, 1963): in addition some of the catecholamine may be taken up into adrenergic storage sites in the liver.

## SUMMARY

1. Pressor responses to the sympathomimetic amines adrenaline, noradrenaline, tyramine and amphetamine were studied in spinal cats and pithed rats.

2. Pretreatment with monoamine oxidase inhibitors (isocarboxazid, mebanazine, nialamide, pargyline, phenelzine or tranylcypromine) resulted in enhancement of the pressor responses to tyramine and amphetamine, but not to adrenaline and noradrenaline.

3. The responses to intraportal injections of sympathomimetic amines were considerably smaller than were responses to equal doses injected into a systemic vein. The responses to intraportal tyramine and amphetamine (but not to to adrenaline or noradrenaline) were enhanced in animals pretreated with nialamide.

4. In eviscerated cats, the responses to tyramine and amphetamine were considerably larger than those observed in intact cats, and the responses were not enhanced by monoamine oxidase inhibitors (nialamide and pargyline).

5. In rats pretreated with $\alpha$-methyldopa, the pressor responses to the four sympathomimetic amines used did not differ from those observed in untreated rats. When pretreatment with a monoamine oxidase inhibitor (nialamide) was superimposed on treatment with $\alpha$-methyldopa, responses to tyramine and amphetamine were enhanced.

6. Pressor responses to $\alpha$-methylnoradrenaline in rats were not affected by pretreatment with a monoamine oxidase inhibitor (nialamide).

7. Pressor responses to tyramine and amphetamine were enhanced in cats treated with SKF 525-A, but responses to adrenaline and noradrenaline were unaffected.

8. The positive inotropic responses in isolated kitten atria to adrenaline, noradrenaline, tyramine and amphetamine were less in atria taken from animals pretreated with a monoamine oxidase inhibitor (nialamide).

9. It is suggested that monoamine oxidase inhibitors potentiate the pressor responses of indirectly acting sympathomimetic amines, not by interfering with the metabolism of

endogenous noradrenaline but by retarding the binding and/or breakdown of these amines within the liver microsomal enzyme system.

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