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# THE EFFECT OF INFUSING NORADRENALINE OR OF STIMULATING THE SYMPATHETIC NERVES ON THE BLOOD PRESSURE RESPONSE TO INDIRECT SYMPATHOMIMETICS

BY

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The present experiments were carried out before those in the preceding two papers and were intended to clarify two points. First, whether the gradual decline in the pressor response to infused noradrenaline was due to a non-specific desensitization—for example, by changes in the internal ionic environment of the smooth muscle cells, so that their capacity to respond was diminished, as suggested by Paton (1961) for guinea-pig ileum. Secondly, was there desensitization to noradrenaline liberated from nerve endings ? The ability of the normal animal to maintain its blood pressure constant suggested that the nerve-liberated transmitter remained effective. On this basis, indirect sympathomimetics whose effects are largely mediated by noradrenaline liberated from nerve stores might show no desensitization. The experiments with direct stimulation of the sympathetic nerves provided more direct information on this point (Gillespie & Muir, 1967a). The results of the experiments in which the cardiac and peripheral effects of noradrenaline on the blood pressure were separated (Gillespie & Muir, 1967b) proved essential to the proper interpretation of the results with the indirect sympathomimetics. In consequence, the order of presentation has been altered.

Some of these results have already been communicated to the British Pharmacological Society (Gillespie & Muir, 1965).

## METHODS

The pithed rat preparation arranged for stimulation of the sympathetic outflow and for perfusion of one hind limb (Gillespie & Muir, 1967a, b) was employed. Blood pressure was recorded from one carotid artery and both femoral veins were cannulated, one for continuous infusion of noradrenaline and the other for injection of drugs. Electrical stimulation of the sympathetic outflow was carried out after injection of atropine (1 mg/kg) and tubocurarine (1–3 mg/kg).

The following drugs, dissolved in normal saline, were used, doses are given in terms of their salts.

Atropine sulphate (B.D.H.), ephedrine hydrochloride (Cockburn), nicotine hydrogen tartrate (B.D.H.), (—)-noradrenaline bitartrate (Koch-Light), (+)-tubocurarine chloride (Burroughs Wellcome), tyramine hydrochloride (Sigma).

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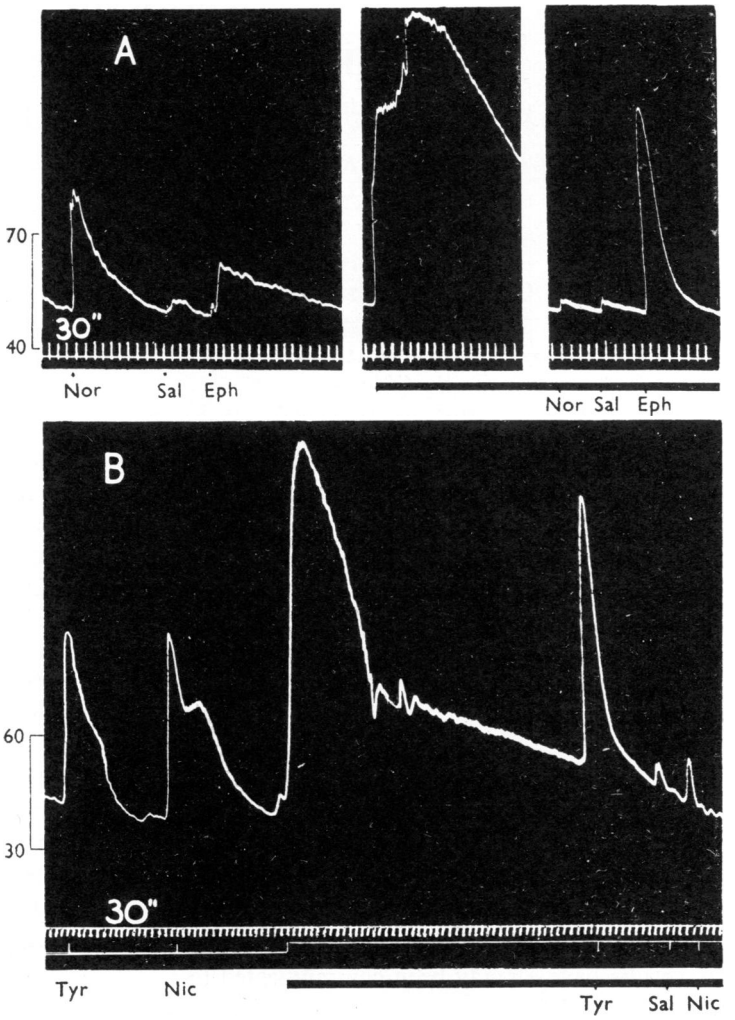


Fig. 1A. The effect of intravenous infusion of noradrenaline (0.01 mg/kg/min) for the period indicated by the black bar, on the response of the blood pressure of a pithed rat (200 g) to intravenous injection of ephedrine (Eph 20 $\mu$g), noradrenaline (Nor 100 ng) and a similar volume of saline (Sal). The time between the first and second panels was 8 min ; that between the second and third panels was 60 min.

Fig. 1B. The effect of intravenous infusion of noradrenaline (0.005 mg/kg/min) for the period indicated by the black bar, on the response of the blood pressure of a pithed rat (270 g) to injection of tyramine (Tyr) 20 $\mu$g and nicotine (Nic) 50 $\mu$g. The response to tyramine remained potentiated at a time when that to nicotine was depressed and comparable to the effect of an equal volume of saline (Sal).

## RESULTS

### *Effect of indirect sympathomimetics and of sympathetic nerve stimulation during infusion of noradrenaline*

Tyramine, ephedrine and nicotine were tested during the infusion of noradrenaline and the pressor response to each was potentiated at a time when the effect of the infused noradrenaline had almost disappeared. This is shown in Fig. 1 (tyramine and ephedrine) and Fig. 2 (nicotine). The response to direct sympathetic nerve stimulation during the infusion of noradrenaline was either unaltered (Fig. 2) or slightly potentiated. Noradrenaline itself could produce large responses if the rate of infusion was greatly increased or if a large (1–10 $\mu$g) single injection of noradrenaline was given.

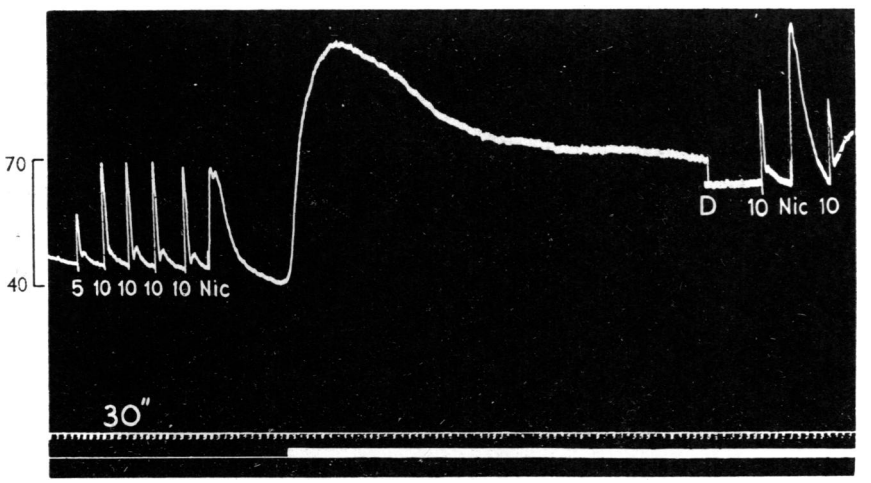


Fig. 2. The effect of intravenous infusion of noradrenaline (0.001 mg/kg/min), during the period indicated by the white bar, on the response of the blood pressure of a pithed rat (250 g) to supramaximal stimulation of the sympathetic nerves and to injection of nicotine (Nic 300 $\mu$g). Submaximal responses to 5 or 10 stimuli, at a frequency of 10/sec, were repeated at 2 min intervals. The drum was stopped at D for 15 min. Tubocurarine (250 $\mu$g) was present throughout.

### *Effect of altering the rate of infusion of noradrenaline*

The ease with which potentiation of the response to these various stimuli could be demonstrated depended both on the type of stimulation and the rate of infusion of noradrenaline. Tyramine potentiation was easily demonstrated and was maintained until very high levels of infusion of noradrenaline (0.2 mg/kg/min) which produced near maximal responses were employed. By contrast, the response to nicotine and to nerve stimulation was potentiated only at lower rates of infusion. As the rate of infusion of noradrenaline was increased, potentiation disappeared, eventually to be replaced by a depression of the response. For example, the response to nicotine was consistently potentiated with infusion rates of noradrenaline of 0.001–0.002 mg/kg/min but usually depressed at 0.005 mg/kg/min. Potentiation of the response to nerve stimulation was still observed at a rate of 0.02 mg/kg/min (Fig. 3) but depressed at higher rates. The

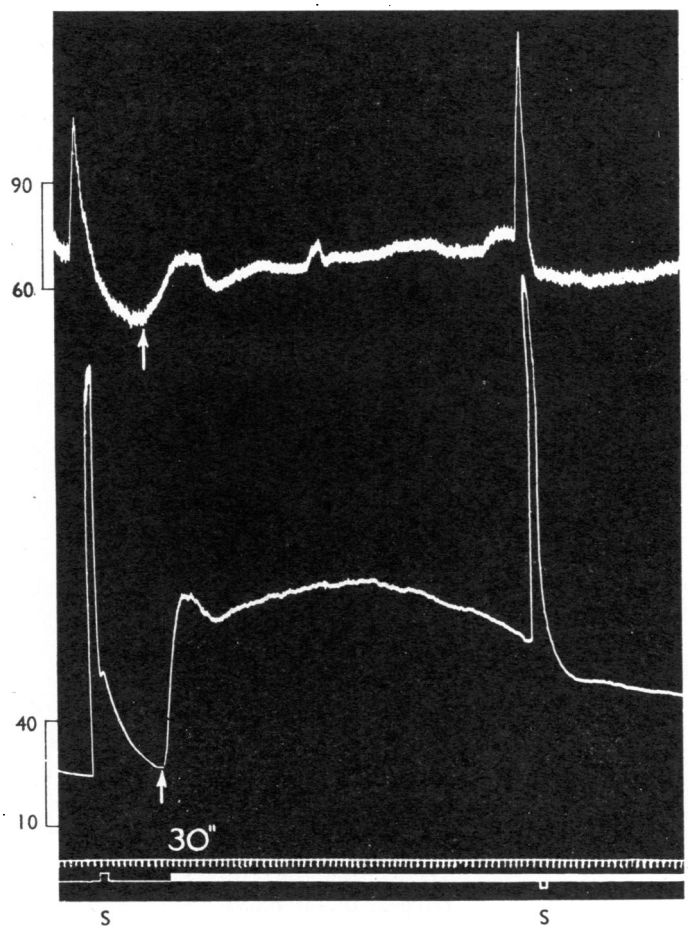


Fig. 3. The effect of intravenous infusion of noradrenaline (0.02 mg/kg/min) on the response of the blood pressure (lower trace) and limb perfusion pressure of a pithed rat (370 g) to supramaximal stimulation of the sympathetic outflow (S) for 30 sec at a frequency of 10/sec. Tubocurarine (400 $\mu$g intravenously) was present throughout. The commencement of infusion of noradrenaline is indicated by the arrows (↑), and its duration by the white bar.

relative resistance of the tyramine potentiation, compared with that of nicotine, to increasing the infusion rate of noradrenaline is shown in Fig. 1B, and its eventual disappearance with high noradrenaline infusion rates in Fig. 4.

*The site of potentiation of the response*

Increased liberation of transmitter by indirect sympathomimetics and by sympathetic nerve stimulation is likely to be responsible for the enhanced responses during the infusion of noradrenaline. This will occur both in the arterial smooth muscle determining the peripheral resistance and in the heart; either or both could be the site responsible for the increased responses. The presence of potentiation at each site was examined in the

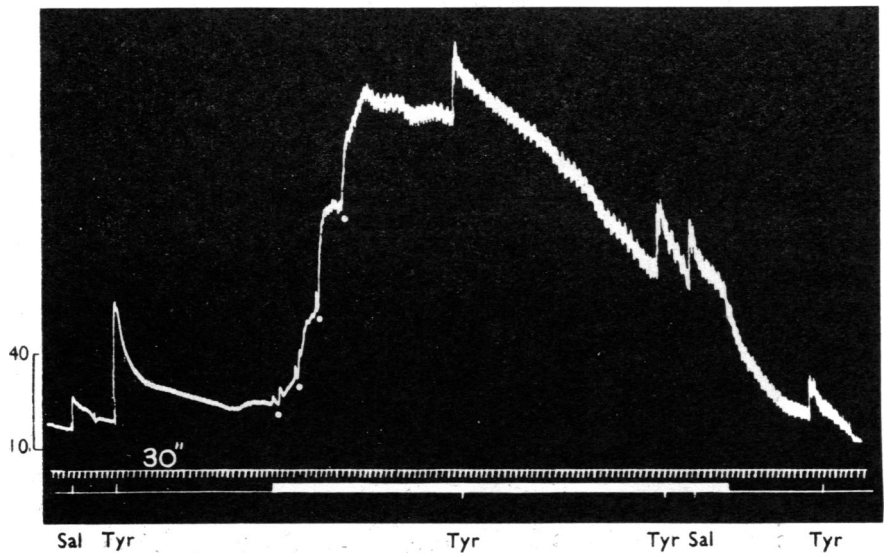


Fig. 4. The effect of intravenous infusion of noradrenaline on the response of the blood pressure of a pithed rat (220 g) to injection of tyramine (Tyr) 200 $\mu$g and an equal volume of saline (Sal). The rate of infusion of noradrenaline was progressively increased from 0.025, 0.05, 0.1 to 0.2 mg/kg/min as shown by the white circles. The duration of infusion is represented by the white bar.

perfused limb preparation (Gillespie & Muir, 1967b). The pressure response in the limb perfused by means of a constant volume output pump gives a measure of the peripheral resistance uncomplicated by changes in cardiac output. Together with the blood pressure response, this may allow the presence of a cardiac component to be recognized. Using this preparation, the effect of an infusion of noradrenaline (0.02 mg/kg/min) on the response to sympathetic nerve stimulation was examined (Fig. 3). Both the effect on blood pressure and on the perfusion pressure in the isolated limb was potentiated. Higher rates of infusion of noradrenaline depress the response to sympathetic nerve stimulation. With tyramine, much higher concentrations of noradrenaline could be used, and potentiation of the response of the blood pressure was still observed, as Fig. 5 shows. The peripheral vascular response to injection of tyramine had disappeared but the effect on blood pressure was enhanced. Under such circumstances the enhanced response is presumably cardiac in origin.

*Effect of indirect sympathomimetics during continuous nerve stimulation*

Continuous infusion of noradrenaline and continuous stimulation of the sympathetic nerves caused similar effects on blood pressure, an initial rise followed by a gradual fall to near the pre-infusion base line. These procedures should produce opposite effects on tissue stores of noradrenaline, infusion will increase stores (Raab & Gigée, 1955; Burn & Rand, 1958) while nerve stimulation will leave them unaltered or depleted (Brown, 1965; Dahlström & Zetterström, 1965). If potentiation of the responses to the sympathomimetics is dependent upon an increase in the stores of transmitter at those sites at which these drugs act, then potentiation should not occur during continuous nerve stimulation. This was tested and the response to tyramine before and during continuous

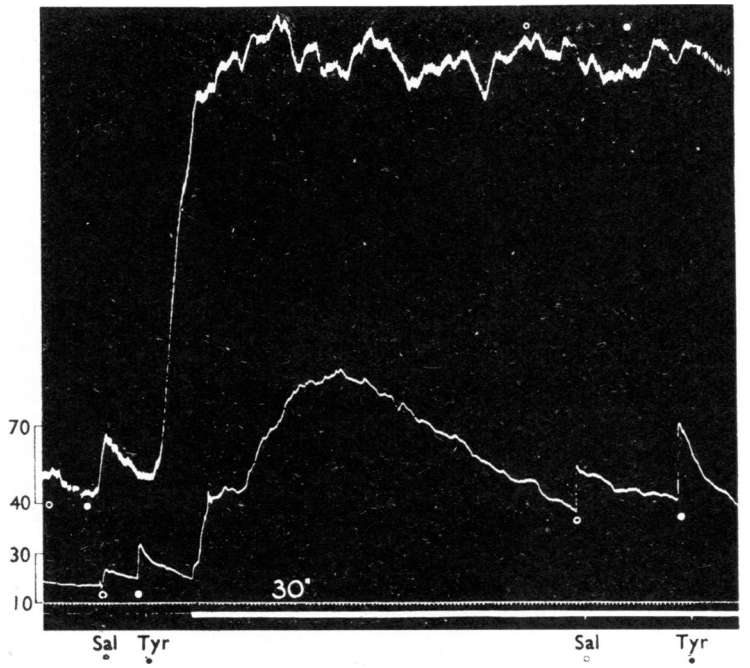


Fig. 5. The effect of intravenous infusion of noradrenaline (0.1 mg/kg/min) on the response of the blood pressure (lower trace) and limb perfusion pressure of a pithed rat (300 g) to injection of tyramine (Tyr) 200 $\mu$g represented by the filled circles (●) and to a similar volume of saline (Sal) indicated by the open circles (○). The period of infusion of noradrenaline is indicated by the white bar. The response of the blood pressure to tryamine is enhanced at a time when that of the peripheral vasculature is not.

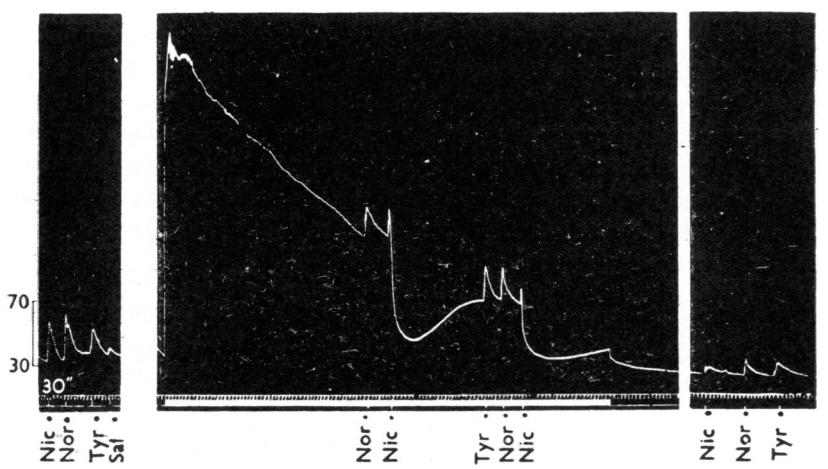


Fig. 6. The effect of continuous, supramaximal stimulation of sympathetic nerves on the response of the blood pressure of a pithed rat (300 g) to injection of nicotine (Nic) 400 $\mu$g, noradrenaline (Nor) 200 ng, tyramine (Tyr) 20 $\mu$g and an equal volume of saline (Sal). The period of stimulation at 10/sec is indicated by the white bar. Early in the stimulation period, nicotine produced a depressor response. Tubocurarine (400 $\mu$g intravenously) was present throughout. Time between the panels is 10 and 14 min respectively.

nerve stimulation is shown in Fig. 6. There was no potentiation. The effect of nicotine was more complex and depended on when the drug was injected during the period of nerve stimulation. During the early phase of continuous nerve stimulation, when the blood pressure was still elevated, nicotine gave a depressor response (Fig. 6). Later, when the pressor effects of nerve stimulation had almost disappeared, the pressor response to nicotine was not only restored but consistently potentiated (Fig. 7).

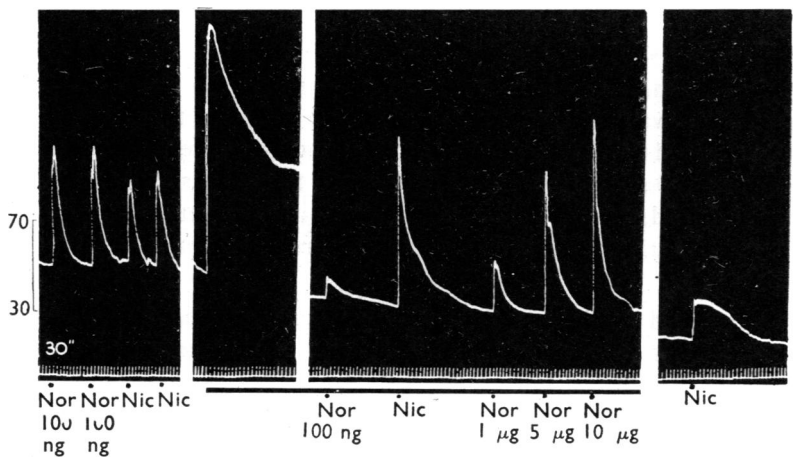


Fig. 7. The effect of continuous supramaximal stimulation of sympathetic nerves on the blood pressure response of a pithed rat (200 g) to injection of nicotine (Nic) 200 μg and noradrenaline (Nor) in the doses shown. The period of stimulation at 10/sec is indicated by the black bar. During stimulation nicotine produced an enhanced response, cf. Fig. 6. Tubocurarine (100 μg) was present throughout. Time between the panels is 40, 50 and 36 min respectively.

## DISCUSSION

When the response to infused noradrenaline has almost disappeared, the pressor effects of tyramine, ephedrine, nicotine and electrical stimulation of the sympathetic nerves were enhanced. A similar potentiation of the pressor effect of tyramine has been reported by Nasmyth (1962) in the pithed rat and in other species by several authors (see Muscholl, 1966). The present experiments with the perfused limb show that both the peripheral vascular smooth muscle and the heart contribute to this potentiation but that the effect on the heart may be the sole mechanism in circumstances in which the vascular smooth muscle is fully contracted. The first objective of these experiments, to determine whether the smooth muscle had lost its capacity to respond, is therefore clearly answered in the negative.

The cause of the potentiation appears to be an increase in the amount of catecholamines liberated from bound stores in the tissues by these agents. The degree of potentiation of the response to nerve stimulation was less than that to the indirect sympathomimetics. This raises the question of whether the stores accessible to the nerve impulse differ from those on which, for example, tyramine acts (De Schaepdryver, Bogaert, Delaunois & Bernard, 1963). Evidence for the existence of several different stores has been put forward (Weiner, Draskóczy & Burack, 1962; Axelrod, 1964; Trendelenburg, 1966). The present results, in which the response to nerve stimulation was potentiated to a lesser

extent than that to tyramine, are consistent with the view that the store available to the nerve impulse is increased to a lesser degree than is that to which tyramine has access. These various stores are usually assumed to be subcompartments within the nerve endings. Whereas in the present experiments the quantities of noradrenaline infused are large, the possibility of binding to a variety of extraneuronal sites arises (Gillespie & Hamilton, 1966). Tyramine might, therefore, owe its greater potentiation to an ability to displace tissue bound noradrenaline from some "silent combinations" (Goldstein, 1949) outside the nerve endings and this noradrenaline would then be available to act on the receptors on the smooth muscle cell.

The effect of tyramine on the blood pressure continues to be potentiated at a time when the peripheral vascular response is maximal, assuming the limb is representative of other vascular beds. This suggests that the noradrenaline dose-response curve for cardiac muscle may be displaced to the right along the abscissa compared with that for vascular smooth muscle and would be consistent with the suggestion made in the previous paper (Gillespie & Muir, 1967b) that the density of receptors in this tissue is less than in smooth muscle.

The potentiation of the response to nicotine during continuous stimulation of the sympathetic nerves is presumably due to a different mechanism than that operating during the infusion of noradrenaline, when the initial depressor response is never seen. The effect may be on transmission in the sympathetic ganglia. Early in the stimulation period large quantities of acetylcholine may be liberated by the preganglionic nerve endings and, summing with the depolarizing effect of nicotine, cause a depolarization block. Later in the stimulation period, the enhanced response to nicotine would indicate that the quantity of acetylcholine liberated is subthreshold for many post-ganglionic neurones and summation with the depolarizing effect of nicotine results in a potentiated response. This potentiation is lost when nerve stimulation ceases. Unlike nicotine, tyramine acts only on the post-ganglionic neuro-effector junction and shows none of these actions during nerve stimulation. Interference with ganglion transmission could also explain the disappearance of the response to either nicotine or nerve stimulation during an infusion of noradrenaline as the rate of infusion is increased. Both adrenaline and noradrenaline are known to be able to block transmission at this site (Marrazzi, 1939; Bülbring & Burn, 1942; Duner & von Euler, 1957).

## SUMMARY

1. The pressor effects of the indirect sympathomimetics tyramine, ephedrine and nicotine were examined before and during the continuous infusion of noradrenaline and during the continuous stimulation of sympathetic nerves. The pressor effect of sympathetic nerve stimulation during the infusion of noradrenaline was also examined.

2. When the pressor effect of the infused noradrenaline had almost disappeared the responses to sympathomimetics were greatly potentiated. The response to sympathetic nerve stimulation was unaltered or slightly increased.

3. The potentiation to tyramine was seen at all rates of infusion of noradrenaline, that to nicotine and nerve stimulation disappeared as the rate of noradrenaline infusion was increased and eventually these responses were depressed. This is probably due to depression of ganglion transmission by the infused noradrenaline.

4. With tyramine the site of potentiation was investigated by measuring the peripheral resistance in one limb in addition to blood pressure. Potentiation of both the cardiac and peripheral responses was seen but at high rates of noradrenaline infusion the peripheral resistance reached a maximum yet potentiation was still seen, presumably entirely by an action on the heart.

5. Continuous stimulation of sympathetic nerves, while producing an effect on blood pressure similar to that of infusing noradrenaline, did not potentiate the response to tyramine. The effect on the response to nicotine was complex: initially this was reversed to depressor but later in the stimulation period the pressor effect reappeared and was then potentiated. These effects are probably due to an action of nicotine on ganglion transmission.

6. An increase in the stores of transmitter available to the drugs and nerve stimulation after infusion of noradrenaline may explain the potentiated responses. The greater potentiation to sympathomimetics than to nerve stimulation may mean that the stores available to the former may be increased to a greater degree. The possibility that some of this store may be extraneuronal and bound to tissues is discussed.

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