Brit. J. Pharmacol. (1963), **21**, 27–38.

THE RESPONSES TO TYRAMINE OF THE NORMAL AND DENERVATED NICTITATING MEMBRANE OF THE CAT: ANALYSIS OF THE MECHANISMS AND SITES OF ACTION

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(*Received December* 7, 1962)

The response to tyramine of the denervated nictitating membrane has been analysed by comparing dose/response curves obtained by injections into the femoral vein and into the carotid artery of the spinal cat while recording contractions of innervated and chronically denervated membranes and those of cats treated with reserpine. It is concluded that the effect of tyramine given by these routes is due primarily to catechol amines released from stores within the nictitating membrane itself. Higher doses of tyramine also cause contraction of the membrane by liberating catechol amines into the circulating blood from stores outside the membrane. The response to tyramine of the nictitating membrane after chronic denervation and/or prior treatment with reserpine is partially due to catechol amines released from unidentified stores within the membrane which resist depletion by reserpine and by postganglionic denervation; evidence is presented that adrenaline rather than noradrenaline is concerned. Because of the high sensitivity of the denervated nictitating membrane to adrenaline and to noradrenaline, the response to these amines released from stores outside the membrane by intravenous injections of tyramine becomes greater than that of the normal membrane. Dose/response curves obtained from responses to intravenous sympathomimetic drugs which act away from the membrane are therefore not fully representative of the effect of the drugs on the membrane itself. Tyramine probably has an affinity both for storage sites and for catechol amine receptors in the smooth muscle of the nictitating membrane, the "intrinsic activity," however, being very weak or even absent.

It is generally accepted that tyramine owes at least the main part of its sympathomimetic effect to the release of the normal sympathetic transmitter from stores within or in the vicinity of adrenergic nerve endings. Experiments published in the last two years have supplied direct proof for such an effect *in vivo* as well as *in vitro* (von Euler & Lishajko, 1960; Schümann & Weigmann, 1960; Lockett & Eakins, 1960; Schümann & Philippu, 1961; Lindmar & Muscholl, 1961; Carlsson & Hillarp, 1961; Cession-Fossion, 1962; Potter, Axelrod & Kopin, 1962; Chidsey, Harrison & Braunwald, 1962; Weiner, Draskoczy & Burack, 1962). Further evidence for this mode of action is provided by the fact that the sympathomimetic effect of

tyramine on different effector organs (for example, arteries, heart, spleen, nictitating membrane and iris) can be reduced either by depleting stores of catechol amine (for example, by chronic denervation, treatment with reserpine or by tyramine itself) or by drugs (such as cocaine, guanethidine, bretylium and imipramine) which block the access of tyramine to catechol amine stores or to cells containing these stores (Schaeppi, 1960 ; Lindmar & Muscholl, 1961 ; Lešić & Varagić, 1961 ; Axelrod, Gordon, Hertting, Kopin & Potter, 1962).

One of the commonly used preparations for studying the effects of sympathomimetic drugs is the nictitating membrane of the cat. With tyramine this preparation, however, has given contradictory results. According to the above-mentioned mode of action of this drug, one would expect tyramine to cause smaller contractions of the chronically denervated than of the innervated membrane. Only a negligible effect has indeed been found by Burn, Leach, Rand & Thompson (1959) working with an isolated, chronically denervated nictitating membrane. Bacq (1936), however, studying the effect of various sympathomimetic amines on the nictitating membrane *in vivo* found that tyramine, in the one dose used, was more active on the denervated than on the normal membrane. This result is explained by the results of Bülbring & Burn (1938), later confirmed by others (Lockett, 1950 ; Fleckenstein & Burn, 1953 ; Innes & Kosterlitz, 1954 ; Fleckenstein & Stöckle, 1955 ; Trendelenburg, 1961 ; Trendelenburg & Weiner, 1962). All these authors, comparing dose/response curves for the action of tyramine on normal and denervated membranes, concluded that the denervated membrane was more sensitive than the innervated to small doses of tyramine, but less sensitive to larger doses.

These facts were not satisfactorily explained until Trendelenburg (1961) suggested that the effect of tyramine on the effector organ is determined by two factors, the amounts of catechol amines available for release by tyramine on the one hand and the sensitivity of the receptors to the liberated catechol amines on the other.

Theoretically, a reduction of the available catechol amines in the membrane would shift the dose/response curve for tyramine to the right and depress the maximum. An increase of the sensitivity to catechol amines, however, would result in a parallel shift of the curve to the left. The curves obtained by Trendelenburg (1961) and by Trendelenburg & Weiner (1962) with innervated membranes of cats previously treated with 0.1 mg/kg of reserpine and with chronically denervated membranes run reasonably parallel, responses of the latter preparation being obtained with 100-times smaller doses than for the former. As the increase in sensitivity to noradrenaline on chronic denervation is approximately 100-fold, it is tempting to conclude that the response of the denervated nictitating membrane to tyramine is determined by the great reduction in stored noradrenaline in the membrane, and by the 100-times increase in effect of the very small amounts of noradrenaline still released from this organ.

In all these investigations tyramine was injected intravenously. The present study compares the effects of intravenous and close-arterial injections of tyramine on innervated and denervated nictitating membranes with or without prior treatment with reserpine. The results confirm that the mode of action of tyramine is rather complex.

METHODS

Sixty cats of either sex weighing 1.5 to 4 kg were used. All were made spinal during deep anaesthesia with ether by cutting the spinal cord between occiput and atlas. After the start of artificial respiration with a Starling pump the adrenal glands were excluded from the circulation by ligatures. In some cats adrenalectomy was performed 1 to 24 hr before the experiment. The blood pressure in the femoral artery was recorded with a mercury manometer. Injections were made into the femoral vein ("intravenous") or by retrograde injection into the cannulated lingual arteries ("intra-arterial"); in the latter instance the drug was injected slowly in 0.1 to 0.2 ml. in order to reach the nictitating membrane directly (Trendelenburg, 1959). The contractions of both nictitating membranes were recorded isotonically and magnified 7-times with a frontal writing lever, the tension on the membranes being 4 g. The frictional distortion of the record was reduced by an electromagnetic vibrator fixed on the rod supporting the levers. The size of contraction is expressed in mm on the smoked drum.

For denervation of the nictitating membrane, the superior cervical ganglion of the right side together with a 1 cm-long piece of the pre- and postganglionic sympathetic nerve was removed aseptically using ether anaesthesia. In some cats, in addition to the ganglionectomy, the right external carotid artery was denervated by stripping away the adventitia and swabbing the artery with 5% phenol. The cats were usually used 9 to 20 days after the operation, and sometimes up to 60 days.

Dose/response curves were obtained from responses to intravenous and intra-arterial injections of increasing doses of tyramine. With intra-arterial injections the drug was given alternately to the innervated and the denervated membrane. Sufficient time for complete relaxation of the membranes was allowed between two injections. The time needed to obtain a full dose/response curve by intra-arterial injections was 4 to 5 hr. Although some tachyphylaxis could not be prevented, the conditions were at least comparable in all experiments.

TABLE 1

CONTRACTIONS CAUSED BY TYRAMINE INJECTED INTRAVENOUSLY OR INTRA-ARTERIALLY ON INNERVATED AND CHRONICALLY DENERVATED NICTITATING MEMBRANES OF UNTREATED AND RESERPINE-TREATED CATS

The mean values, their standard errors and the number of observations (in parentheses) are given. The contractions are indicated as mm on the kymograph. Reserpine was given in a dose of 3 mg/kg intraperitoneally 48 and 24 hr before the experiment. i.a., intra-arterial; i.v., intravenous

Dose of tyramine	Innervated membranes				Chronically denervated membranes			
	Untreated		Reserpine		Untreated		Reserpine	
(mg/kg)	i.a.	i.v.	i.a.	i.v.	i.a.	i.v.	i.a.	i.v.
0·003	2±1·5 (5)		0 (3)		1±1·2 (3)			
0·001	5·4±1·9 (19)		1±1 (2)		5·7±1·3 (14)		4±5 (3)	
0·003	6·8±1·4 (31)		0·6±0·65 (5)		7·5±1·8 (19)		2·5±3·5 (2)	
0·01	11±1·5 (34)	0 (5)	5±2 (5)	0 (2)	8·5±1·5 (23)		8±3·3 (4)	3·3±4 (3)
0·03	28±2·6 (33)	2·6±1·4 (6)	5·1±2·4 (5)	0 (3)	8·4±1·5 (19)	0 (1)	14±5 (4)	4·7±3·2 (3)
0·1	32±2·4 (28)	3·5±1·5 (6)	11±3·5 (5)	0·5±0·2 (3)	9·6±1·6 (17)	8·5±5 (2)	13±1 (4)	6·3±3·7 (3)
0·3	38±4·2 (12)	9·3±2·3 (13)	17±3·4 (4)	1±0·8 (4)	14±4 (7)	18·4±1·7 (7)	22±6 (4)	7±1·9 (4)
1	38±5·6 (8)	22·6±3 (6)	18±5 (3)	1·8±1·4 (3)	14·5±2·8 (5)	21±1·4 (2)	22±4·7 (3)	13±1·8 (3)
3	34±6·4 (3)	47·3±5·5 (7)		10·3±4 (3)	22±1·9 (3)	22·5±3 (2)	21±4·3 (3)	18±3·4 (3)
10		55±2·3 (5)		16·3±6 (3)		27 (1)		23±6 (3)

The sensitivity of both membranes to noradrenaline injected intravenously and intra-arterially was, as a rule, tested at the end of each experiment and, in a few animals, before and after the dose/response curve for tyramine had been obtained. The mean values, their standard errors and the number of experiments are given in Table 1.

Reserpine was injected intraperitoneally in a dose of 3 mg/kg, 24 and 48 hr before the experiment. The animals were protected from cold, and milk was given by stomach-tube.

The drugs used were: L-noradrenaline hydrochloride (Arterenol; Hoechst), reserpine (Serpasil; Ciba), and tyramine hydrochloride (F. Hoffmann-La Roche, Basel). The given doses of tyramine refer to the hydrochloride.

RESULTS

General effects of chronic denervation on the nictitating membrane. Immediately after denervation the nictitating membrane was paralysed and covered about half of the eye. This relaxation was still easily observed 10 days after the operation, but then slowly began to disappear. After 40 days the tone of all denervated membranes had increased, and in most resting animals equal areas of the membrane were visible on both sides. When the animals were slightly disturbed only the innervated membrane contracted. The stress of anaesthesia and the following surgery, however, caused a maximal contraction of the denervated membrane; relaxation did not occur at all in about half of the animals and only slowly in the others. Only fully relaxed, denervated membranes could be used for the study of the tyramine effect. The influence of emotion and of anaesthesia on several sympathetically denervated organs such as the heart, the iris, the salivary glands and the blood vessels was described over thirty years ago by Cannon & Bacq (1930).

A characteristic of most denervated nictitating membranes was the spontaneous activity which we never observed with normal membranes. This activity was either twitches at regular intervals (Fig. 1) or in volleys, or sustained increased tone. The

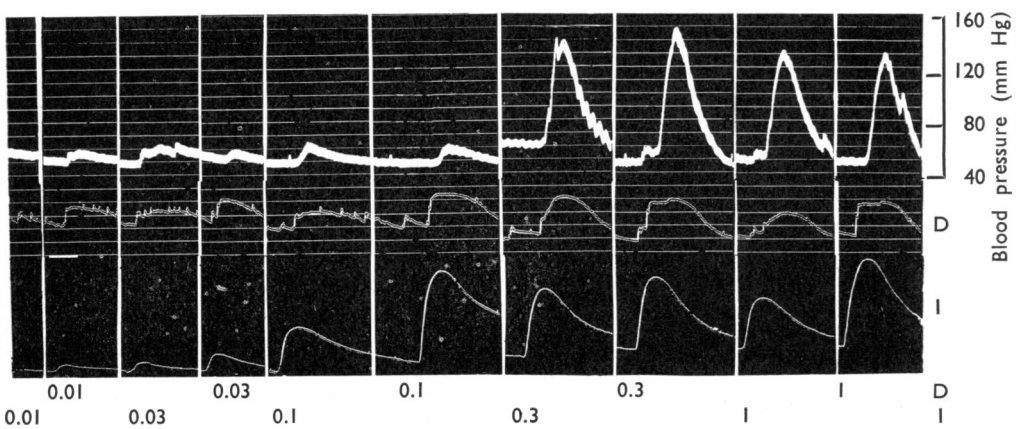

Fig. 1. Spinal cat, 2 kg. Adrenal glands excluded from the circulation. Right nictitating membrane chronically denervated (14 days). Intra-arterial injections. From top to bottom: arterial blood pressure, contractions of chronically denervated (D) and of innervated (I) membrane. The figures indicate the doses of tyramine (mg/kg) injected into the lingual artery on the side of the denervated (upper row) and of the innervated membrane (lower row).

size of these contractions varied considerably from animal to animal, but it was often big enough to make it impossible to distinguish them from small drug effects.

The increased tone and spontaneous activity of the nictitating membrane after denervation also occurs with the isolated *in vitro* preparation (Burn *et al.*, 1959) and was the greatest practical difficulty met in our experiments. We made several attempts to avoid this increased tone. Adrenalectomy performed 1 hr to 1 day before the experiment had some effect but did not prevent completely the increase in tone due to operational stress.

Chronic denervation increased the sensitivity of the nictitating membrane to noradrenaline by a factor of 30 to 300 (usually of about 100) which accords with the results of Innes & Kosterlitz (1954), Kirpekar, Cervoni & Furchgott (1962) and Trendelenburg & Weiner (1962).

The response of the normal nictitating membrane to intravenous and intra-arterial injections of tyramine. The slope of the dose/response curve for tyramine injected intravenously corresponds well with that obtained by Trendelenburg & Weiner

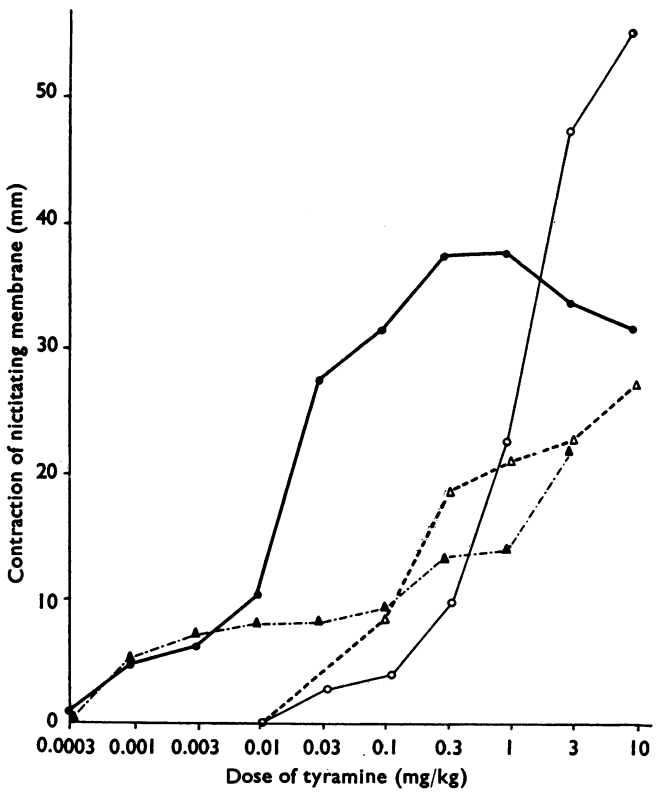

Fig. 2. Dose/response curves for innervated and chronically denervated nictitating membranes with intravenous and intra-arterial injections of tyramine. Ordinate: height of contraction in mm on the kymograph. Abscissa: dose of tyramine (mg/kg log scale). ● — ● innervated membrane; tyramine intra-arterially. ○ — ○ innervated membrane; tyramine intravenously. ▲ - - - ▲ chronically denervated membrane; tyramine intra-arterially. △ - - - △ chronically denervated membrane; tyramine intravenously.

(1962). The curve for intra-arterial tyramine runs parallel to our curve for intravenous injection, identical effects in the lower half being obtained with doses about 30-times smaller. The maximum contraction, however, was much smaller than that after intravenous injections, and with the highest doses the contractions were less than maximal (Fig. 2).

The response of the denervated nictitating membrane to tyramine injected intravenously and intra-arterially. The effect of denervation in increasing the sensitivity of the membrane to small doses of tyramine reported by the above-mentioned authors using intravenous injections was fully confirmed, as was also the reduced sensitivity to higher doses of tyramine.

The responses to intra-arterial injections of tyramine were not as uniform as in the intravenous series. Thirteen out of twenty-four denervated membranes also showed an increased sensitivity, compared with the normal membrane, to small doses (Fig. 1), and in one experiment both membranes had the same sensitivity. But in contrast to the intravenous series, in which all denervated membranes were supersensitive to small doses of tyramine, ten out of twenty-four denervated membranes were much less sensitive to intra-arterial injections of tyramine over the whole dose range. The animals which had had the external carotid artery denervated showed reduction in sensitivity of their denervated membranes.

The decreased sensitivity to medium and larger doses of tyramine was greater with intra-arterial injections. In all experiments pronounced tachyphylaxis occurred for the denervated membrane, even after small doses of tyramine.

With the higher doses of tyramine we made the interesting observation that the response of the nictitating membrane was smaller when the drug reached it promptly after intra-arterial administration than when it was injected intravenously or intra-arterially into the contralateral lingual artery (Fig. 3). With the last route of administration the contraction started after a longer latency (Fig. 1). In one animal the normal membrane also responded with greater contractions to the tyramine given on the side of the denervated membrane (Fig. 1).

The response of the normally innervated nictitating membrane after treatment with reserpine to intravenous and intra-arterial injections of tyramine. After treatment with 3 mg/kg of reserpine on 2 consecutive days, the effect of tyramine was greatly reduced over the whole dose range (Figs. 4 and 5). The curves for the intravenous and the intra-arterial series run approximately parallel over the whole dose range, the latter route requiring doses about 30-times smaller for equal contractions.

The effect of prior treatment with reserpine on the response of the denervated nictitating membrane to tyramine. All denervated membranes of cats treated with reserpine were well-relaxed and had little spontaneous activity. After two doses of reserpine the animals were in a poor state, were heavily sedated, refused any food and were usually unable to walk. Three out of eight died within 48 hr after the first dose. Surprisingly they seemed to tolerate much better the stress of anaesthesia and section of the spinal cord. Their blood pressure varied around 60 mm Hg and remained so for more than 5 hr, whereas the untreated spinal cats had blood

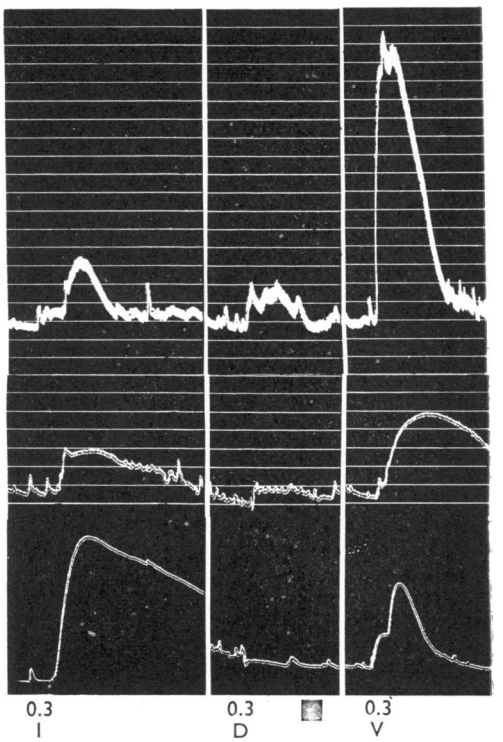

Fig. 3. Spinal cat, 2 kg. Adrenal glands excluded from the circulation. Right nictitating membrane chronically denervated (14 days). Three injections of 0·3 mg/kg of tyramine, the first made into the left (I), the second into the right lingual artery (D) and the third into a femoral vein (V). From top to bottom: arterial blood pressure, contractions of right (denervated) membrane and of left (normal) membrane.

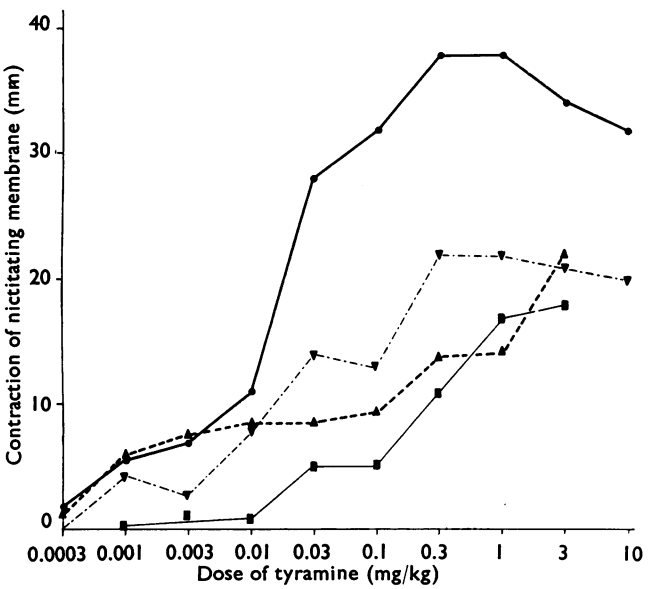

Fig. 4. Dose/response curves for innervated and chronically denervated nictitating membranes with tyramine injected intra-arterially. Ordinate: height of contraction in mm on the kymograph. Abscissa: dose of tyramine (mg/kg, log scale). ● — ● innervated membrane of untreated cats. ▲ - - - ▲ chronically denervated membrane of untreated cats. ■ — ■ innervated membrane of cats treated with 3 mg/kg of reserpine 48 and 24 hr before the experiment. ▼ - - - ▼ chronically denervated membrane of cats treated with reserpine.

pressures between 30 and 50 mm Hg. In the intra-arterial series prior treatment with reserpine did not significantly influence the effect of tyramine (Fig. 4); the slightly greater responses for the upper dose range may be due to better relaxation of the membranes at the time of administering tyramine.

In the intravenous series reserpine depressed the responses to all but threshold doses of tyramine (Fig. 5).

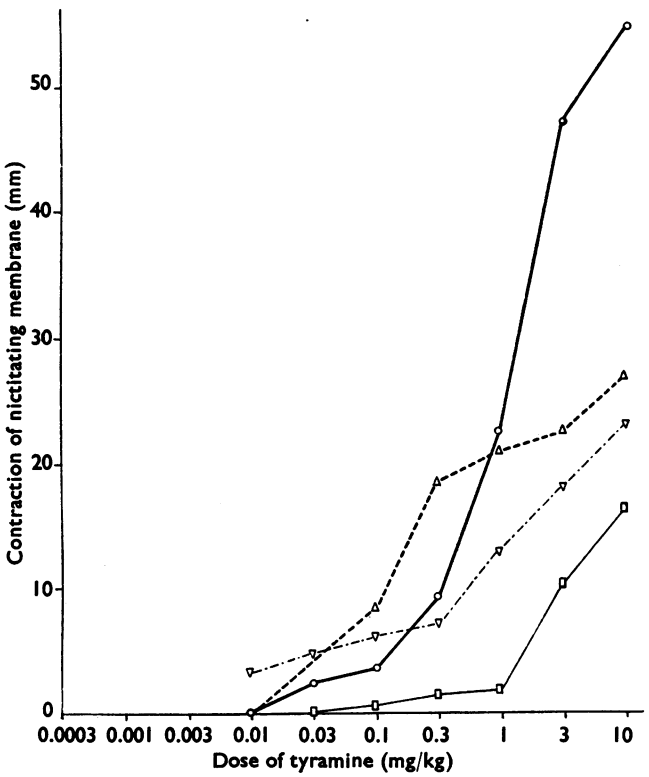

Fig. 5. Dose/response curves for innervated and chronically denervated nictitating membranes with tyramine injected intravenously. Ordinate: height of contraction in mm on the kymograph. Abscissa: dose of tyramine (mg/kg, log scale). ○ —— ○ innervated membrane of untreated cats. △ - - - △ chronically denervated membrane of untreated cats. □ —— □ innervated membrane of cats treated with 3 mg/kg of reserpine 48 and 24 hr before the experiment. ▽ - - - ▽ chronically denervated membrane of cats treated with reserpine.

The effect of denervation of the external carotid artery. This was performed on three cats at the end of our investigations. In each experiment the denervated membrane was markedly less sensitive than was the innervated one to intra-arterially injected tyramine over the whole dose range.

Observations concerning the "adrenolytic," adrenergic "sensitizing" and "desensitizing" effects of tyramine. Some other observations seem worth mentioning. Some denervated membranes in a state of moderate contraction were relaxed by tyramine in small doses, whereas higher doses caused contraction of the same membranes.

In several experiments the sensitivity to noradrenaline of both membranes was tested before and after the dose/response curves for tyramine had been obtained. In denervated membranes, the sensitivity to noradrenaline was decreased after tyramine. Sometimes repeated application of noradrenaline restored its effectiveness. The opposite result was observed with the normal membrane, which was very sensitive to the first dose of noradrenaline given after large amounts of tyramine. This sensitivity always decreased gradually after repeated injections of noradrenaline.

DISCUSSION

Evidence that the response of the nictitating membrane to tyramine is partially an effect of catechol amines released remotely from the membrane

With noradrenaline, intra-arterial doses about 30-times smaller cause responses identical to those on intravenous administration. We may therefore conclude that for identical contractions the number of tyramine molecules reaching the nictitating membrane is the same whether the drug is injected intra-arterially or in 30-times larger amounts intravenously. The dose/response curves for intravenous and intra-arterial injections of tyramine on the nictitating membrane run parallel until the size of contraction is about 60% of the maximum, but greater contractions can be obtained by injections into the femoral vein. It has already been mentioned that tachyphylaxis cannot be avoided if a full dose/response curve is to be obtained on the same animal. Nevertheless, tachyphylaxis should follow similar courses with each mode of administration and, since the catechol amine stores within the membrane are the same, noradrenaline is likely to be liberated from them to the same extent whether the drug is given intra-arterially or in 30-times larger amounts into the femoral vein. The greater effect of high intravenous doses and the more marked tachyphylaxis after intra-arterial administration can therefore be explained only by assuming that the membrane also reacts to catechol amines released from outside the membrane when tyramine is given intravenously.

After removal of the superior cervical ganglion the noradrenaline content of the nictitating membrane falls below measurable levels (Kirpekar *et al.*, 1962). On such depleted membranes tyramine, injected intravenously in doses up to the ED40 for the normal membrane, always caused a greater response than on normal membranes. As suggested by Trendelenburg & Weiner (1962), this is best explained by an increased sensitivity of the denervated membrane to the small amounts of catechol amines still released from the membrane and, as we may now conclude, also from other sites in the body.

The results with intra-arterial injections show clearly that the nictitating membranes are not uniformly influenced by the usual method of denervation. About half of the denervated membranes were still supersensitive to small doses of tyramine, as were all the membranes with intravenous injections. Half of the membranes, however, responded much less to small doses of the drug and gave dose/response curves which were very similar to those for the intra-arterial series on innervated membranes of animals previously treated with reserpine. But even when the means of all values for the intra-arterial series are considered together, the curve is flattened compared with that for the intravenous series, and the maximum contraction is much

smaller. Tachyphylaxis occurred very rapidly when tyramine was injected intra-arterially. A most interesting finding is illustrated in Fig. 3 where the same dose, which was ineffective when given intra-arterially because of tachyphylaxis, still caused a contraction of the membrane when given intravenously. Again the only reasonable explanation seems to be that the small stores of catechol amines still present in the membrane after denervation are rapidly exhausted by relatively small doses of tyramine given intra-arterially, and that the contraction following intravenous injections of tyramine is due to catechol amines released at a site remote to the membrane.

Nature of the catechol amine stores sensitive to tyramine within the nictitating membrane but resisting chronic denervation and treatment with reserpine

The lack of complete inactivity of tyramine on the denervated nictitating membrane has been a matter of much controversy (Vane, Wolstenholme & O'Connor, 1960). Burn *et al.* (1959) have shown that a very weak effect can still be obtained by electrical stimulation of the nerve in an isolated nerve-membrane preparation following careful destruction of the cervical sympathetic nerve. It was thought that some fibres of obscure origin might escape denervation. Evidence for this is provided in our experiments in which cutting the nerves in the adventitia of the external carotid artery, in addition to ganglionectomy, further decreased the effect of tyramine. The short treatment with reserpine diminished the effect of intravenously administered tyramine on the chronically denervated nictitating membrane by decreasing the amounts of noradrenaline outside the membrane available for the release of tyramine. The dose/response curve for intra-arterially injected tyramine on the denervated nictitating membrane was not influenced by acute treatment with reserpine, indicating that the stores resisting denervation were also insensitive to the depleting action of reserpine. Whether these " resistant " stores are special compartments or whether tyramine liberates an amine different from noradrenaline and more resistant to both procedures cannot be decided at present.

However, a comparison of the curves obtained after treatment with reserpine alone and after reserpine plus chronic denervation provides evidence for a predominant release of adrenaline rather than of noradrenaline, since on the denervated membrane after reserpine tyramine is only 3- to 10-times more active than on the innervated one after reserpine. Further support for this assumption is given by the results of Trendelenburg & Weiner (1962) who, after giving a single dose of 3 mg/kg of reserpine, found no measurable amounts of noradrenaline in the nictitating membrane whereas there was still about half of the adrenaline originally present, and those of von Euler & Purkhold (1951), who found adrenaline less reduced than noradrenaline after sympathetic denervation of the spleen

Interactions of tyramine with tissue binding sites and specific receptors

Nasmyth (1962) has challenged the view that tyramine is entirely an indirectly acting sympathomimetic drug and it may well be that the ratio of direct to indirect mode of action of a sympathomimetic drug varies at different effector sites. However, under our experimental conditions, the weak effect of high intra-arterial doses of tyramine is best explained by an indirect effect on noradrenaline and especially

adrenaline still present in small amounts within the nictitating membrane even after chronic denervation and treatment with reserpine. In addition, a remote effect of tyramine occurs even with intra-arterial administration of higher doses. Therefore, only a study on the isolated, denervated and reserpine-treated nictitating membrane showing full tachyphylaxis to the indirect effect could answer the question whether tyramine in high doses can stimulate directly catechol amine receptors.

An affinity of tyramine for such receptors of the smooth muscle of the nictitating membrane is, however, very likely because of the occurrence of an adrenolytic effect on contracted, denervated membranes which is compatible with the action of a weak agonist.

The transient increased sensitivity to noradrenaline of the normally innervated nictitating membrane after repeated large doses of tyramine may be explained by the accumulation of the drug at tissue binding sites. Exogenous noradrenaline then enters these stores less rapidly than under normal conditions and therefore becomes more active on the receptor sites. It may thus be assumed that tyramine not only displaces catechol amines from tissue stores but also interferes with their subsequent uptake. This could explain a similar "sensitizing" effect of tyramine observed by Lindmar & Muscholl (1961) with rabbit isolated perfused hearts, where the chronotropic effect of tyramine was greater than that of dimethylphenylpiperazinium at doses of each drug which released the same amount of noradrenaline. The antagonism of tyramine at tissue binding sites as well as at catechol amine receptors is a competitive one and rapidly disappears with repeated injections of noradrenaline.

The careful technical assistance of Mr M. Fischer and Miss F. Steimer is gratefully acknowledged.

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