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RESPONSE OF NORMAL, DENERVATED, AND RESERPINE-TREATED ARTERIES TO SYMPATHOMIMETIC AMINES AND NICOTINE IN DOGS

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The sensitivity of normal, denervated carotid arterial segments, and of carotid arteries of dogs previously treated with reserpine, to sympathomimetic amines and nicotine has been compared using isolated perfused segments of these vessels. Arteries from animals and arterial segments treated with reserpine, denervated by peri-arterial stripping or by re-anastomosing of segments removed and reversed, both showed an increased sensitivity to noradrenaline which correlated well with a decrease in tissue noradrenaline content. Tyramine did not produce vasoconstriction in denervated vessels, but some constriction was observed when the vessels had been pretreated with noradrenaline or with dopamine or dopa. The effect of nicotine upon dog carotid arterial segments was recorded 130 times. The most frequent response was vasodilatation. This involved both the arterial wall *per se* and the vasa vasorum. The vasodilatation in response to nicotine was seen in arteries from normal and reserpine-treated animals and in denervated vessels.

In 1903 Meltzer & Meltzer reported that removal of the superior cervical ganglion of the rabbit rendered the vessels of the ear more sensitive to adrenaline. Since that time many similar observations have been made on other species, including man, which confirm an increased sensitivity to both adrenaline and noradrenaline following chronic denervation. Von Euler & Purkhold (1951) measured the amount of extractable noradrenaline from the spleen following degeneration of the sympathetic fibres, and observed a significant decrease in the tissue noradrenaline. Burn & Rand (1958) observed a similar increased sensitivity of vascular smooth muscle to noradrenaline following treatment of animals with the alkaloid reserpine. They correlated this increased sensitivity with a decrease in the tissue noradrenaline content and suggested that the hypersensitivity of denervated arteries could be accounted for by a decrease in noradrenaline content of this tissue. They further suggested that the tissue noradrenaline stores played an important role in the production of the sympathomimetic activity of substances such as tyramine and nicotine.

The present experiments, carried out on isolated perfused segments of the dog carotid artery using the technique of Smith, Syverton & Coxe (1951), were designed to determine whether the altered sensitivity of the reserpine-treated and denervated tissues to noradrenaline, tyramine and nicotine was correlated with changes in the noradrenaline content of the artery.

METHODS

Mongrel dogs of either sex were used in all experiments. Dogs were given 0.5 mg/kg reserpine (dissolved in 20% ascorbic acid) intraperitoneally on two successive days and sacrificed on the third day. Denervation of the right carotid artery was performed by two different methods: first, by arterial stripping; secondly, by removal and reversal of an arterial segment with subsequent anastomosis using the method described by Jacobson & Suarez (1960). The vessels were removed for study twelve to seventeen days after denervation on the assumption that nervous tissue in the arterial wall had by this time undergone complete degeneration.

In all animals both carotid arteries from the aortic arch to the thyroid artery were removed under pentobarbitone anaesthesia. The reactivity of the vessels was measured by the angioplethysmokymographic technique described by Smith, Syverton & Coxe (1951).

Segments of the excised carotid arteries 18 to 20 mm in length were each mounted in a simple plethysmograph and were perfused through the lumen with Tyrode solution containing 0.2% sodium bicarbonate. The perfusion was maintained for several hours under constant conditions (temperature 37.5° C; input pressure 100 cm saline; flow rate 3.6 ml./min). Volume changes during perfusion were recorded continuously using a drum camera. The artery was perfused for 45 min without recording to allow it to distend to a constant volume. Recording was then started and after 10 min the volume of the artery was determined. This was accomplished by interrupting perfusion momentarily and applying a positive pressure (50 mm Hg) to the open pipette end of the plethysmograph so that the artery collapsed. The positive pressure was then released and perfusion was restarted. After 10 to 15 min the artery was ready for testing. Individual doses of noradrenaline, tyramine or dopa were injected into the perfusion fluid through a modified T-tube in the quantities indicated. The time-concentration curve of these injections was calibrated by injecting Evans blue dye into the flowing perfusate and recording the dilution curve of the dye by a cuvette densitometer (Model 103 of the Colson Corp.). The cuvette was inserted into the perfusion system where the arterial specimen was normally mounted. The time-concentration curves recorded conformed with those previously obtained by Emmel & Smith (1951). Other drugs studied were added to the perfusate in the concentrations indicated, and the test artery was then perfused with these dilutions of the drug and the reactions were recorded.

Some observations were also made on the effect of nicotine on arterial segments from normal and reserpine-treated domestic swine.

Pressor substances in the arterial segments were extracted with acid saline. The activity of the extract was estimated by its effect on the pithed rat blood pressure in comparison with noradrenaline (Burn & Rand, 1959a). The activity was taken to represent noradrenaline, but the effect of other substances in the extract was not excluded.

Noradrenaline bitartrate (l-isomer) and tyramine hydrochloride and L-dihydroxyphenylalanine (dopa), expressed as the free base, were used. Nicotine doses are expressed in terms of the dihydrogen tartrate salt. Hexamethonium chloride, atropine sulphate, and 3-4-dihydroxyphenylethylamine hydrochloride (dopamine) were used as the salts.

RESULTS

Sensitivity to noradrenaline. In the normal arteries, noradrenaline in doses of 0.5, 5 and 50 μg produced a graded constriction. In both the reserpine-treated and denervated preparations, the duration of the response to 0.5 and 5 μg of noradrenaline was significantly increased ($P<0.02$). The duration of the response to 50 μg doses of noradrenaline in the reserpine-treated carotid arteries did not differ significantly from the control values ($P<0.8$). Results are shown in Table 1. To test whether sensitivity of the denervated arterial segments rose as tissue noradrenaline content fell, noradrenaline was estimated in the denervated right carotid

TABLE 1

EFFECT OF NORADRENALINE ON ARTERIAL SEGMENTS

Time in min for segments to recover ¾ of the total volume decrease (mean values). Figures in parentheses refer to the number of trials

Dose of noradrenaline (μg)	Control	Denervated	Reserpine
0·5	2·27 (22)	5·5 $P<0·01$ (9)	3·81 $P<0·02$ (16)
5	3·58 (23)	10·5 $P<0·01$ (12)	7·80 $P<0·01$ (12)
50	11·0 (14)	24·2 $P<0·01$ (5)	12·25 $P<0·8$ (5)

arteries and the control left arteries of 7 dogs. The mean value for the concentration of noradrenaline in the control arteries was 0.52 μg/g of tissue ; in the denervated arteries it was less than 0.11 μg/g ; the difference between the means of the two groups was highly significant (see Table 2). Since no difference was observed in the sensitivity to sympathomimetic amines of arterial segments which had been

TABLE 2

EXTRACTABLE PRESSOR SUBSTANCES IN DOG ARTERIES EXPRESSED AS NORADRENALINE IN μG/G TISSUE

Dog no.	Normal	Denervated	Dog no.	Reserpine-treated
D 1	0·85	<0·15	R 2	<0·12
D 2	0·75	<0·10	R 3	<0·14
D 3	0·40	<0·15	R 4	<0·08
D 4	0·50	<0·10	R 6	<0·06
D 5	0·17	<0·12	R 7	<0·10
D 7	0·60	<0·07		
D 8	0·38	<0·10		
Mean	0·52	<0·11		<0·10

denervated by stripping or by reversal anastomosis, and since denervation by both methods caused similar loss of noradrenaline, all denervated tissues have been considered as one group.

Sensitivity to tyramine. Fig. 1 shows the response of a control arterial segment to a 50 μg dose of tyramine in comparison with the response to 0.5, 5 and 50 μg of noradrenaline. In some arteries the initial doses of tyramine produced a very small vasoconstriction ; however, after subjecting the tissues to repeated doses of noradrenaline, the response to tyramine was increased. Denervated arterial segments and segments from reserpine-treated animals failed to respond to tyramine in doses which produced constriction in the control arterial segments (Table 3).

Sensitivity to nicotine. In the anaesthetized dog, nicotine raises the arterial blood pressure. The drug might therefore be expected to constrict the isolated perfused normal arterial segment. When single injections of nicotine were made in concentrations of up to 1 mg, no effect was observed. When nicotine (4 μg/ml.) was perfused through normal arterial segments for periods of 15 min, a constrictor action of 5 to 10 sec duration was observed 2 times in the 18 vessels studied ; in these experiments nicotine was administered 58 times. Vasodilatation of both the arterial wall

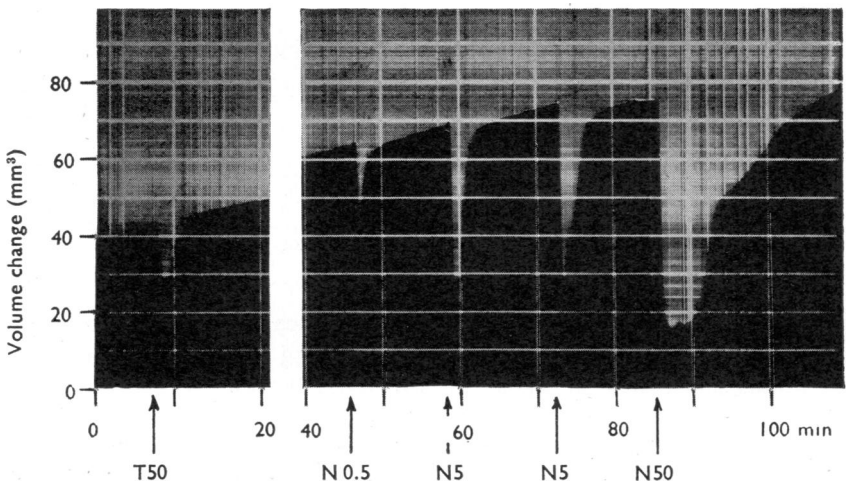

Fig. 1. Response of a normal dog carotid arterial segment to multiple doses of noradrenaline (N) showing the increase of both magnitude and duration of the vasoconstriction produced by increasing doses (0.5 μg, 5 μg, 5 μg and 50 μg) of noradrenaline. The vasoconstriction of the artery to similar 50 μg doses of tyramine (T50) and noradrenaline (N50) is shown; the area of the noradrenaline curve being 20 times greater than that of the tyramine response. (Initial volume of segment 82 mm³). The rise in base-line seen in these figures is produced by an outflow of perfusate through the vasa vasorum of the arterial segment into the plethysmograph, not by a spontaneous increase in arterial volume.

TABLE 3

THE EFFECT OF TYRAMINE ON DENERVATED AND CONTROL ARTERIAL SEGMENTS OF THE DOG

Dog no.	Artery	Volume mm³	Vol. decrease with 50 μg tyramine mm³	% vol. decrease	
				Control	Denervated
D 22	Denervated	54	0		0
	Control	67	19·5	29	
D 23	Denervated	30	0		0
	Control	70	9·5	14	
D 20	Denervated	30	0		0
	Control	54	15	28	
D 28	Denervated	56	0		0
	Control	66	15	23	
D 29	Denervated	34	0		0
	Control	43	1	2	
D 30	Denervated	42	0		0
	Control	87	3	3	
		Mean % volume decrease		16·5	0

and of the vasa vasorum was observed (Fig. 2). The action of nicotine was studied on 11 denervated vessels; 4 vessels constricted once, but subsequently responded to nicotine by dilatation (see Table 4). In this series, nicotine was administered 27 times, vasodilatation being seen 23 times. Fourteen vessels from reserpine-treated dogs were studied. Vasoconstriction alone from repeated nicotine perfusion was seen in only one vessel. In 2 other vessels perfusion with nicotine caused

TABLE 4

EFFECTS OF NICOTINE (4 μG/MIN) ON PERFUSED ARTERIAL SEGMENTS OF THE DOG

	Number		Incidence of effects		
	Arterial segments	Exposure to nicotine	Constriction	Constriction followed by dilatation	Dilatation
Control	18	58	2	0	56
Reserpine-pretreated	14	45	10	2	33
Denervated	11	27	4	0	23
Total	43	130	16	2	112

a brief vasoconstriction followed by a more prolonged dilatation. In 6 vessels simple vasoconstriction was observed once, vasoconstriction being followed subsequently by vasodilatation. In the remaining 5 vessels vasodilatation only was observed. In a total of 45 nicotine perfusions of reserpine-treated vessels, dilatation was recorded 33 times (see Table 3).

The effects of atropine and hexamethonium on the vasodilator action of nicotine were investigated. Atropine sulphate (1 μg/ml.) or hexamethonium (10 μg/ml.) was perfused through normal arterial segments for 5 min before and during the perfusion of nicotine. Neither of these drugs alone altered the volume of the normal arterial segments. In no single experiments during which either atropine or hexamethonium was perfused did we observe vasoconstriction, nor was the vasodilator action of nicotine altered by either blocking agent (Figs. 2 and 3).

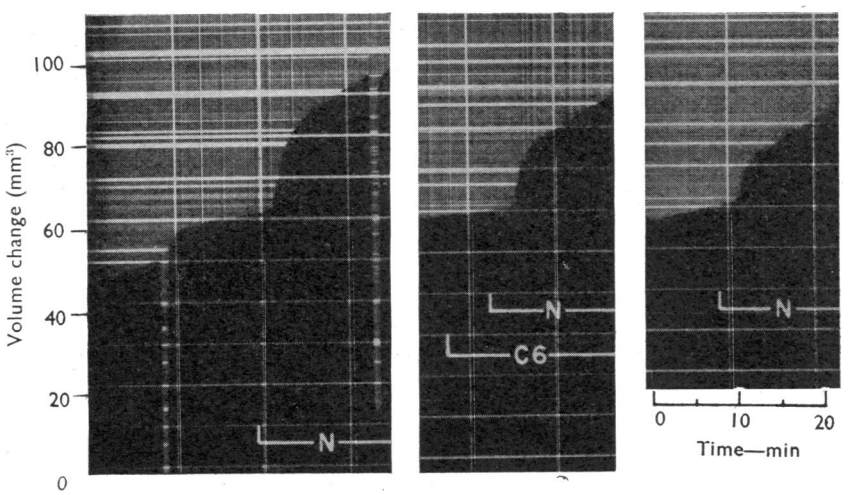

Fig. 2. Vasodilatation of a normal dog carotid arterial segment to nicotine (N) and failure of hexamethonium (C6) to block this response. This figure shows three consecutive vasodilatations to nicotine perfusion (4 μg/ml. for 15 min). In the first reaction the initial volume of the artery, shown by first interruption of record, was 58 mm³; following perfusion with nicotine for 13 min the arterial volume was 81 mm³, shown by second interruption in record. Flow through the vasa vasorum during control period was 4 mm³/10 min; during perfusion with nicotine the vasa flow rate was 12 mm³/10 min.

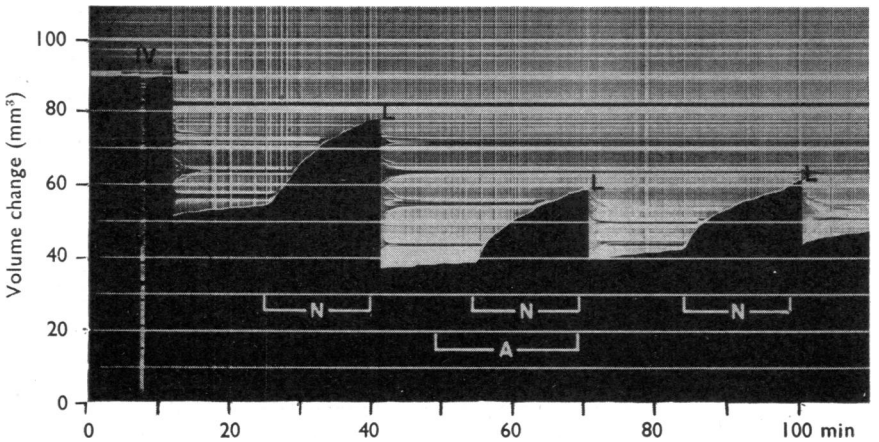

Fig. 3. Vasodilatation of a carotid artery from a normal dog to nicotine (N) and failure of atropine (A) to block this response. This chart shows three consecutive vasodilatations to nicotine (4 μg/ml.). In the first reaction the initial volume (IV) of the artery was 88 mm³; following perfusion with nicotine for 10 min the arterial volume was 110 mm³. The meniscus was lowered at points on the record labelled " L " in order to keep the record on the photographic paper.

The dilator action of nicotine in the dog as reported here is in contrast to the action of nicotine on the perfused carotid artery of the domestic swine. In this species, vasodilatation is not observed during perfusion of the normal artery or the artery from reserpine-treated swine; only a vasoconstrictor response has been recorded.

Effect of dopa, dopamine and noradrenaline on the response to tyramine. Dopa and dopamine are natural precursors in the biosynthesis of noradrenaline. Burn & Rand (1960) have recently reported that the administration of these two agents will restore the sympathomimetic actions of tyramine in the reserpine-treated cat, rabbit and rat. The effect of these drugs was therefore followed on the response of the denervated carotid artery to tyramine and on the response of the carotid artery of dogs which had been previously treated with reserpine. Reserpine-treated vessels were usually insensitive to tyramine. However, when they were subjected to dopamine (100 μg), a transient vasoconstriction was observed, and the vessels recovered their initial volume. When tyramine (50 μg) was added to the perfusion fluid its constrictor action was partially restored (Fig. 4). Similar results were obtained with dopa (2 mg).

In denervated arterial segments, the constrictor response to tyramine was absent or significantly less than that observed in control segments. Failure of reserpine-treated and denervated arterial segments to respond to tyramine in these experiments was not a reflection of the technique employed, since large doses of tyramine produced constriction. In three denervated segments an initial dose of tyramine (50 μg) failed to produce constriction; however, the constrictor effect of tyramine became evident after treatment of the segments with dopamine (Fig. 5). In tissues pretreated with dopamine, repeated doses of tyramine produced tachyphylaxis. The effect of dopa (2 mg) in restoring the tyramine response was less evident in denervated

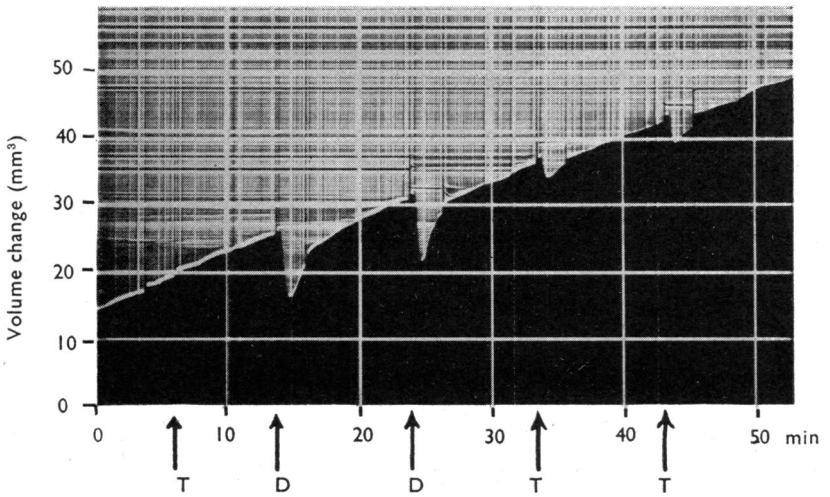

Fig. 4. Reactions of an arterial segment from a dog treated with reserpine to tyramine and dopamine. This artery initially failed to respond to a 50 μg dose of tyramine (T). Two 100 μg doses of dopamine (D) produced transient vasoconstrictions in the preparation. Subsequent 50 μg doses of tyramine then produced vasoconstriction. (Initial volume of specimen 105 mm³.)

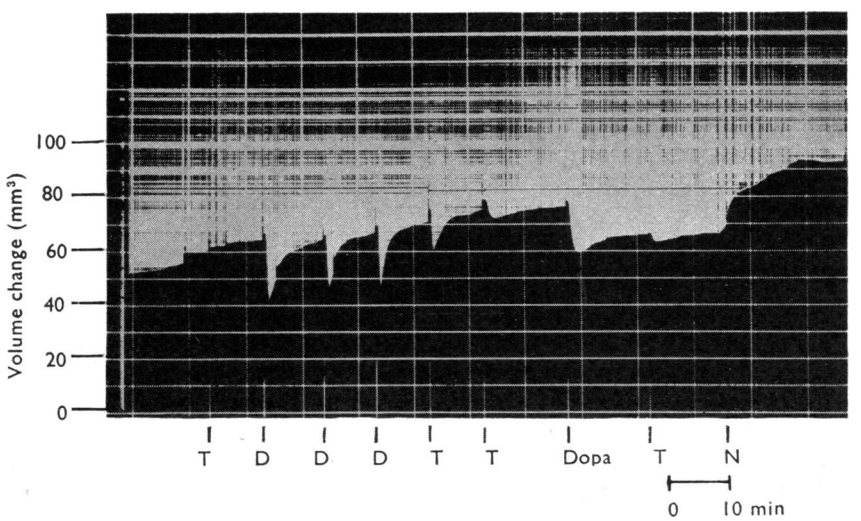

Fig. 5. Reactions of a denervated carotid arterial segment. This segment initially failed to respond to a 50 μg dose of tyramine (T). Three 100 μg doses of dopamine (D) produced transient vasoconstriction. Subsequent 50 μg doses of tyramine then produced vasoconstriction. Dopa, 2 mg, did not further restore the tyramine response. Nicotine (N) produced vasodilatation of this artery.

tissues than in tissues from reserpine-treated animals. Three other denervated arterial segments showed a reduced response to initial injections of tyramine. After treatment of these tissues with noradrenaline the response to tyramine was greater

than that observed before treatment (Fig. 6). As in the restoration of the tyramine response by dopamine, so the responses to tyramine after noradrenaline showed tachyphylaxis.

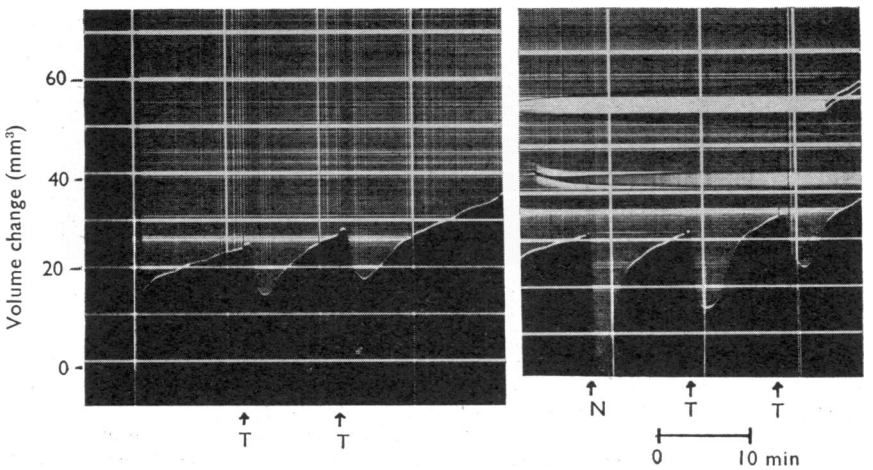

Fig. 6. Reactions of a denervated carotid artery. Initial 50 μg doses of tyramine (T) produced a transient vasoconstriction. The vessels responded to 5 μg noradrenaline (N). The following 50 μg doses of tyramine produced a greater constriction than the controls. The second dose of tyramine (50 μg) produced a vasoconstriction of smaller magnitude equal to the control values.

Reserpine sensitivity of swine. Since dogs withstood reserpine in doses of 0.5 mg/kg, swine were initially subjected to this dosage. However, this proved to be a fatal dose, two animals not surviving more than 24 hr after the initial injection. The dose was then reduced to 0.05 mg/kg, which still proved to be excessive and necessitated the use of a single injection of reserpine and the use of the animal on the second day.

DISCUSSION

Studies have already been made on the change in the sensitivity of sympathetically denervated structures such as the spleen, nictitating membrane, iris and heart to sympathomimetic agents (Burn & Rand, 1959a & b). These studies have indicated a correlation between the amount of noradrenaline in the tissue and sensitivity to noradrenaline. As the noradrenaline in the tissues fell, the sensitivity to catechol amines increased and the actions of phenylethylamines and of nicotine decreased. However, studies have not so far been undertaken to correlate the sensitivity of denervated arterial smooth muscle to noradrenaline with changes in the amount of noradrenaline extractable from arterial tissue.

Arterial segments after chronic denervation and reserpine-treatment were found to have an increased sensitivity to noradrenaline which was accompanied by a diminution in the amount of extractable noradrenaline and which, therefore, resembled the sensitivity seen in other sympathetically innervated structures after denervation and after reserpine-treatment. This altered sensitivity may be explained

in the following manner. Under normal conditions the peripheral store of noradrenaline continuously discharges noradrenaline to maintain vascular tone. If the store is reduced there is less noradrenaline available to be discharged and hence to occupy the receptor sites. As a consequence vascular tone is reduced, and since more receptor sites become vacant the action of noradrenaline increases. An example of this effect was provided by a study of the action of reserpine on the spontaneous rate of isolated rabbit atria. Macmillan (1959) found that atria from normal rabbits had a spontaneous rate of 136 beats/min, while atria from rabbits previously treated with reserpine had a spontaneous rate of 115 beats/min. Noradrenaline, when added to the bath, increased the atrial rate more in atria from the reserpine-treated rabbits than in atria from normal rabbits.

Burn & Rand (1958) have demonstrated that the pressor action of tyramine is absent in the reserpine-treated cat, but that its pressor action can be restored by an infusion of noradrenaline and adrenaline and by precursors of noradrenaline, dopamine, (−) dopa, *m* tyrosine and phenylalanine (Burn & Rand, 1960). They have suggested that tyramine exerts its pressor action through a release of noradrenaline from a store in organs receiving sympathetic innervation. Von Euler & Lishajko (1960) have shown that tyramine will release noradrenaline from adrenergic granules isolated from the splenic nerve of a sheep. Wherever this store may be, results from the work of Burn & Rand (1960), von Euler & Lishajko (1960) and from those presented here strongly indicate that tyramine does not act directly on tissue noradrenaline receptors, but rather indirectly through the release of noradrenaline. The ability of dopa and dopamine to restore the pressor activity of tyramine in the vessels from reserpine-treated dogs is in confirmation of work previously reported.

Our observations on denervated vessels, however, are different from those of Burn & Rand (1960). They found that an infusion of noradrenaline into the spinal cat was not able to restore the action of tyramine either in the denervated iris or in the denervated vessels of the foreleg, and that in this respect denervated vessels behaved quite differently from the vessels of an animal treated with reserpine. We found that noradrenaline, dopamine and dopa were able to restore the constrictor effect of tyramine in the denervated vessels.

Since in the intact animal the administration of nicotine caused a pressor response, its vasodilator action was not expected. As the vasodilator effect was observed in arterial segments denervated by periarterial stripping and by reversal anastomosis, it is unlikely that sympathetic or parasympathetic ganglion stimulation could have caused the effect. Nicotine also produced vasodilatation in the presence of the ganglionic blocking agent hexamethonium. Our results do not support the conclusion that the vasodilator response is mediated through a release of acetylcholine since it occurred in the presence of atropine, and Smith (1950) has shown that acetylcholine produces a constrictor action in the isolated perfused artery of man and swine. Similar vasodilator effects with nicotine were obtained in reserpine-treated tissues which did not respond to tyramine.

Apparently there is a species difference in the action of nicotine on isolated perfused arterial segments, for in the normal and reserpine-treated domestic swine

nicotine caused only a vasoconstrictor response. The marked sensitivity of the domestic swine to reserpine is unusual among experimental animals and deserves comment. Rats survive, without much difficulty, the administration of 8 mg/kg reserpine, cats and rabbits 2 mg/kg and dogs 1 mg/kg for two or three successive days. Swine present a peculiar sensitivity which more closely resembles that of the human.

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