Brit. J. Pharmacol. (1960), **15**, 47.

THE EFFECT OF PRECURSORS OF NORADRENALINE ON THE RESPONSE TO TYRAMINE AND SYMPATHETIC STIMULATION

BY

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(RECEIVED AUGUST 6, 1959)

Previous observations have shown that the effects of sympathetic stimulation and of tyramine were absent in the organs of animals treated with reserpine, but that they were restored by an infusion of noradrenaline. Observations are described showing that an infusion of adrenaline did not restore the pressor action of tyramine in the cat or in the rat, but that in the rat the pressor action was restored by an infusion of dopamine, or of (−)-dopa, or of *m*-tyrosine, or of phenylalanine. Observations are also described showing that the effect of postganglionic stimulation of the fibres to the nictitating membrane and to the iris was restored by an infusion of dopamine or of (−)-dopa; it was restored less well by an infusion of noradrenaline. An infusion of noradrenaline did not restore the action of tyramine on the denervated iris or on the denervated vessels of the cat's foreleg. An infusion of noradrenaline appeared to increase the effect of sympathetic stimulation of the hypogastric nerves to the uterus of the virgin cat about as much as an infusion of adrenaline. An infusion of noradrenaline restored the constrictor action of nicotine on the perfused vessels of the rabbit ear.

The pressor action of tyramine was shown by Carlsson, Rosengren, Bertler, and Nilsson (1957) to be absent in animals treated with reserpine, and we observed (Burn and Rand, 1958b) that it was restored by an intravenous infusion of noradrenaline. We also found that in normal dogs (not treated with reserpine), or in the perfused dog hindleg, the vasoconstrictor action of stimulation of the lumbar sympathetic chain was increased by an intravenous infusion of noradrenaline (Burn and Rand, 1960).

We have now carried out experiments to see if similar effects followed the infusion of adrenaline and of the precursors of noradrenaline, and have tested their effect on stimulation of sympathetic fibres to organs other than the blood vessels.

METHODS

In treating animals with reserpine a solution of reserpine 5 mg./ml. was prepared in a solution of ascorbic acid 20%. Cats received 0.6 ml. on two successive days; male rats of weight 200 g. received 0.3 ml. on two successive days. Observations were made on the blood pressure in the spinal preparation of cats and in the rats prepared as described by Muscholl and Vogt (1957), in which the brain and spinal cord of the rat are destroyed by pithing (Shipley and Tilden, 1947), and the rat receives 1 to 2 mg. atropine. In making intravenous infusions a motor-driven syringe (C. F. Palmer London, Ltd.) was used and a polythene tube was inserted into a vein other than the vein which was cannulated for injections. In the rat all infusions were made for 25 min. In the cat the duration varied from 18 to 30 min.

In experiments on the virgin cat uterus, the cat was anaesthetized with chloralose, after which the abdomen was opened and the cat was eviscerated. The ligament between the right ovary and the kidney was divided and a thread was tied to the ovary and connected to a lever writing on the drum. Sometimes stimulation was applied to both hypogastric nerves and sometimes to the right nerve only, using electrodes of a pattern already described (Burn and Rand, 1960). The nerves were dissected below the inferior mesenteric ganglion and were irrigated through the electrode-holder by a slow stream of oxygenated Krebs solution. The abdominal cavity was filled with Locke solution at 37° and maintained from a slow drip. Rabbit ears were perfused by the method described by Burn (1952) using the outflow recorder of Stephenson (1948).

RESULTS

Restoration of the Action of Tyramine.—Observations on the restoration of the pressor

TABLE I

INCREASE OF PRESSOR ACTION OF TYRAMINE IN SPINAL PREPARATIONS OF CATS TREATED WITH RESERPINE

Dose of tyramine=2 mg.

Substance Infused	Amount Infused	Pressor Response (mm. Hg)		Increase	Mean
		Initial	After Infusion		
Noradrenaline	0·1 mg.	20	48	28	19
		10	30	20	
		18	42	24	
		18	22	4	
	0·5 ,,	12	70	58	48
		10	52	42	
		8	70	62	
		20	60	40	
		2	46	44	
		18	64	46	
		19	65	46	
		22	72	50	
Adrenaline..	0·5 ,,	38	20	18	7
		12	20	8	
		32	32	0	
		6	22	16	
		12	32	18	
		16	32	16	
Dopamine ..	5 ,,	10	34	24	13
		20	26	6	
		16	26	10	
	10 ,,	26	72	44	34
		24	48	24	

action of tyramine were made in the cat and in the rat. The results for a series of experiments in the cat are given in Table I, in which the effect of infusing noradrenaline, adrenaline, and dopamine is seen. Adrenaline had little or no effect. This is illustrated in Fig. 1 in which a series of injections of 2 mg. tyramine was given before and after the intravenous infusion of 0.5 mg. adrenaline, and of 0.5 mg. noradrenaline, repeated twice. The pressor action of tyramine was increased only after the infusion of noradrenaline, and not after the infusion of adrenaline.

The increase in the pressor action after the infusion of noradrenaline was greatest for the first injection after the infusion, and then rapidly disappeared. This is shown in Fig. 2 in which the mean results of eight experiments are shown; from these it appears that even the second injection had little more effect than the injection before the infusion.

The effect of dopamine was more lasting, as is shown in Fig. 2, although the amount of dopamine infused was 10 mg. as compared with 0.5 mg. noradrenaline, that is to say was less than an equipressor amount.

Observations in rats have been summarized in part in Table II, where the increase in the pressor action of tyramine has been expressed, not in terms of the height of the rise, but in terms of the area of the pressor response recorded on the drum. Thus the first line of Table II gives the results of three experiments in which 5 μg. noradrenaline was infused. In the first of these the area of the pressor response to the first injection of 0.5 mg. tyramine after the infusion was 9.5 times as great as it was before the infusion. Table II shows that dopamine when given in only 10 times the dose was more effective than noradrenaline. The action of dopamine and of (−)-dopa was much more lasting than that of noradrenaline, as is shown in Figs. 3 and 4.

We were not successful with tyrosine; it was dissolved with difficulty, and a trial with the N-acetyl derivative of tyrosine was also ineffective. However, an infusion of 2 mg. meta-tyrosine (having the –OH in the *meta* position in the ring) was effective in increasing the pressor action of tyramine, though rather less so than 2 mg. (−)-dopa.

TABLE II

RATIO OF PRESSOR RESPONSE TO TYRAMINE BEFORE AND AFTER INFUSION IN THE PITHED RAT TREATED WITH RESERPINE

The response was taken as the area of the rise of pressure on the kymograph.

Substance Infused	Amount Infused	Ratio, Taking Response Before Infusion=1
Noradrenaline ..	5 μg.	9·5, 8·4, 4·9
	10 ,,	12·3, 24·7, 7·3
	20 ,,	12·4, 15·9
Dopamine	50 ,,	16·4
	100 ,,	24·6
	200 ,,	37·0
(−)-Dopa	1 mg.	8·8, 6·4
	2 ,,	8·6
(±)-*m*-Tyrosine ..	2 ,,	6·4
(−)-Phenylalanine	25 ,,	9·6
(−)-Adrenaline ..	20 μg.	0·3, 3·2
(−)-N-Acetyl Tyrosine ..	10 mg.	0·25
(−)-Tyrosine ..	1·6 ,,	0·9
	3·0 ,,	0·5

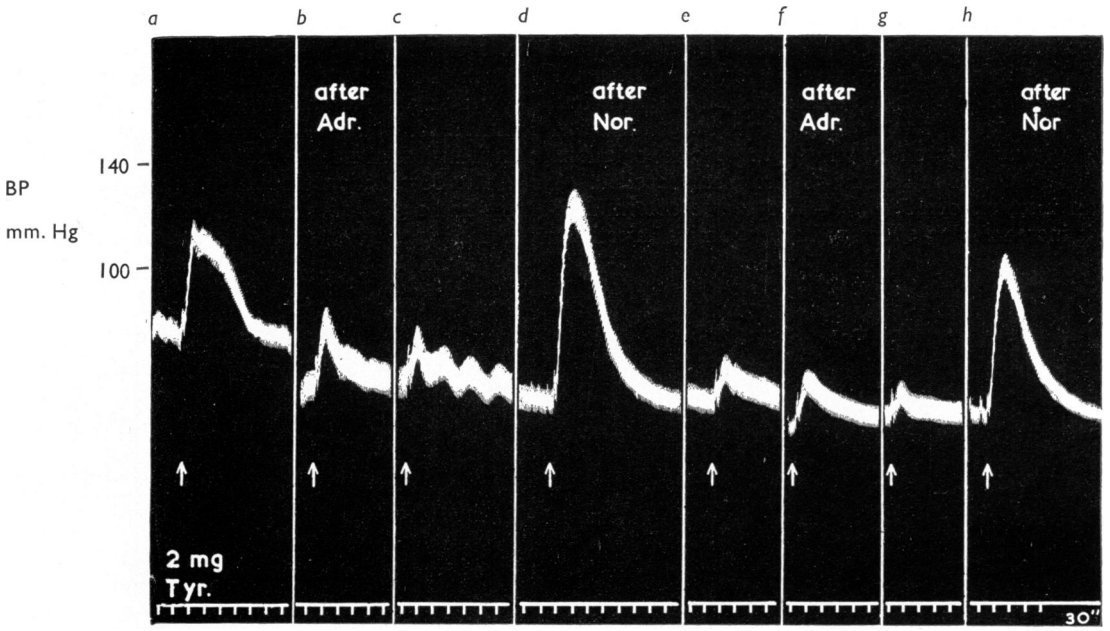

FIG. 1.—Blood pressure in spinal preparation of reserpine-treated cat. 2 mg. tyramine injected at each arrow. Between (*c*) and (*d*), and between (*g*) and (*h*), infusions of 0.5 mg. noradrenaline, after which the responses to tyramine were increased. Between (*a*) and (*b*), and between (*e*) and (*f*), infusions of 0.5 mg. adrenaline, which did not increase the responses.

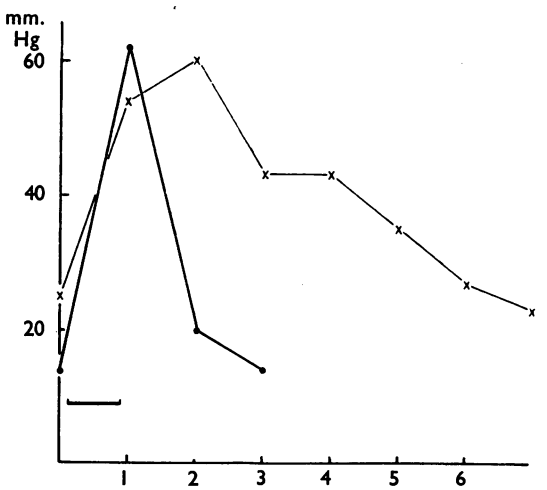

FIG. 2.—Mean rise of blood pressure in spinal preparation of reserpine-treated cat due to injection of 2 mg. tyramine. Black circles show increase after infusion (marked by horizontal line) of 0.5 mg noradrenaline in 8 cats. The increase was evident with the first injection of tyramine after the infusion, but had almost disappeared with the second injection. Crosses show increase after infusion of 10 mg. dopamine (2 cats). This increase persisted for several injections. Abscissae: successive injections of tyramine. Ordinates: rise in blood pressure (mm.Hg).

Finally we observed that an infusion of 25 mg. phenylalanine increased the pressor action of tyramine, having a prolonged effect.

Effects on Sympathetic Stimulation. — The observations on the effect of a noradrenaline infusion on sympathetic stimulation which have been made so far have been confined to vasoconstriction. We therefore turned to the iris of the cat's eye and to the nictitating membrane. An example of an experiment is given in Fig. 5. At the beginning, stimulation of the postganglionic fibres had no effect on the diameter of the pupil, and had a very small effect on the nictitating membrane. An intravenous infusion of 10 mg. dopamine was then given during a period of 15 min. About 7 min. after the end of the infusion the postganglionic fibres were stimulated again, using the same stimulus as before. The pupil dilated 5 mm. and the contraction of the nictitating membrane was 16 mm. on the drum. Stimulation at intervals during the next 40 min. continued to elicit similar large responses.

In one experiment (Fig. 6) a comparison was made between the effect of dopamine, noradrenaline, and (−)-dopa in restoring the response to sympathetic stimulation in the reserpine-treated cat. After the response of the iris had been restored by an infusion of 8 mg. dopamine,

injections of 4 mg. tyramine were given so that both the effect of sympathetic stimulation and of tyramine on the iris became very small. Noradrenaline was then infused during 20 min.; this increased the effect of tyramine greatly, but had much less effect on sympathetic stimulation. After further injections of tyramine the effect of an infusion of 30 mg. (−)-dopa was determined. It increased the effect of both tyramine injections and of sympathetic stimulation, though the effect of sympathetic stimulation was not increased as much as it had been by the infusion of dopamine.

We also observed that the effect of sympathetic stimulation on the iris was increased after giving an infusion of adrenaline.

The observation that an infusion of noradrenaline had a greater effect in increasing the response of the iris to tyramine than it had in increasing the

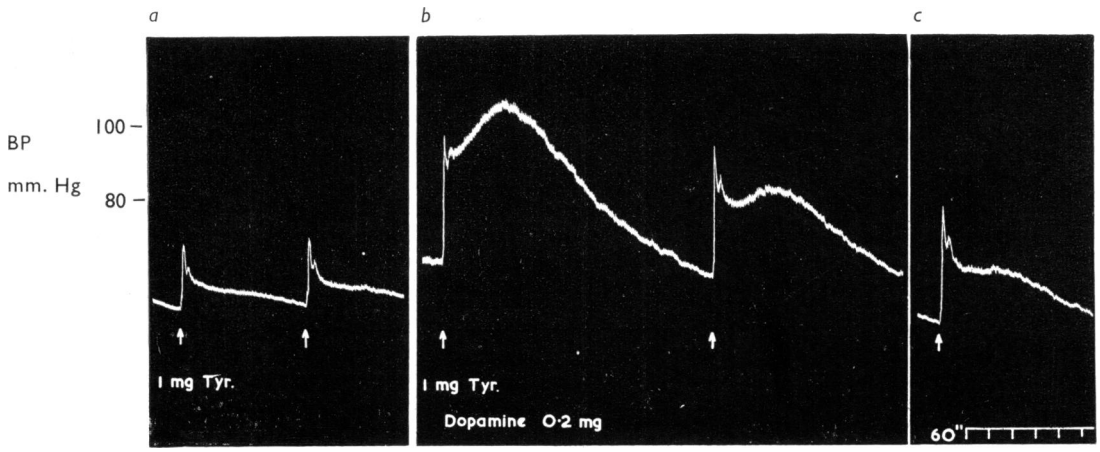

FIG. 3.—Blood pressure of pithed, reserpine-treated rat. Injections of 1 mg. tyramine into right jugular vein at each arrow. Between (*a*) and (*b*) 200 μg. dopamine was infused at 8 μg./min. into the left jugular vein. (*b*) The response to the 1st and 2nd injections of tyramine after the infusion of dopamine, and (*c*) the 3rd injection.

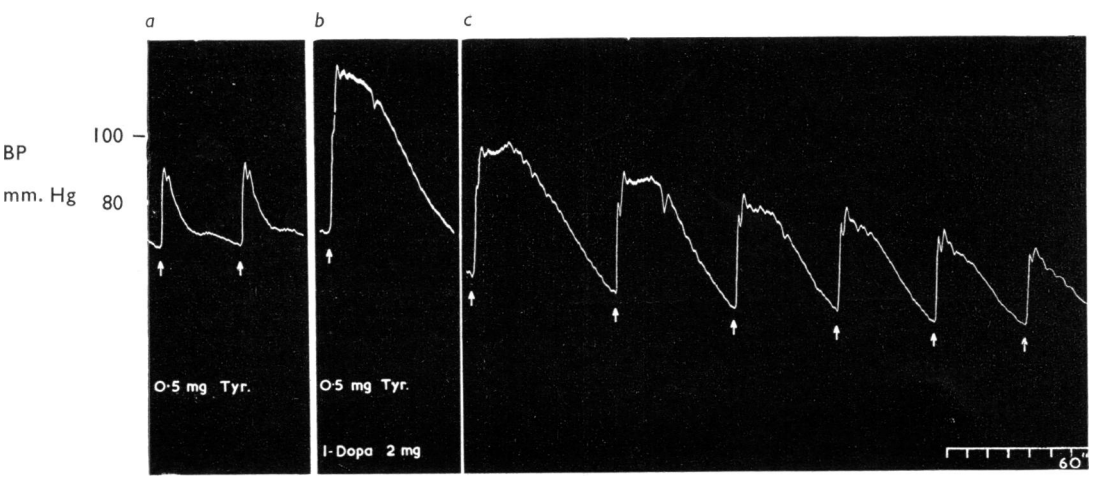

FIG. 4.—Responses to 0.5 mg. of tyramine in pithed, reserpine-treated rat (*a*). Between (*a*) and (*b*), 0.5 mg. of (−)-dopa was infused. The enhanced responses to tyramine after the infusion of (−)-dopa are shown in (*b*) and (*c*). Note that the response to the seventh injection of tyramine after the infusion of (−)-dopa was still greater than the response before the infusion.

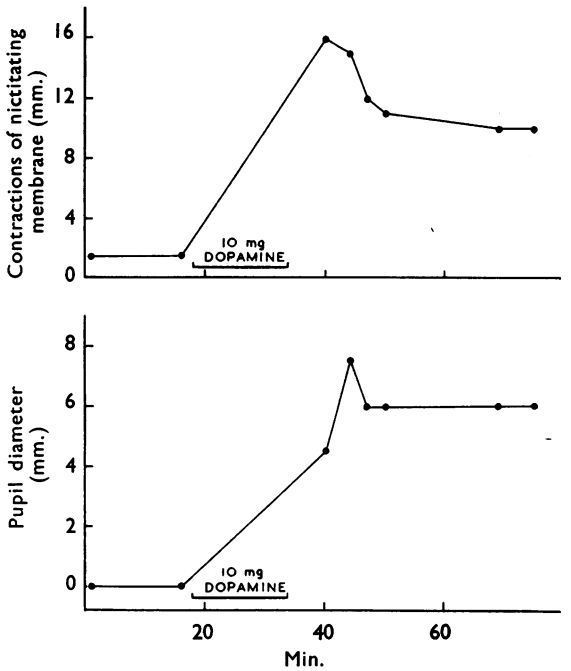

FIG. 5.—Reserpine-treated cat. Upper graph shows contractions of the nictitating membrane (mm. on kymograph record). The lower graph shows dilatation of the pupil measured directly. Stimulation of the postganglionic fibres leaving the superior cervical ganglion for 20 sec. with 5 mA. square wave pulses, 2 msec. duration, 25/sec. After the infusion of 10 mg. dopamine the responses were greater.

response to sympathetic stimulation was also made in experiments on the spleen. Before the infusion of noradrenaline was made, stimulation of the splenic nerves (in the presence of atropine) failed to cause any contraction of the spleen, and an injection of tyramine was also ineffective. After an infusion of 0.5 mg. noradrenaline, stimulation of the splenic nerves caused a very slight contraction of the spleen, while an injection of tyramine caused a large contraction.

Cat Uterus.—We made observations in which we compared the effect of an infusion of noradrenaline with that of an infusion of adrenaline on the inhibitory response to stimulation of the hypogastric nerves of the uterus of the virgin cat. An example of such a comparison is given in Fig. 7, in which the infusion of 100 μg. noradrenaline and the infusion of 100 μg. adrenaline increased the effect of sympathetic stimulation almost equally.

Effect of Infusion in Denervated Tissues.—Since noradrenaline is almost absent in denervated tissues (Euler and Purkhold, 1951; Burn and Rand, 1959), and since tyramine has no action in these tissues (Burn, 1932a), we carried out experiments to see if the infusion of noradrenaline would restore the response to tyramine. We denervated the right iris in two cats by removing the superior cervical ganglion, and after two weeks we determined the effect of an infusion of 1 mg. noradrenaline on the response of the iris to an intravenous injection of tyramine. In neither experiment did the infusion restore the response to tyramine, although the dose injected was as large as 4 mg. The pupil did not dilate at all, though the left pupil dilated maximally.

We made two experiments on the denervated vessels of the foreleg, after removal of the stellate ganglion of one side two weeks previously. The result in one of these is shown in Fig. 8, in which the changes in volume of the normally innervated foreleg, recorded by a plethysmograph, are seen at the top. The middle record is that of the denervated foreleg. The injection of 2 mg. tyramine caused constriction in the normal foreleg, and a slight passive dilatation in the denervated foreleg. These responses remained unchanged after the infusion of 1 mg. noradrenaline. The second experiment gave a similar result.

Infusion of Noradrenaline in the Rabbit Ear.—Finally experiments were made in the perfused rabbit ear in which nicotine normally has a constrictor effect. This constriction is absent if the ear is taken from a rabbit which has been treated with reserpine (Burn and Rand, 1958a), and we wished to know if the constrictor response could be restored by an infusion of noradrenaline. Fig. 9 is taken from an experiment of this kind. At the beginning the injection of 10 μg. nicotine acid tartrate into the fluid perfusing the ear caused only an increase in outflow. During the night noradrenaline was added to the fluid perfusing the ear in a concentration of 0.1 μg./ml. On the next morning a series of four injections of 10 μg. nicotine acid tartrate had a small but increasing constrictor effect as shown by the fall in outflow, and then the injection of 20 μg. caused a large constriction. Thereafter two injections to 10 μg. nicotine were each without effect. It appeared that the infusion of noradrenaline had restored some constrictor action of nicotine, but that after the larger dose of nicotine further doses were then ineffective.

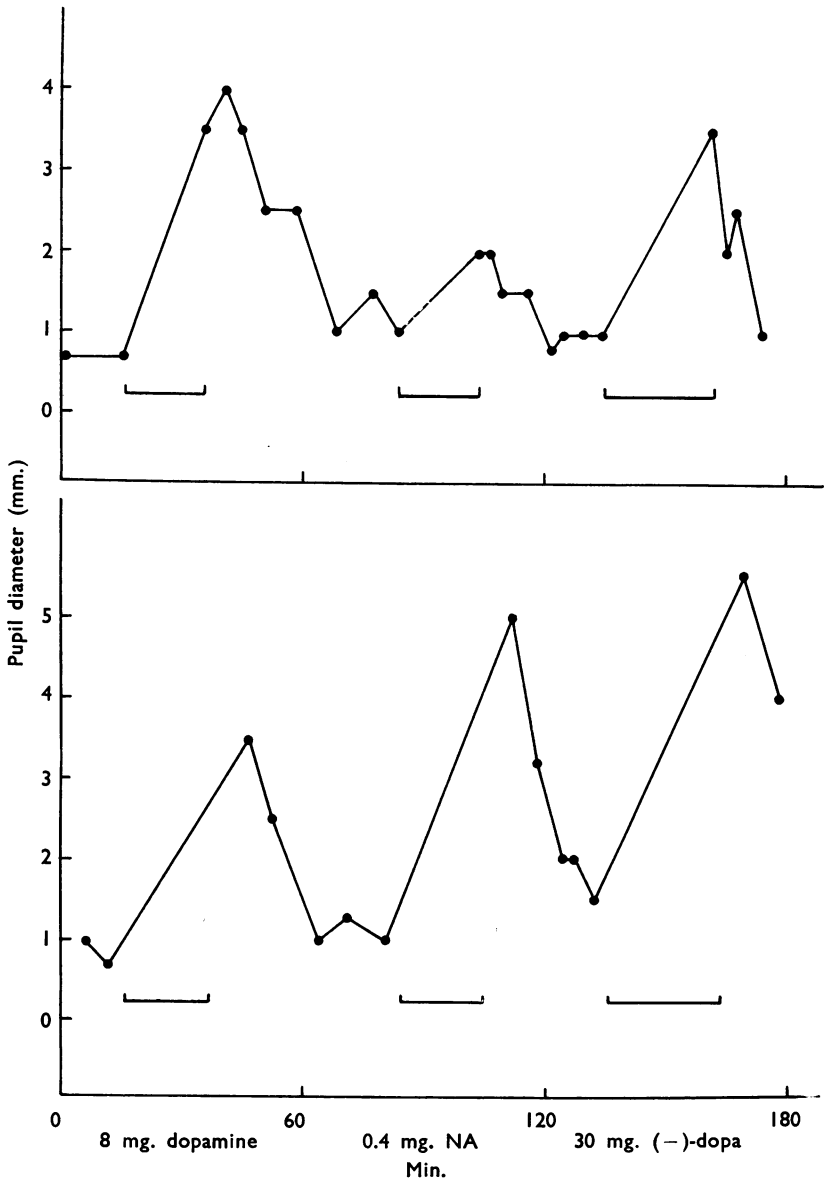

FIG. 6.—Reserpine-treated cat. Upper graph shows dilatation of the pupil in mm produced by postganglionic sympathetic stimulation. Lower graph shows dilatation produced by injection of tyramine (4 mg.). An infusion of noradrenaline (0.4 mg.) was less effective than an infusion of dopamine (8 mg.) or of (−)-dopa (30 mg.) in increasing the response to stimulation, but not less effective in restoring the response to tyramine.

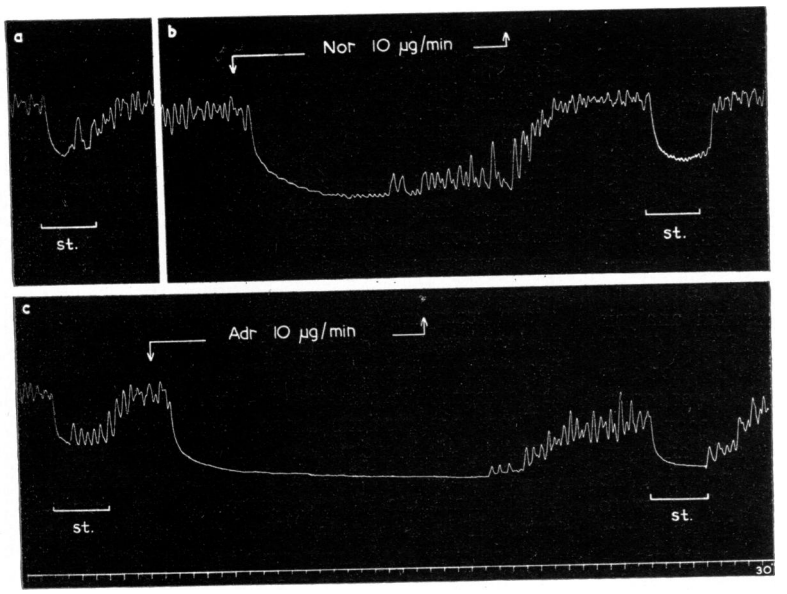

FIG. 7.—Right horn of uterus of virgin cat *in situ*. Right hypogastric nerve stimulated (St) for 2 min. with 20 mA. square wave pulses, 2 msec. duration, 25/sec. After an infusion of 100 µg. of noradrenaline, stimulation produced a greater and more complete inhibition than before. This increased response was only temporary, and about 30 min. later there was only a partial inhibition on stimulation. Then, after an infusion of 100 µg. adrenaline, stimulation was again more effective.

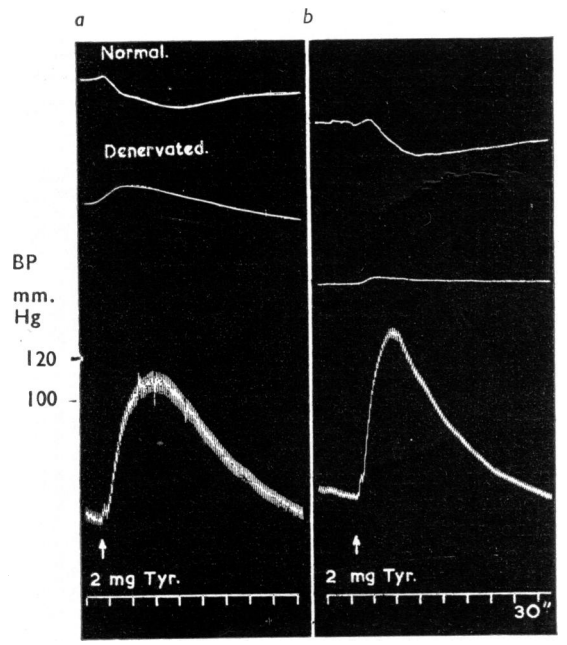

FIG. 8.—Normal (upper) and denervated (lower) foreleg plethysmograph records from cat in which right stellate ganglion had been removed 28 days previously. Spinal preparation. Tyramine (2 mg.) had no constrictor action on the vessels of the denervated foreleg (*a*) initially, or (*b*) after the infusion of 1 mg. noradrenaline.

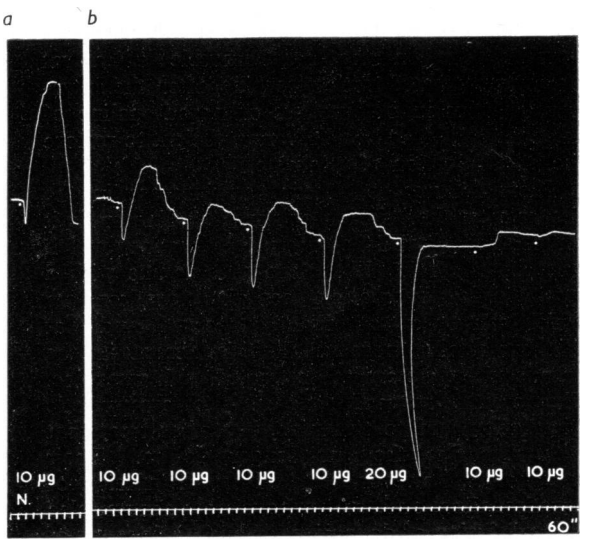

FIG. 9.—Perfused ear from reserpine-treated rabbit. In (*a*) 10 µg. nicotine acid tartrate produced dilatation. Noradrenaline (10^{-7}) was added to the perfusing solution overnight between (*a*) and (*b*). Then, during perfusion with fresh Locke solution, nicotine acid tartrate 10 µg. and 20 µg. produced constriction. After the injection of the larger dose the constrictor action of nicotine was lost.

Discussion

The observations described support our earlier suggestion (Burn and Rand, 1958b) that there is a store in the neighbourhood of sympathetic nerve endings which can take up noradrenaline from the blood stream and that the effect of sympathetic stimulation and the effect of an injection of tyramine and related amines depends on the size of the store. The earliest observations of this kind were made by Burn (1932a and b), but more recently efforts have been made to detect the uptake of catechol amines by chemical analysis of the tissues. Thus Raab and Gigee (1955) observed that the heart muscle of the dog took up noradrenaline and adrenaline after intraperitoneal injection of these substances; they recorded a 12-fold increase of noradrenaline and an even greater increase of adrenaline. However, the amounts they injected into a dog of 10 kg. were 38 mg. noradrenaline together with 75 mg. adrenaline. v. Euler (1956) infused amounts of noradrenaline between 2.5 and 7.8 μg./kg./min. into cats for over 30 min., and gave intraperitoneal injections of noradrenaline and adrenaline up to 2 mg./kg. He failed to observe any increase in the catechol amine content of the heart, the spleen, the liver, the kidney, and skeletal muscle. Recently Muscholl (private communication) has failed to observe any increase of noradrenaline in the heart muscle of a rat previously treated with reserpine after the intravenous infusion of amounts of noradrenaline of the order of 14 μg. which were sufficient to restore the pressor action of tyramine.

These failures to observe an increase in the tissues after the infusion of noradrenaline make it important to recall the precise observations which have been made. These are that organs with a sympathetic innervation contain noradrenaline which can be extracted and measured, and that this extractable noradrenaline is absent in the organs when the animal is treated with reserpine. The organs normally respond to sympathetic stimulation and to tyramine, but after treatment with reserpine they no longer do so. Their response is, however, restored by an infusion of noradrenaline, and, as we have now shown, also by an infusion of dopamine, or of (−)-dopa, or of meta-tyrosine or of phenylalanine. We have seen indications that dopamine is indeed more efficient in restoring the effect of sympathetic stimulation than is noradrenaline itself.

It is clear from studies of the restoration of the tyramine response that it is a very partial restoration. That is to say in the normal animal repeated injections of tyramine can be given for many hours without much diminution in the size of the response. In the reserpine-treated animals, after the infusion of noradrenaline, two or three injections of tyramine are sufficient to cause the restored response to disappear. The response to sympathetic stimulation appears to persist for periods of an hour or so, but we have as yet no evidence that it persists longer. Quite small amounts of noradrenaline are sufficient to restore the pressor action of tyramine; thus in one experiment the injection of 10 μg. noradrenaline into a reserpine-treated cat was enough to cause an increase, and amounts such as 0.25 mg. in a dog produced a considerable fall in the threshold for sympathetic vasoconstriction.

These observations suggest that much smaller amounts of noradrenaline can be shown to be effective by our physiological tests than can be demonstrated to have accumulated by chemical tests. It is likely that there are several stages between uptake of noradrenaline from the blood and incorporation into granules such as Blaschko and his colleagues have demonstrated in the adrenal medulla (Blaschko, 1959). Incorporation into granules of noradrenaline taken from the blood may indeed not occur at all, for what is present in the granules may be the result of synthesis only. But that the increase in the effect of tyramine or of sympathetic stimulation or of nicotine (in the rabbit ear vessels) as a result of an infusion of noradrenaline is due to retention of noradrenaline in some form and in some situation we think is most likely. In Fig. 9 an infusion of noradrenaline restored the constrictor action of nicotine in the rabbit ear vessels. When a larger dose of nicotine was given which produced a large constriction, the smaller doses then lost all effect. The observation is consistent with the constrictor action of nicotine being due to release of noradrenaline taken up by the vessels from the perfusing fluid. After the large dose constriction was no longer seen because all had been released.

We often observed that an infusion of noradrenaline restored the action of tyramine to a greater extent than it restored the effect of sympathetic stimulation. A difference exists between them since the effect of sympathetic stimulation is blocked by bretylium whereas the action of tyramine is not. Tyramine may compete by mass action for the sites to which noradrenaline is attached, while sympathetic impulses may act differently. If noradrenaline is taken up into a store, tyramine may release it before it has reached the sites from which it is released by sympathetic stimulation.

Do the precursors restore the action of tyramine themselves, or must they first be converted to noradrenaline ? It is difficult to see how phenylalanine can act directly, and some conversion in the direction of noradrenaline seems more likely. If so, it is evidence that the conversion can occur very quickly.

The evidence that noradrenaline restored the pressor action of tyramine much more effectively than adrenaline was clear ; however, an infusion of adrenaline was found to restore the action of sympathetic stimulation in causing pupil dilatation, and an infusion of adrenaline seemed to be as effective as one of noradrenaline in increasing the inhibitory action of hypogastric stimulation on the uterus. A sharp distinction between noradrenaline and adrenaline in relation to the store at sympathetic nerve endings therefore cannot be made.

We wish to thank Miss Roneen Hobbs for the experiments in which rabbit ears were perfused. We also wish to thank Dr. H. Blaschko for a supply of *m*-tyrosine. This work has been done during the tenure by one of us (M. J. R.) of a Fellowship from the Life Insurance Medical Research Fund of Australia and New Zealand.

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