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TACHYPHYLAXIS TO SOME SYMPATHOMIMETIC AMINES IN RELATION TO MONOAMINE OXIDASE

BY

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Tachyphylaxis to the effects of indirectly acting sympathomimetic amines has been studied on the blood pressure of the cat, rabbit and rat, on the cat spleen and nictitating membrane and on the rabbit heart. The pressor responses to tyramine and to phenethylamine declined slowly with repeated injection; the extent of tachyphylaxis induced by these amines depended on the dosage and on the frequency of injection. The pressor responses to α-methyltyramine and to dexamphetamine (α-methylphenethylamine) declined rapidly with successive injections. The tachyphylaxis induced by one indirectly acting sympathomimetic amine is crossed to others, but not to directly acting amines, such as noradrenaline. In animals treated with nialamide, a drug which inhibits monoamine oxidase, the tachyphylaxis induced by tyramine and by phenethylamine was similar to that produced by their α-methyl derivatives in normal animals. Similar results were obtained when the responses to indirectly acting sympathomimetic amines were studied on the cat spleen *in situ* and on the rabbit heart *in vitro*. Indirectly acting sympathomimetic amines impaired the responses of the cat nictitating membrane to sympathetic nerve stimulation; this effect was most evident with α-methylated amines.

Some sympathomimetic amines which are not catechol derivatives, such as tyramine and amphetamine, exert their effects indirectly by releasing noradrenaline from tissue stores (Burn & Rand, 1958; Axelrod, Gordon, Hertting, Kopin & Potter, 1962). Euler & Lishajko (1960) and Schümann (1960) found that these amines displaced noradrenaline from chromaffin granules isolated from the splenic nerve: using granules from the adrenal medulla Schümann & Philippu (1962) found that noradrenaline was released almost stochiometrically by the indirectly acting sympathomimetic amines.

Responses to successive doses of indirectly acting sympathomimetic amines usually become smaller; that is tachyphylaxis develops. Blaschko (1962) said about the indirectly acting sympathomimetic amines: "The simplest picture we can make of the action of tyramine is that of an exchange of the stored catechol amine with tyramine. Once the tyramine has replaced the catechol amine, it has probably exerted its main effect; it is bound and then presumably slowly removed. The binding of amine at the site of storage may be responsible for the phenomenon of tachyphylaxis: while some of the tyramine is still present, a second dose is less effective. Tachyphylaxis is most marked in substances like amphetamine or ephedrine, amines that are immune from attack by amine oxidase."

Alles (1933) stated that responses to amphetamine (α-methylphenethylamine) declined more rapidly than did responses to phenethylamine, and responses to α-methyltyramine declined more rapidly than those to tyramine. Winder, Anderson & Parke (1948) studied the occurrence of tachyphylaxis to phenethylamine and five of its derivatives: there was least sign of tachyphylaxis with phenethylamine and most with derivatives having an α-methyl group. Cowan, Koppanyi & Maengwyn-Davies (1961) found that in the cat the responses to successive injections of ephedrine and amphetamine, each of which contains an α-methyl group, rapidly diminished whilst the responses to tyramine, which is not α-methylated, declined only gradually. Blaschko, Richter & Schlossman (1937) showed that the introduction of an α-methyl group into the molecule of a sympathomimetic amine rendered the amine immune to destruction by monoamine oxidase.

The purpose of our investigation was to determine the influence of the enzyme monoamine oxidase on the development of tachyphylaxis to some indirectly acting sympathomimetic amines.

METHODS

Blood pressure. Spinal cats were prepared by the method of Burn (1952). Other cats were anaesthetized with a mixture of chloralose and pentobarbitone (80 and 6 mg/kg respectively) given intraperitoneally. Rabbits were anaesthetized with urethane (1.5 g/kg) given intravenously. Rats were injected with atropine (1 mg/200 g) and pithed by the method of Shipley & Tilden (1947). Blood pressure was recorded by a mercury manometer from a carotid artery and drugs were injected into a femoral or jugular vein.

Spleen volume. The spleen volume of anaesthetized cats was recorded by the method described by Burn (1952).

Contractions of the nictitating membrane. Contractions of the nictitating membrane were recorded with a frontal writing lever with magnification of five- to six-times, exerting a tension of about 1.5 g. The postganglionic cervical sympathetic nerves were stimulated by rectangular wave pulses from an electronic stimulator via bipolar platinum electrodes. Stimulus parameters were 2 msec duration, 5 V and frequencies of 0.1 to 100 shocks/sec, applied for 10 sec at 2 min intervals.

Isolated heart. Rabbit hearts were perfused by Langendorff's method with McEwen's (1956) solution bubbled with 5% carbon dioxide and 95% oxygen and maintained at 37° C.

Treatment with nialamide. Solutions of nialamide containing 10 mg/ml. in 0.9% saline were prepared by gently warming. Intraperitoneal injections of nialamide were given to animals 4 to 24 hr before an acute experiment. Doses were: cats, 15 mg/kg; rabbits, 20 mg/kg; and rats, 50 mg/kg.

Note on the use of nialamide. In these experiments we were concerned with observations on the actions of indirectly acting sympathomimetic amines. Many monoamine oxidase inhibitors possess sympathomimetic activity, and the use of these drugs would have obscured the purpose of our experiments. Such drugs include phenelzine, pheniprazine and tranylcypromine; nialamide is free from sympathomimetic actions (Ryall, 1961).

RESULTS

Blood pressure

Spinal cats

Effect of tyramine on the blood pressure. The pressor responses to successive injections of tyramine given to a spinal cat usually increased slightly for one or two injections and then subsequently declined. The rate at which the responses

diminished depended on the dose of tyramine and the interval between injections. Fig. 1 is from an experiment in which tyramine (4 mg at T) was injected intravenously every 15 min: the pressor responses to the second, third and fourth injections are slightly greater than that to the first. Thereafter the responses became progressively smaller until by the eighth injection the response was 60% of that to the first injection. The ninth injection was 4 mg of α-methyltyramine (αMT); it produced a very much smaller response than was usually seen in cats not rendered tachyphylactic to tyramine, which suggests that the tachyphylaxis was not specifically against tyramine but extended to the α-methyl derivative.

Effect of α-methyltyramine. The first injection of α-methyltyramine (4 mg) into a spinal cat produced a pressor response that was considerably more prolonged than that to a similar dose of tyramine. This has been reported for pressor effects in

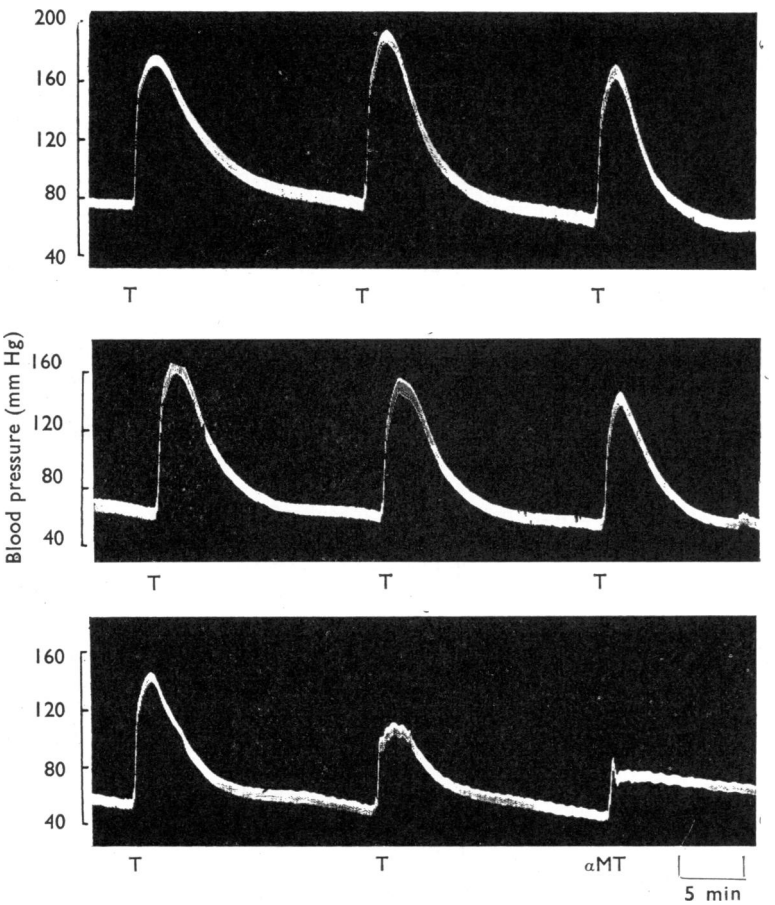

Fig. 1. Pressor responses of a spinal cat (3.0 kg) to 4 mg doses of tyramine (T), repeated at 15 min intervals. The records are continuous. The responses gradually declined and the eighth injection (lowest record) caused a response only 60% of the size of the first response. The ninth injection was of 4 mg of α-methyltyramine (αMT); the response to this substance, after partial tachyphylaxis to tyramine, was much smaller than that seen in control cats (cf. Fig. 2).

dogs by Alles (1933). However, the responses to successive doses of α-methyltyramine declined much more rapidly than those to tyramine. Fig. 2 (upper tracing) shows the initial pressor responses of a spinal cat to noradrenaline (2 and 4 μg), tyramine (4 mg) and α-methyltyramine (4 mg). Between the two parts of the experiment illustrated in Fig. 2 two further doses of α-methyltyramine (4 mg) were administered. The lower tracing shows that the response to the fourth injection of α-methyltyramine was only 23% of the initial response to this amine. Then a second dose of tyramine (4 mg) caused a response only 20% of its initial response. Thus, the tachyphylaxis induced by repeated injections of α-methyltyramine extended to tyramine. The responses to the directly acting sympathomimetic amine, noradrenaline, in Fig. 2 were enhanced after the production of tachyphylaxis to the indirectly acting amines. Thus the reduced responses elicited by successive injections of an indirectly acting sympathomimetic amine cannot be explained by a decreased sensitivity of the cardiovascular system to pressor substances.

Effect of tyramine after a monoamine oxidase inhibitor. The α-methyl group in α-methyltyramine renders the drug immune to destruction by monoamine oxidase. Thus, after inhibition of monoamine oxidase it might be expected that the effects of tyramine would more closely resemble those of its α-methyl derivative. This

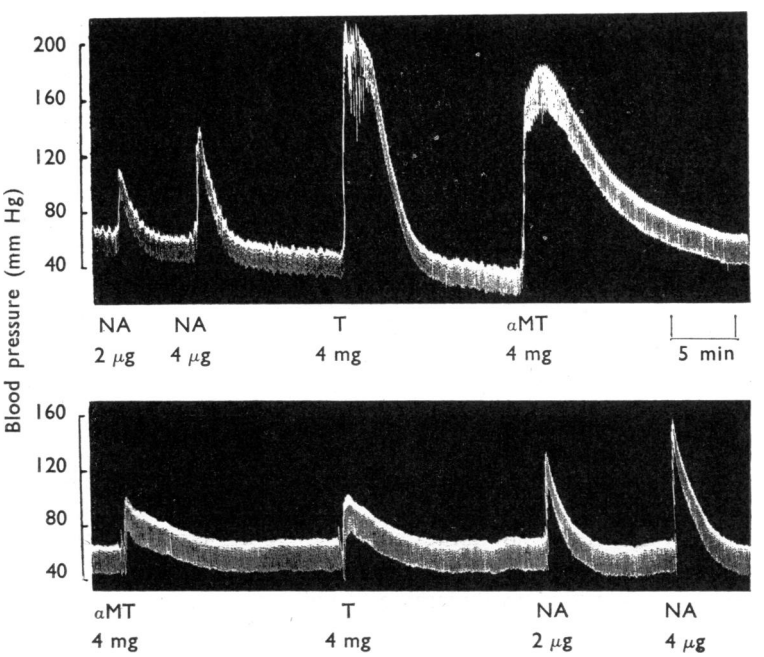

Fig. 2. Pressor responses of a spinal cat (4.0 kg). The upper record shows responses to noradrenaline (NA, 2 and 4 μg), tyramine (T, 4 mg) and α-methyltyramine (αMT, 4 mg). Between the two records two further injections of α-methyltyramine (4 mg) were administered during 45 min. The lower record shows the reduced response elicited by the fourth injection of α-methyltyramine. The tachyphylaxis was crossed to tyramine (4 mg) but the responses to noradrenaline were somewhat enhanced.

was tested by treating cats with nialamide (15 mg/kg), a potent inhibitor of monoamine oxidase. The results of such an experiment are shown in Fig. 3. In Fig. 3A the injection of tyramine (4 mg) caused a more prolonged pressor response than in an untreated cat. The response resembled that caused by the injection of the same dose of α-methyltyramine into an untreated cat. Fig. 3B shows the very small pressor effect produced by a second injection of tyramine, 30 min after the first. The tachyphylaxis was again crossed to α-methyltyramine (Fig. 3D) and the response to tyramine did not increase after leaving the animal without any further injections for 100 min (Fig. 3D).

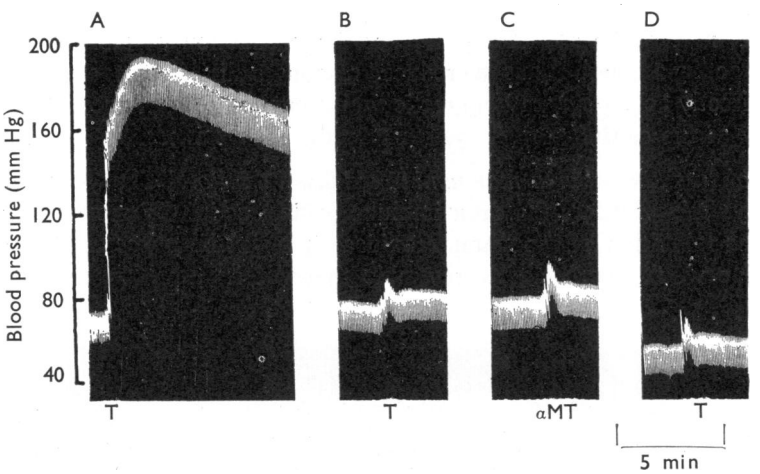

Fig. 3. Pressor responses of a spinal cat, treated 4 hr before the acute experiment with nialamide (15 mg/kg). In A the injection of 4 mg of tyramine (T) produced a very large prolonged rise in blood pressure. In B is shown the response to a second injection of tyramine (4 mg) and in C is the small response elicited by injection of 4 mg of α-methyltyramine (αMT) 15 min after the injection in B. Between C and D the animal was left for 100 min without any injections, but the response to tyramine in D was still very small.

Thus, after inhibition of monoamine oxidase in the spinal cat the pressor response to tyramine more closely resembled that to α-methyltyramine, the pressor response to the first injection was more prolonged, and tachyphylaxis to successive injections was rapid.

Tachyphylaxis to phenethylamine and dexamphetamine. Phenethylamine and its α-methylated derivative dexamphetamine had similar effects on the blood pressure of the spinal cat, although the duration of the initial pressor response to dexamphetamine was longer than that to phenethylamine, as reported by Alles (1933) in studies on dogs. When phenethylamine (2 mg) was injected at regular intervals into cats the pressor responses declined only slightly. However, the responses to the same dose either of dexamphetamine, or of phenethylamine in cats treated with nialamide, declined rapidly. These results are shown graphically in Fig. 4. The pressor responses to successive doses of either phenethylamine (2 mg) or dexamphetamine (2 mg) are expressed as a percentage of the first response obtained in each instance. The pressor response to phenethylamine (2 mg) declined to approximately 80% by the eighth injection, whilst the response to its α-methyl

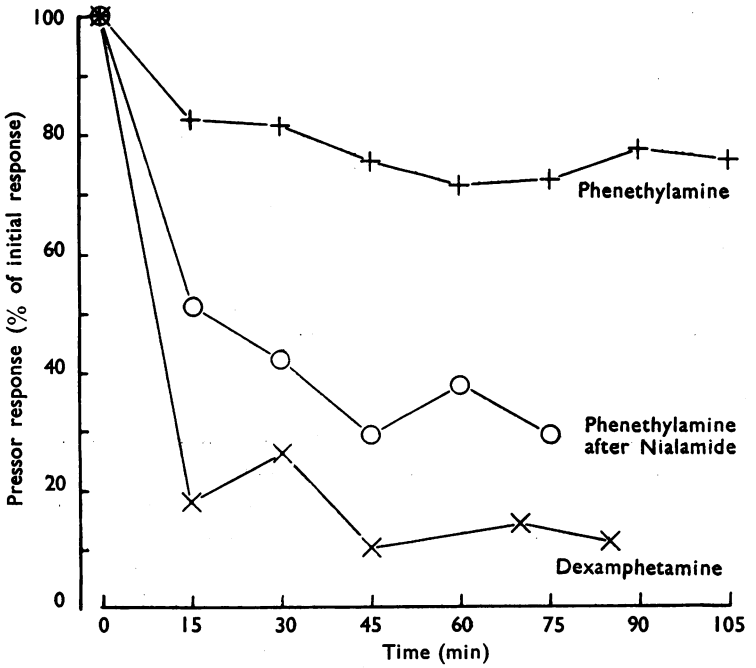

Fig. 4. Pressor responses of spinal cats to phenethylamine (+) and to dexamphetamine (×) in untreated cats and to phenethylamine in cats treated with nialamide (○). Each dose of sympathomimetic amine was 2 mg and each point is the mean of observations from three cats. The pressor responses are expressed as percentages of the initial pressor response to that amine in the particular cat. In untreated cats tachyphylaxis is slow to phenethylamine and rapid to dexamphetamine. In cats treated with nialamide the responses to phenethylamine declined rapidly.

derivative (dexamphetamine) declined to about 10% after six doses. In cats previously treated with nialamide the pressor response to phenethylamine declined rapidly on successive injections, so that the response had decreased to 30% after six doses.

The pressor effect of guanethidine. Guanethidine causes an initial pressor effect which is believed to be due to an acute release of catechol amines. Bartlet (1962) showed that there was tachyphylaxis to this pressor response in spinal cats and also that after guanethidine the pressor response to tyramine was reduced. There is other evidence that indirectly acting sympathomimetic amines and guanethidine act at the same sites (Day, 1962 ; Day & Rand, 1963). In our experiments when the pressor response to any of the indirectly acting sympathomimetic amines had declined as a result of repeated dosage then the pressor response to guanethidine (1 to 3 mg/kg) was very much reduced or was abolished. Thus there is cross tachyphylaxis between individual indirectly acting sympathomimetic amines, and between these substances and guanethidine.

Anaesthetized rabbits

Tachyphylaxis to the effects of tyramine on the blood pressure developed more rapidly in the rabbit than in the spinal cat. Thus after four injections of 4 mg

of tyramine at 15 min intervals the mean response (for three rabbits) was 60% of the initial response. However, the pressor responses to successive injections of α-methyltyramine declined much more rapidly. Thus the fourth injection of α-methyltyramine (4 mg) caused a mean response of 17% of the initial response and the fifth injection produced no effect at all on the blood pressure in each of three rabbits. In rabbits treated with nialamide the initial injection of tyramine (4 mg) caused a larger pressor response than in untreated rabbits and it produced heart arrhythmias in three of four rabbits, one of which did not survive. However, in these rabbits subsequent injections of tyramine produced rapidly diminishing responses and did not cause any irregularities of the heart beat. The fourth injection caused a mean response of 19% of the initial response.

Pithed rats

Effect of tyramine on blood pressure. Single intravenous injections of tyramine (100 μg) produced pressor responses which declined slowly on repeated injection at intervals of 5 to 7 min. Thus in one experiment the pressor response to the fifteenth injection of tyramine (100 μg) was 54% of the pressor response to the first injection. In another experiment in which the interval between doses was extended to 10 min the response to the fifteenth injection was 95% of that to the first.

Effect of infusions of tyramine. After tyramine (1 to 2 mg) had been infused into a vein during 20 to 40 min the response to a single dose of tyramine exhibited a greater degree of tachyphylaxis than that produced after giving the same amount of tyramine in single doses.

Recovery from tachyphylaxis to tyramine. When the response to a single injection of tyramine had declined as a result of giving repeated single doses or of giving an infusion of tyramine, the response could be partly restored by resting the preparation for 20 to 40 min, and even further restored by administration of noradrenaline (10 to 20 μg in 20 min).

Effect of α-methyltyramine. The pressor response to α-methyltyramine in the spinal rat was more prolonged than that to the same dose of tyramine. The responses to successive doses declined more rapidly than those to tyramine. After tachyphylaxis to α-methyltyramine had been induced by repeated injections then the responses were not increased either by resting the preparation or by infusing noradrenaline (20 μg in 20 min) into a vein.

Effect of tyramine after nialamide. After nialamide (40 mg/kg) had been injected subcutaneously into a pithed rat the pressor responses to repeated injections of tyramine (100 μg) diminished more rapidly than in an untreated rat. This increased rate of development of tachyphylaxis was much more evident in rats treated with nialamide (50 mg/kg) on the day before the experiment. In these rats tyramine (100 μg) produced a greater and longer rise in blood pressure than in untreated rats, and caused irregularities of the heart beat; for this reason the doses of tyramine were reduced to 50 μg. The responses to successive injections of tyramine (50 μg) declined much more rapidly than did responses to tyramine (100 μg) in untreated rats. Similar degrees of tachyphylaxis were produced by the same total doses of

tyramine in rats treated with nialamide and of α-methyltyramine in untreated rats. After tachyphylaxis to tyramine had been induced in rats treated with nialamide the tachyphylactic state persisted despite either 40 min rest from drug injection or infusion of 20 μg of noradrenaline during 20 min.

The mean results of experiments on the pressor responses of spinal rats are plotted graphically in Fig. 5. After a total dose of 900 μg of tyramine the response to a single injection of 100 μg was reduced to 90% of the initial response. Repeated injections of α-methyltyramine reduced responses to a single injection to 20% of the initial responses after a total dose of 600 to 700 μg had been given. Similar results were obtained with tyramine in rats treated with nialamide.

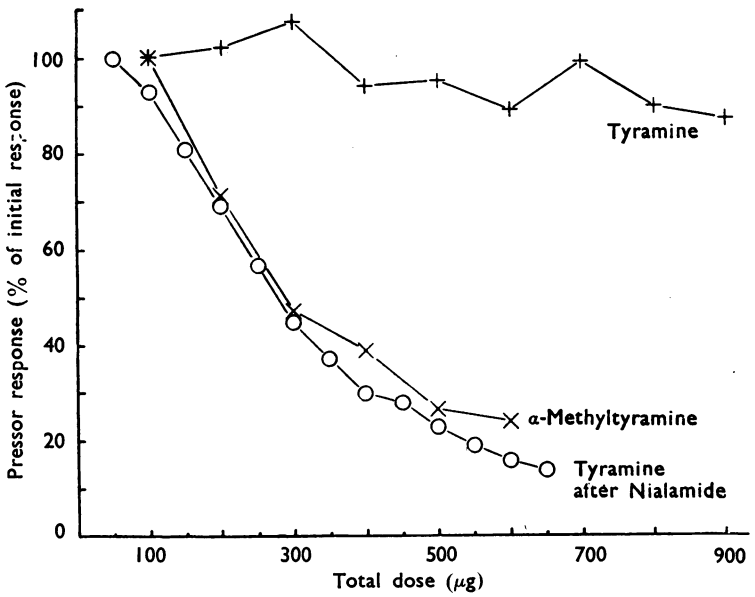

Fig. 5. Graph showing mean responses of blood pressure of pithed rats to repeated injections of tyramine (100 μg/dose) and of α-methyltyramine (100 μg/dose) in untreated rats and of tyramine (50 μg/dose) in rats treated with nialamide. Tachyphylaxis to tyramine was slow in untreated rats and that to α-methyltyramine was rapid. In rats treated with nialamide, tyramine produced tachyphylaxis similar to that produced by the same total dose of α-methyltyramine in untreated rats.

Response to a single injection of noradrenaline. In untreated rats, the response to a single injection of noradrenaline (5 to 10 ng) was potentiated after the first few doses of tyramine. However, when some degree of tachyphylaxis to tyramine had been induced the response to noradrenaline was reduced. Rats treated with nialamide responded to noradrenaline in the same way as untreated rats; that is when some degree of tachyphylaxis had occurred to tyramine the response to noradrenaline was diminished. These results are in contrast to the effect of α-methyltyramine which always enhanced the pressor effect of noradrenaline even when the response to α-methyltyramine itself was greatly reduced. In rats which had been treated with reserpine and then pithed, the pressor response to noradrenaline was

potentiated after giving α-methyltyramine. In these rats α-methyltyramine was without its usual pressor action.

Tachyphylaxis in other tissues

The rate of onset of tachyphylaxis varied somewhat between different tissues, but the responses to α-methyltyramine always declined more rapidly than did the responses to tyramine. Prior treatment with nialamide greatly increased the tachyphylaxis to tyramine.

Isolated perfused rabbit heart. The increases in heart rate produced by successive injections of either tyramine (0.5 mg) or α-methyltyramine (0.5 mg) given at 15 min intervals were studied with hearts taken from untreated rabbits. There was a more rapid tachyphylaxis to α-methyltyramine. The increases in heart rate produced by successive injections of tyramine (0.5 mg) at 15 min intervals into hearts from rabbits treated with nialamide decreased more rapidly than did responses to tyramine in hearts from untreated rabbits. Arrhythmias were regularly produced by the first injection of α-methyltyramine into normal hearts and by tyramine into the hearts from rabbits treated with nialamide.

Our findings on the more rapid tachyphylaxis to tyramine in perfused hearts from rabbits treated with nialamide confirm the report by Davey, Farmer & Reinert (1963) of a more rapid tachyphylaxis to tyramine in perfused hearts from guinea-pigs treated with nialamide.

Cat spleen volume. When the effects of tyramine were measured on blood pressure and spleen volume in the same cat the contractions of the spleen to successive injections of tyramine (4 mg) declined much more rapidly than did the pressor responses. Thus in one experiment the response of the spleen to injections of tyramine (4 mg) declined by 90% after only six injections although the blood pressure responses were at this time quite undiminished. In this experiment a single injection of noradrenaline (20 μg) greatly increased the contraction of the spleen to the next injection of tyramine.

α-Methyltyramine (2 to 4 mg) produced a large prolonged contraction of the spleen when injected into cats. When the spleen volume had returned to the level before injection subsequent injections of α-methyltyramine produced only very small effects. In a cat treated with nialamide (15 mg/kg) on the day before the experiment, tyramine (2 mg) produced a greatly potentiated contraction of the spleen, but subsequent injections were virtually ineffective.

Effect of indirectly acting sympathomimetic amines on sympathetic nerve function

Cat nictitating membrane. Both indirectly acting sympathomimetic amines and sympathetic nerve stimulation ultimately owe their effects to release of noradrenaline from the storage site. When tachyphylaxis to indirectly acting sympathomimetic amines has developed, the responses to sympathetic nerve impulses may be impaired.

Day & Rand (1963) showed that after dexamphetamine (5.5 mg/kg), given in divided doses, there was a considerable block of the responses of the nictitating membrane to sympathetic nerve stimulation, which was especially evident at low stimulus frequencies. We have now shown that when α-methyltyramine was

administered in small divided doses, to avoid large contractions of the nictitating membrane, then a total dose of 4 to 12 mg/kg produced considerable block of the response to sympathetic nerve stimulation which was especially evident at low stimulus frequencies. Similarly in a cat treated with nialamide, tyramine (total dose 1.4 mg/kg) reduced the size of the contractions of the nictitating membrane to sympathetic nerve stimulation. Repeated injections of tyramine (2 mg) into untreated cats produced only very slight tachyphylaxis on the nictitating membrane, and this was associated with only a slight reduction in the height of contraction of the membrane in response to sympathetic nerve stimulation.

DISCUSSION

In cats, rabbits and rats pressor responses to tyramine declined relatively slowly with successive injections. However, in animals treated with the monoamine oxidase inhibitor, nialamide, the pressor responses to tyramine rapidly waned and the rate of onset of tachyphylaxis was similar to that produced by repeated injections of α-methyltyramine into untreated animals. Tachyphylaxis to tyramine in untreated animals was reversed by resting the preparation or more rapidly by an infusion of noradrenaline. This observation has been made by other workers (Cowan, Cannon, Koppanyi & Maengwyn-Davies, 1961). However, the reduced responses to α-methyltyramine in untreated animals or to tyramine in animals treated with nialamide, caused by repeated injections, were not much affected either by rest or by an infusion of noradrenaline.

Schümann & Philippu (1962) showed that tyramine and other indirectly acting sympathomimetic amines released noradrenaline from a suspension of isolated chromaffin granules from the adrenal medulla and themselves replaced the released noradrenaline in almost stochiometric proportions within the granules. If this also occurred with noradrenaline in storage sites at sympathetic nerve endings then it would explain the finding of Davey *et al.* (1963) that the content of noradrenaline was decreased after repeated injections of tyramine. It may be that if sufficient time is allowed between successive injections of tyramine then the tyramine would be removed from the store, probably as a result of its destruction by monoamine oxidase. Tachyphylaxis to tyramine, which is a substrate for monoamine oxidase, presumably only occurs when the ability of the monoamine oxidase to remove tyramine from the store is exceeded by giving large or frequent doses of tyramine. The indirectly acting sympathomimetic amines which are not substrates for monoamine oxidase, such as amphetamine and α-methyltyramine, also act by releasing noradrenaline from the storage site. However, these amines may remain bound within the store for prolonged periods and thus prevent the entry of subsequent doses of indirectly acting sympathomimetic amines into the store. Therefore tachyphylaxis to these substances would be rapid. Thus our results fully support the views of Blaschko (1962, quoted in Introduction). After inhibition of monoamine oxidase, tyramine behaved in a similar manner to α-methyltyramine and tachyphylaxis was consequently very rapid.

The decline of responses caused by giving repeated injections of an indirectly acting drug such as tyramine is not due solely to loss of noradrenaline from storage

sites. Nasmyth (1960) found that there was no depletion of noradrenaline from the guinea-pig heart after tachyphylaxis to tyramine. However, more recently Davey *et al.* (1963) found that after giving sufficient tyramine so that the heart no longer responded with increased contractions the noradrenaline content of the hearts was reduced by 40%. However, this is not a large depletion and would not in itself be expected to cause any marked impairment of responses to tyramine; in fact Crout, Muskus & Trendelenburg (1962) found that depletion of 50% of the noradrenaline from guinea-pig atria by treatment with reserpine had little effect on the responses to tyramine. Davey *et al.* (1963) showed that in hearts taken from guinea-pigs treated with nialamide the noradrenaline content was higher than in hearts from control animals even after sufficient tyramine had been given to cause complete loss of responses. Weiner, Draskoczy & Burack (1962) reported that large doses of tyramine (100 mg/kg) injected into rats diminished the content of noradrenaline in the spleen and the heart. Davey & Farmer (1963) measured the noradrenaline contents of guinea-pig perfused hearts after producing tachyphylaxis to a number of indirectly acting sympathomimetic drugs. After establishing tachyphylaxis to tyramine (total dose, 3 mg) there was a 55% depletion; after amphetamine (1.2 mg) there was no depletion; after ephedrine (0.8 mg) there was an increase in noradrenaline content.

Winder *et al.* (1948) considered that tachyphylaxis to derivatives of phenethylamine arose because of their persistent occupation of receptor sites. The "receptor" site for indirectly acting sympathomimetic amines is the site at which noradrenaline is bound, and from which it can be displaced. Our results show that monoamine oxidase plays a part in removal from receptor sites; when the amine is immune from attack by monoamine oxidase, or when monoamine oxidase is inhibited by nialamide, the persistence of occupation of receptor sites is increased. Our concept is that noradrenaline is displaced from the superficial regions of the store and the displacing amine, for as long as it persists there, prevents access of further doses to the remainder of the noradrenaline deeper within the store. Axelrod *et al.* (1962) found that injections of tyramine released [^{3}H]-noradrenaline which had been taken up by the rat isolated perfused heart, and that the amount of noradrenaline released by each successive injection of tyramine decreased as tachyphylaxis occurred. They also observed that the amount of tyramine taken up by the heart exceeded the amount of noradrenaline released. An explanation for this may be that not all the noradrenaline binding sites were occupied by noradrenaline initially and tyramine occupied these free sites as well as displacing noradrenaline from occupied ones.

Davey *et al.* (1963) suggested that the explanation for the more rapid tachyphylaxis to tyramine after treatment with nialamide was because nialamide reduced the re-entry of released noradrenaline into the storage site. In our view, it is the accumulation of tyramine in the presence of nialamide which would hinder the re-entry of noradrenaline. Dengler, Spiegel & Titus (1961) found that amphetamine reduced the uptake of noradrenaline by tissues, and Axelrod, Hertting & Potter (1962) showed that treatment with amphetamine greatly reduced the uptake of [^{3}H]-noradrenaline by rat heart; when given after the [^{3}H]-noradrenaline, amphetamine depleted the bound [^{3}H]-noradrenaline by about 40%. We found noradrenaline

infusions to be ineffective in reversing the tachyphylaxis produced by the α-methylated amines or by the amines without an α-methyl group in animals treated with nialamide. However, tachyphylaxis to amines without an α-methyl group in untreated animals was reversed by infusion of noradrenaline. These observations suggest that monoamine oxidase has a role in regulating the amines in the store.

The observation that indirectly acting sympathomimetic amines can cause sympathetic nerve blockade is not new. Aström (1949) demonstrated this phenomenon in the rabbit isolated innervated ileum and we have confirmed its existence in whole animals and in isolated tissues (Day, 1962 ; Day & Rand, 1963). Cowan *et al.* (1961a) noticed only a slight impairment of sympathetic nerve transmission to the cat nictitating membrane after tachyphylaxis to amphetamine ; however, they did not describe the stimulus parameters in their experiments. We have shown previously (Day & Rand, 1963) and in the present experiments that the effect of the indirectly acting sympathomimetic amines in blocking sympathetic nerve transmission is most marked at low frequencies of nerve stimulation.

These observations may throw some light on the way in which monoamine oxidase inhibitors exert their anti-hypertensive action. These inhibitors do not themselves block sympathetic nerve transmission. However, they may predispose the sympathetic nerve to an indirect block. We have shown previously that there are marked similarities in the actions of amphetamine (and related drugs) and guanethidine: They both attach to the storage sites for noradrenaline and displace the drug. They both block the responses to sympathetic nerve stimulation, perhaps by preventing further release of noradrenaline from its stores ; this effect may be due to occupancy of some of the noradrenaline storage sites by amphetamine and guanethidine.

Phenethylamine and tyramine are derivatives of the naturally occurring amino acids, phenylalanine and tyrosine. It can be expected that the action of amino acid decarboxylases will produce the amines, and that the amines are then destroyed by monoamine oxidase. When the action of monoamine oxidase is blocked, the amines accumulate and as a consequence of this the release of noradrenaline from stores at the sympathetic nerve endings would be impaired. Patients receiving monoamine oxidase inhibiting substances excrete greatly increased amounts of tyramine formed as a result of the decarboxylation of tyrosine but not broken down further (Sjoerdsma, Lovenberg, Oates, Crout & Udenfriend, 1959).

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REFERENCES

ALLES, G. A. (1933). The comparative physiological actions of dl-β-phenylisopropylamines. 1. Pressor effect and toxicity. *J. Pharmacol. exp. Ther.*, **47**, 339–354.

ASTRÖM, A. (1949). Anti-sympathetic action of sympathomimetic amines. *Acta physiol. scand.*, **18**, 295–307.

AXELROD, J., GORDON, E., HERTTING, G., KOPIN, I. J. & POTTER, L. T. (1962). On the mechanism of tachyphylaxis to tyramine in the isolated rat heart. *Brit. J. Pharmacol.*, **19**, 56–63.

AXELROD, J., HERTTING, G. & POTTER, L. (1962). Effect of drugs on the uptake and release of ^{3}H-norepinephrine in the rat heart. *Nature (Lond.)*, **194**, 297.

BARTLET, A. L. (1962). The pressor action of guanethidine in the spinal cat. *J. Pharm. Pharmacol.*, **14**, 91–95.

BLASCHKO, H. (1962). Formation of pressor amines. In *Hypertension, Recent Advances*, ed. BREST, A. N. & MOYER, J. H., pp. 321–329. London: Kimpton.

BLASCKHO, H., RICHTER, D. & SCHLOSSMAN, H. (1937). The oxidation of adrenaline and other amines. *Biochem. J.*, **31**, 2187–2196.

BURN, J. H. (1952). *Practical Pharmacology*. Oxford: Blackwell.

BURN, J. H. & RAND, M. J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol. (Lond.)*, **144**, 314–336.

COWAN, F. F., CANNON, C., KOPPANYI, T. & MAENGWYN-DAVIES, G. D. (1961a). Reversal of phenylalkylamine tachyphylaxis by norepinephrine. *Science*, **134**, 1069–1070.

COWAN, F. F., KOPPANYI, T. & MAENGWYN-DAVIES, G. D. (1961b). Tachyphylaxis of some sympathomimetic amines. *Georgetown med. Bull.*, **14**, 199–203.

CROUT, J. R., MUSKUS, A. J. & TRENDELENBURG, U. (1962). Effect of tyramine on isolated guinea-pig atria in relation to their noradrenaline stores. *Brit. J. Pharmacol.*, **18**, 600–611.

DAVEY, M. J. & FARMER, J. B. (1963). The mode of action of tyramine. *J. Pharm. Pharmacol.*, **3**, 178–182.

DAVEY, M. J., FARMER, J. B. & REINERT, H. (1963). The effects of nialamide on adrenergic functions. *Brit. J. Pharmacol.*, **20**, 121–134.

DAY, M. D. (1962). Effect of sympathomimetic amines on the blocking action of guanethidine, bretylium and xylocholine. *Brit. J. Pharmacol.*, **18**, 421–439.

DAY, M. D. & RAND, M. J. (1963). Evidence for a competitive antagonism of guanethidine by dexamphetamine. *Brit. J. Pharmacol.*, **20**, 17–28.

DENGLER, H. J., SPIEGEL, H. E. & TITUS, E. O. (1961). Effect of drugs on uptake of isotopic norepinephrine by cat tissues. *Nature (Lond.)*, **191**, 816–817.

EULER, U. S. VON & LISHAJKO, F. (1960). Release of noradrenaline from adrenergic transmitter granules by tyramine. *Experientia (Basel)*, **16**, 376–377.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol. (Lond.)*, **131**, 678–689.

NASMYTH, P. A. (1960). Some observations on the effects of tyramine. In *Adrenergic Mechanisms*, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 337–344. London: Churchill.

RYALL, R. W. (1961). Effects of cocaine and antidepressant drugs on the nictitating membrane of the cat. *Brit. J. Pharmacol.*, **17**, 339–357.

SCHÜMANN, H. J. (1960). Über die Freisetzung von Brenzcatechinaminen durch Tyramin. *Naunyn-Schmeideberg's Arch. exp. Path. Pharmak.*, **238**, 41–43.

SCHÜMANN, H. J. & PHILIPPU, A. (1962). Release of catechol amines from isolated medullary granules by sympathomimetic amines. *Nature (Lond.)*, **193**, 890–891.

SHIPLEY, R. E. & TILDEN, J. J. (1947). Pithed rat preparation suitable for assaying pressor substances. *Proc. Soc. exp. Biol. (N.Y.)*, **64**, 453–455.

SJOERDSMA, A., LOVENBERG, W., OATES, J. A., CROUT, J. R. & UDENFRIEND, S. (1959). Alterations in the pattern of amine excretion in man produced by a monoamine oxidase inhibitor. *Science*, **130**, 225.

WEINER, N., DRASKOCZY, P. R. & BURACK, W. R. (1962). The ability of tyramine to liberate catecholamines in vivo. *J. Pharmacol. exp. Ther.*, **137**, 47–55.

WINDER, C. V., ANDERSON, M. M. & PARKE, H. C. (1948). Comparative properties of six phenethylamines, with observations on the nature of tachyphylaxis. *J. Pharmacol. exp. Ther.*, **93**, 63–80.