Br. J. Pharmac. Chemother. (1967), **30**, 88–98.

THE ORIGIN OF THE DECLINE IN THE VASOPRESSOR RESPONSE TO INFUSED NORADRENALINE IN THE PITHED RAT

BY

J. S. GILLESPIE* AND T. C. MUIR

From the Institute of Physiology and Division of Experimental Pharmacology, The University, Glasgow

(*Received November* 29, 1966)

In several species, including man, the continuous infusion of noradrenaline always results in an eventual steady decline from the initial pressor level (Nickerson, 1962; Rosenthale & Dipalma, 1963; Beyth & Gutman, 1965). This reduction in response is usually attributed to desensitization or tachyphylaxis. Our interest in this phenomenon arose during attempts to modify that preparation of the anaesthetized rat described by Straughan (1958) as an assay method for acetylcholine. To overcome the large reflex depressor artefact which accompanies any intravenous injection in this preparation, the rat was pithed and the normal neurally maintained vascular tone mimicked by infusing noradrenaline continuously in amounts sufficient to raise the blood pressure to about 120 mm Hg. A highly sensitive and artefact-free preparation in which the effect of 0.25 ng of acetylcholine could be recognized was obtained. However, within 5–10 min of beginning the infusion both the blood pressure and the sensitivity to acetylcholine began to fall. Two points seemed of interest. First, if this were due to desensitization to noradrenaline, it was not specific for one substance. Secondly, although the noradrenaline infused was limited to that required to match the neurally released transmitter in restoring the blood pressure to normal levels, desensitization still occurred. Previous investigations of this phenomenon have employed animals with an intact vasoconstrictor outflow in which the infused noradrenaline raised the blood pressure above normal.

The present paper reports the results of an investigation into the causes of these changes. The literature contains few reports which allow the contribution of the peripheral vascular smooth muscle to the fall in blood pressure to be separated from that of the heart. To do this, a modification of the technique of Bygdeman (1963) was used, in which the isolated vascular bed of one lower limb was perfused *in situ* with the animal's own arterial blood. The perfusion pressure gave a measure of the response of the vascular smooth muscle uncomplicated by cardiac efficiency. The blood pressure response of the whole animal, excluding the perfused limb, gave an integrated picture of both the peripheral vascular response and the ability of the heart to maintain its output in face of any increase in peripheral resistance.

* Henry Head Fellow.

METHODS

The pithed rat preparation (Gillespie & Muir, 1967a) was used and blood pressure recorded from one carotid artery. An external jugular vein was cannulated for the administration of drugs and a femoral vein for the infusion of noradrenaline. Arterial blood from the other carotid artery was pumped by means of a Watson–Marlow constant output pump into the distal end of one femoral artery cannulated in the upper thigh. The vascular bed of the perfused limb was then isolated by tying tight ligatures around the entire limb above the femoral cannula but excluding the femoral vein and nerve. The perfusion pressure was measured by a Condon mercury manometer. The output of the pump was adjusted to give an initial perfusion pressure of about 50–60 mm Hg. This usually corresponded to a flow rate of 1.5 ml./min. The perfusion pressure fluctuated; these variations were found to be due to small platelet emboli. Large amounts of heparin given to the animal and soaking the tubing and cannula in a solution of heparin helped to reduce their occurrence.

Noradrenaline was infused *via* a femoral vein (0.01–0.5 mg/kg/min, at flow rates from 0.05–0.4 ml./min commonly 0.1 ml./min), using a Palmer Slow Injection apparatus. Direct, supramaximal electrical stimulation of the sympathetic nerves was carried out as described in the preceding article, after atropine (1 mg/kg) and tubocurarine (0.5–2 mg/kg) had been given.

The following drugs were used, and with the exception of pitressin doses refer to their salts: (−)-noradrenaline bitartrate (Koch-Light), ; amyl nitrite B.P.C. (Martindale Samoore); phentolamine (Ciba); atropine sulphate (B.D.H.); (+)-tubocurarine chloride (Burroughs Wellcome); pitressin (vasopressin B.P. Parke-Davis).

RESULTS

Effect of prolonged intravenous infusion of noradrenaline

The effect on the blood pressure of the whole animal is shown in Fig. 1. In these experiments the rate of infusion of noradrenaline was adjusted so that the initial rise in pressure was to 120–150 mm Hg. This pressure was not maintained, declining at first

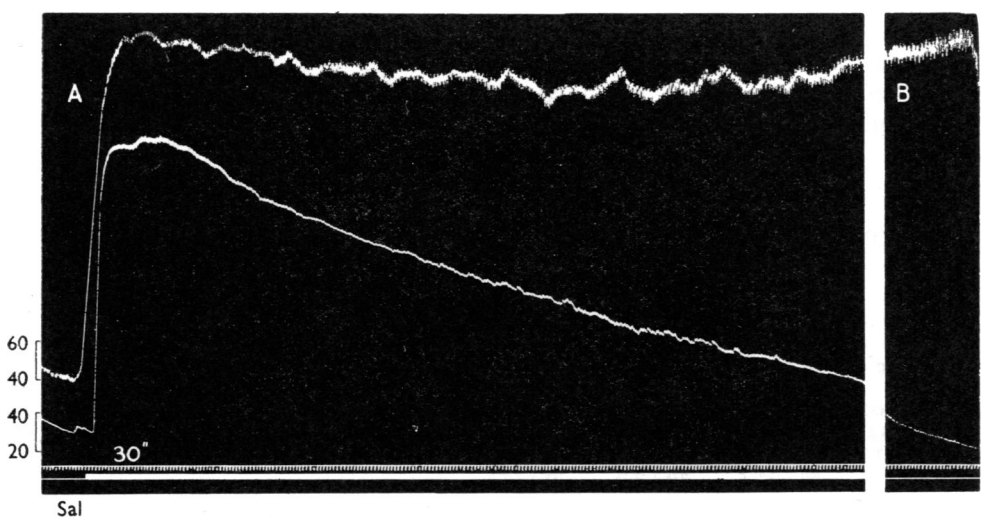

Fig. 1. The response of the blood pressure (lower trace) and the perfusion pressure in one hind limb of a pithed rat (310 g) to intravenous infusion of noradrenaline (0.1 mg/kg/min, white bar). Tubocurarine (310 μg intravenously) was present throughout. The effect of stopping the infusion is seen in B. The peripheral vascular response is maintained. The blood pressure falls even further. The time between panels is 27 min.

rapidly and later more slowly so that after ½–2 hr it had returned to or near the pre-infusion level in spite of the continuing infusion of noradrenaline. Stopping the infusion of noradrenaline caused a further fall, although the level of the blood pressure had already returned to pre-infusion values. The decline in response to noradrenaline was not specific for this substance; the pressor response to vasopressin and the depressor effect of acetylcholine were also reduced (Fig. 2).

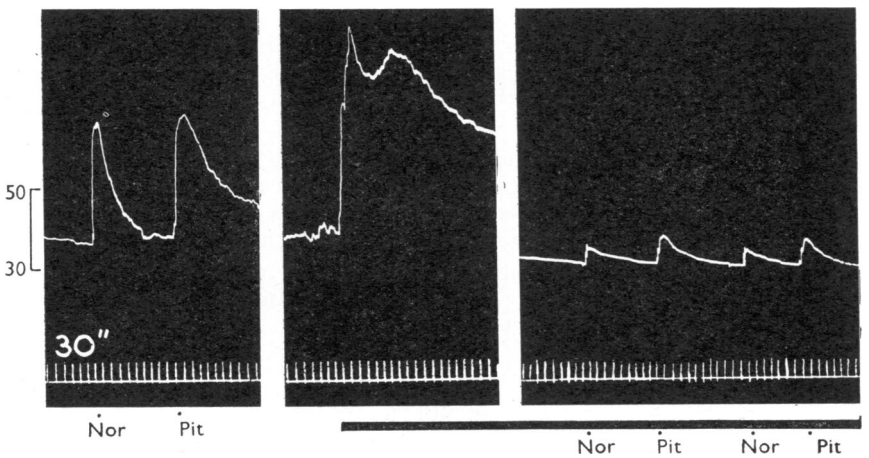

Fig. 2. The effect of intravenous infusion of noradrenaline (0.01 mg/kg/min; black bar) for 75 min on the response of the blood pressure of the pithed rat (250 g) to injection of noradrenaline (Nor) 200 ng, and pitressin (Pit) 0.001 IU. In the 60 min between the second and third panels the infusion was continued. The time between the first and second panel was 12 min.

The response of the blood-perfused limb was quite different. The perfusion pressure rose, as did the arterial pressure, but there was little or no subsequent decline. On the contrary, on several occasions, the initial rapid rise was followed by a secondary gradual rise throughout the period of infusion. These differences are illustrated in Fig. 1 and Table 1.

TABLE I

THE RESPONSE OF THE BLOOD PRESSURE AND THE PERFUSION PRESSURE OF ONE HIND LIMB TO CONTINUOUS INFUSION OF NORADRENALINE

			Mean pressor response (mm/Hg)				Mean % change in the response	
			Limb pressure		Blood pressure			
Expts. (no.)	Rate of infusion (mg/kg/min)	Mean duration of infusion (min)	At beginning of infusion	At end of infusion	At beginning of infusion	At end of infusion	Limb pressure	Blood pressure
5	0·01	28±11·5	30±2·8	39±13·8	58± 8·3	33±8·1	+18±38·7	−46±10·4
1	0·02	46	17	12	49	21	−29	−57
5	0·03	115±27·8	115± 9·2	138± 9·6	109±13·9	11±6·5	+25±17·0	−88± 7·8
2	0·04	102±11·9	115±25·1	115±25·6	135±30·1	4±1·5	−1± 1·0	−103± 0·5
3	0·05	72±10·4	98±31·2	132± 5·7	100±25·9	18±2·8	+7±35·8	−81± 3·4
10	0·1	60± 8·5	165± 8·9	160± 9·1	110± 8·7	26±7·3	−3± 2·24	−76± 6·4
1	1·6	60	184	174	180	38	−5	−79
Mean							+2± 6·6	−76± 7·2

The possibility that, in spite of these large rises in blood and perfusion pressure, these amounts of noradrenaline were insufficient to produce desensitization of the vascular smooth muscle was investigated by progressively increasing the amount of noradrenaline infused, until this greatly exceeded that required for a maximum response, and, indeed, until enormous quantities of catecholamines had been given (for example, 50 mgm in 100 min). In no case was there any significant fall in perfusion pressure. One such experiment is illustrated in Fig. 3, and the results of all our experiments are analysed in Table 1.

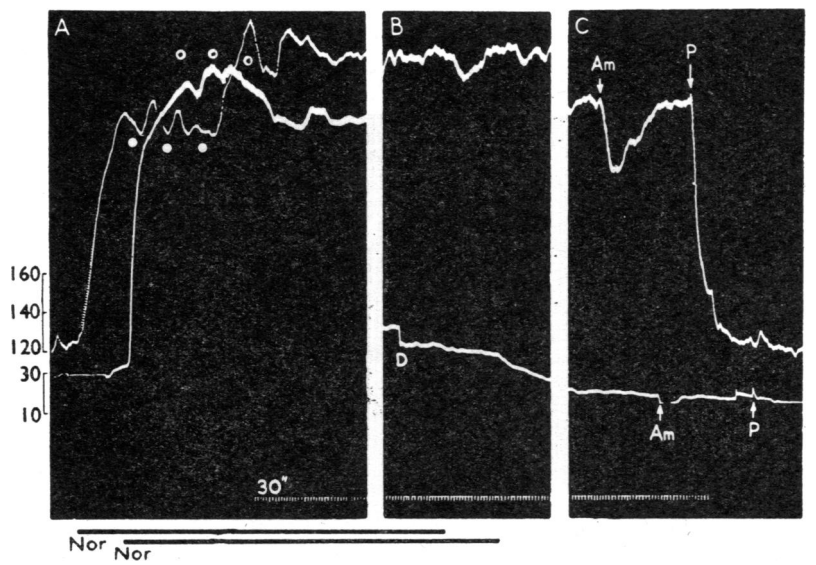

Fig. 3. The response of the blood pressure (lower trace) and the perfusion pressure of one hind limb of a pithed rat (300 g) to infusion of noradrenaline (Nor). The traces are displaced in time with regard to one another as shown by the position of the black bars representing the infusion of noradrenaline. The rate of infusion was progressively increased from 0.2 mg/kg/min (initial) to 0.4, 0.4 to 0.8 then back to 0.4 mg/kg/min as shown by the open circles (blood pressure) and filled circles (perfusion pressure). After the infusion the perfusion pressure in the limb does not fall but is reversibly lowered by amyl nitrite (Am) inhaled and abolished by phentolamine (P) 1 mg intra-arterially. The interval between A and B is 33 min and that between B and C 51 min. The drum was stopped at D for 15 min. Tubocurarine (350 μg) was present throughout.

The effect of stopping the infusion of noradrenaline on the blood pressure and on the perfusion pressure of the limb also differed. The blood pressure started to fall almost immediately, irrespective of the quantity of noradrenaline infused, and often ended in the death of the animal. The effect on the perfused limb varied with the rate of noradrenaline infusion. At 0.01 mg/kg/min (total 1 mg or less of noradrenaline), there was a short delay followed by a decline in pressure to the pre-infusion value. At higher rates of infusion the pressure did not fall when the infusion stopped, and, with very high rates, death intervened with no measurable reduction in peripheral resistance. In some of these animals, the pressor response persisted without decline, for periods up to 1 hr after the end of the noradrenaline infusion. That the vascular contraction underlying

this effect was not due to some irreversible terminal spasm, was shown (Fig. 3c) by the introduction of amyl nitrite vapour into the inspired air. This produced a dramatic fall in pressure which was reversed on removal of the drug. The close intra-arterial injection of phentolamine (1 mg) into the perfused limb caused an immediate, permanent fall in the perfusion pressure to the pre-existing base line (Fig. 3c), suggesting that contraction was due to a continuing effect of noradrenaline.

Effect of prolonged continuous stimulation of sympathetic nerves

Continuous stimulation of the sympathetic nerves caused a rise both in the arterial blood pressure in the whole animal, and in the perfusion pressure of the isolated limb. The pressor response in the whole animal declined as described previously (Gillespie

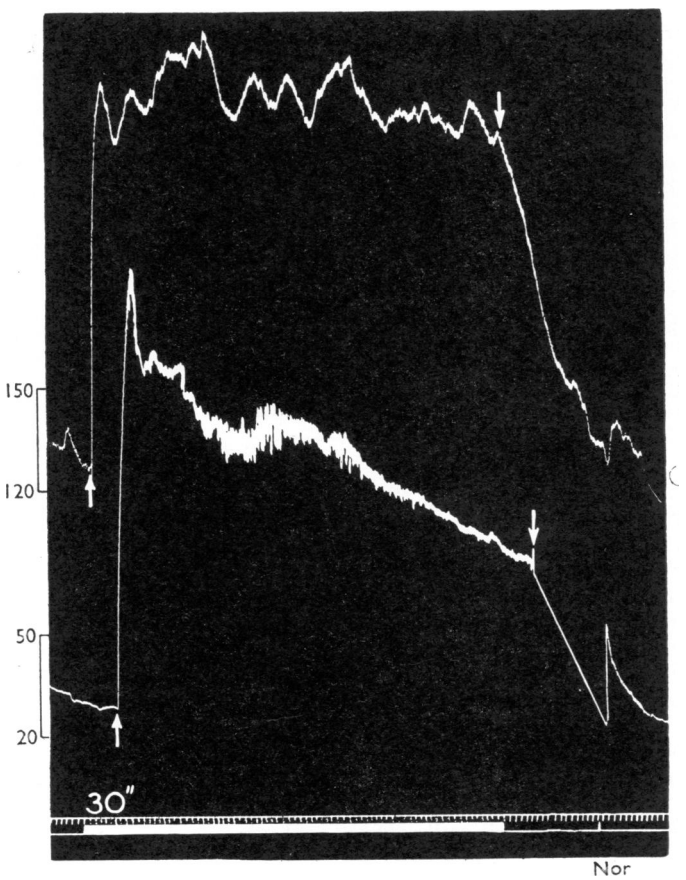

Fig. 4. The response of the blood pressure (lower trace) and the perfusion pressure of one hind limb of a pithed rat (300 g) to prolonged continuous stimulation of sympathetic nerves at a frequency of 10/sec as indicated by the white bar and to noradrenaline 500 μg (Nor). The beginning and end of stimulation is indicated on the traces by the arrows. The peripheral resistance is maintained, the blood pressure response declines. Tubocurarine (400 μg intravenously) was present throughout. Part of the record of blood pressure after the end of stimulation was missing, this has been arbitrarily replaced by a straight line.

TABLE 2

THE RESPONSE OF THE BLOOD PRESSURE AND THE PERFUSION PRESSURE OF ONE HIND LIMB TO CONTINUOUS STIMULATION OF THE SYMPATHETIC OUTFLOW

The experiments fell into two groups. In one (four animals) there was no fall in perfusion pressure; in the other (nine animals) there was a fall but less than the fall in blood pressure.

		Mean pressor response (mm Hg)					
		Limb pressure		Blood pressure		Mean % change in the response	
Expts. (no.)	Rate of stimulation (pulses/sec)	At beginning of stimulation	At end of stimulation	At beginning of stimulation	At end of stimulation	Limb pressure	Blood pressure
4	10	86±16·5	88±15·8	110±8·6	18±5·9	+3±2·4	−85±8·3
9	10	100±5·4	60± 2·8	101±3·0	27±7·9	−42±7·0	−74±6·3

& Muir, 1967a), but rather more quickly in the present experiments, possibly because of the extracorporeal circulation. The course of the pressor response in the perfused limb varied. In four animals the initial rise was maintained throughout the period of stimulation with no evidence of desensitization (Fig. 4)—that is, resembling the response to infused noradrenaline. In a further nine experiments there was some decline in the pressor response with time, but in no instance was this as great or as rapid in development as that in the arterial blood pressure of the whole animal (Table 2). The fall in arterial blood pressure in both groups was similar, with no evidence of resistance to desensitization in the four animals in which the limb perfusion pressure was maintained constant throughout. One difference between nerve stimulation and infusion of noradrenaline was that, on stopping nerve stimulation, blood pressure began to fall after a short latent period, even when this had been maintained undiminished until the end of stimulation. There was never a prolonged maintenance of vasoconstriction in the post-stimulation period. The pressure response from infusing noradrenaline never fell so rapidly on stopping the infusion. The rate of fall varied with the previous infusion rate,

TABLE 3

THE DECLINE IN THE LIMB PERFUSION PRESSURE FOLLOWING THE END OF CONTINUOUS STIMULATION OF THE SYMPATHETIC VASOCONSTRICTOR OUTFLOW OR THE INFUSION TO NORADRENALINE

Some experiments included in Tables 1 and 2 have had to be omitted as the animals died almost immediately after the stimulation or infusion was stopped.

		Mean pressor response in perfused limb (mm/Hg)		
Expts. (no.)	Rate of stimulation (pulses/sec) or of infusion (mg/kg/min)	At end of stimulation or infusion	10 min after stimulation or infusion	Mean % change
		Nerve stimulation		
10	10	65± 9·6	11± 4·6	−98±16·8
		Noradrenaline infusion		
5	0·01	43±15·0	−3± 9·8	−134± 6·7
4	0·03	148± 5·1	90±13·7	−55±17·8
4	0·05	124± 6·0	39±14·1	−68±11·9
6	0·1	157±13·6	156±13·9	−1± 1·5
1	1·6	180	175	−3

and up to 0.05 mg/kg/min it was sufficient to produce a considerable fall by the end of 10 min. At higher rates of infusion there was no observable fall either on stopping the infusion or 10 min later (Table 3 and Fig. 1). The graded relation between the rate of infusion of noradrenaline and the rate of decay of the response on stopping the infusion would have been better expressed by the half times of decay, but with the higher rates of infusion these could not be calculated as the response hardly declined before the animal died.

The role of anoxia

The maintained intense vasconstriction in the perfused limb suggested that tissue anoxia might be playing some part in the observed decline in arterial blood pressure. This was investigated during the infusion of noradrenaline by changing from air to 100% oxygen as the respiratory gas. The result was a rise in pressure both in the perfused limb and in the whole animal. In neither case was the rise maintained, and subsequently the pressures in the whole animal and in the hind limb fell at approximately the same rate. Respiring rats with 100% oxygen from the beginning of an experiment did not prevent the fall in arterial blood pressure during continuous infusion of noradrenaline.

DISCUSSION

It has frequently been reported that the continuous infusion of adrenaline or noradrenaline results in a rise of systemic blood pressure which is not maintained despite the continuation of the infusion, and that when the infusion is stopped there is a long-lasting fall in pressure below the pre-infusion level (Bainbridge & Trevan, 1917; Erlanger & Gasser, 1919; Freeman, Freedman & Miller, 1941; Blacket, Pickering & Wilson, 1950; Duner & von Euler, 1957). In most instances intact animals were used, so that the infusion caused a rise in pressure above, often far above, normal. The present experiments show that a similar decline in pressure is seen in a pithed rat even when the initial rise is adjusted so as to restore what would be the normal pressure in this animal.

Prolonged infusion of these amines produces a shock-like condition and a great variety of changes has been reported, most of which have at one time or another been causally related to the fall in blood pressure. Chief among these are a reduction in circulating blood volume with haemoconcentration (Bainbridge & Trevan, 1917; Erlanger & Gasser, 1919; Freeman *et al.*, 1941); liberation of vasodilator substances, including histamine, or the vasodilator action of the infused sympathomimetic itself (Blacket *et al.*, 1950; György & Dóda, 1960; Coppola & Dipalma, 1962); acidosis (Small, Weitzner & Nahas, 1959); and cardiac failure (Elliott, 1905; Chen & Schmidt, 1925; Chen, 1928; Mügge, 1932; Chappel, Rona, Balázs & Gaudry, 1959; Szakács & Mehlman, 1960). It is also true that each of these mechanisms at some other time has been excluded as the cause of the loss in response. More specifically pharmacological explanations have also been offered. One view is that the pressor amines accumulate on their specific receptors, presumably followed by some form of "accommodation," so that the drug receptor complex loses its ability to initiate a response (Winder, Anderson & Parke, 1948; Huidobro, Croxatto, Allende & del Río, 1950; Rosenthale & Dipalma, 1963; Perez-Reyes & Lipton, 1963; Pohle & Mathies, 1963). A second explanation, based on a

theory of drug receptor interaction which assumes that the energy for the initiation of a response is derived only at the moment of association, proposes that the rate of drug receptor combinations declines with time as the receptors become occupied (Croxatto & Huidobro, 1956 ; Paton, 1961).

In most investigations arterial blood pressure has been the sole measure of cardiovascular changes, and so it was not possible to say whether the pressure fall was due to a fall in peripheral resistance or a decline in cardiac output. Nevertheless, two of the explanations of the fall in pressure—namely, the action of vasodilator substances or receptor saturation—imply that the vascular smooth muscle relaxes. In the present experiments this is clearly not so in the pithed rat. The peripheral resistance in the perfused limb did not decline with time ; on the contrary, there was often a slow secondary rise (Fig. 1). In only three reports in the literature have we been able to find information allowing the peripheral and central components to be evaluated. Erlanger & Gasser (1919) measured the regional flow through the femoral and mesenteric arteries and found that in both the flow declined during the infusion of adrenaline, indicating vasoconstriction, and that this effect persisted and might continue up to 2 hr beyond the end of the infusion. Horita, West & Dille (1953) measured blood flow in the femoral, carotid and mesenteric arteries and found that it was maximally reduced by repeated injections of amphetamine and remained so, so that subsequent injections of the drug produced only vasodilatation. They observed a similar prolonged vasoconstriction outlasting the end of the drug infusion in the perfused, isolated rabbit ear. Seager (1952), on the other hand, in a short note, reported that with a " Gibbs artificial heart " in the circulation the failure in the pressor response to infused sympathomimetics was still seen, and concluded that the failure was in the peripheral blood vessels. Neither adrenaline nor noradrenaline was included in the group of sympathomimetics he used.

If vascular smooth muscle cells do not show desensitization, this would eliminate the anomaly that smooth muscle cells in other sites (nictitating membrane, spleen) showed no evidence of desensitization to infused noradrenaline at a time when the blood pressure was steadily falling (György & Dóda, 1960 ; Pohle & Matthies, 1963). In some situations, of course, the prolonged maintenance of large contractions may result in a change in the ionic environment inside the cell and a reduction in the maximum response possible. Sub-maximal responses would be proportionately reduced. This type of non-specific desensitization has been reported (Paton, 1961). There was no evidence of this in the present experiments.

It is not possible to say whether the diminished cardiac output is the result of a diminished venous return or of failure of the cardiac musculature. Both factors may be operating. The appearance of arrhythmia during the infusion, together with the frequent reports in the literature of the damaging effect of sympathomimetics on the heart (Elliott, 1905 ; Freeman *et al.*, 1941 ; Chappel *et al.*, 1959 ; Szakács & Mehlman, 1960) suggest that cardiac damage is likely to be important. Indirect support for this is provided by the sudden worsening in the animal's condition immediately the noradrenaline infusion is stopped, suggesting that maintenance of the cardiac output is dependent on a continuous supply of the amine. This is consistent with a stimulating effect on a weakened heart, but difficult to relate to any of the peripheral changes said to be responsible for the diminished venous return, none of which would be expected to change

rapidly. The cardiac damage cannot be due solely to the mechanical strain imposed by the rise in peripheral resistance, since in some experiments the increase was restricted to the level normal for the animal. It is probable that the effect on the heart is a direct one, as was suggested by Chappel *et al.* (1959), since of the amines they tested for cardiac toxicity isoprenaline was the most potent yet did not cause a rise in peripheral resistance.

Paton (1961) has developed a theory of drug receptor interaction in which the effector cell stimulation is a quantal event at the moment of combination of drug and receptor. The present observation that with high rates of noradrenaline infusion the vasoconstriction does not diminish on stopping the infusion is difficult to reconcile with this theory. It is true that the circulating blood concentration will rise to high levels during such an infusion and that this will take time to disappear when the infusion is stopped. Nonetheless, the blood pressure begins to fall almost immediately the infusion ends, suggesting that the level of circulating amines acting on the heart is falling, yet the vascular constriction may persist unaltered till the animal's death. Experiments in progress in this laboratory have shown a similar persistence of constriction in isolated perfused arteries on returning to saline after high concentrations of noradrenaline. The results are consistent both with occupation theories of drug action and also with the modification of rate theory suggested by Paton & Rang (1966), in which the stimulus occurs not on association of drug and receptor but on dissociation.

In general, the results with prolonged nerve stimulation and with noradrenaline infusion are in agreement. It is true that in only four of the 13 experiments with nerve stimulation was the peripheral constriction maintained without decline, but in all others the decline in blood pressure was much greater than could be accounted for by the change in peripheral resistance. Experiments with nicotine to be reported (Gillespie & Muir, 1967c) suggest that the decline may partly be due to failure of ganglion transmission because of inadequate acetylcholine release. The residual twitching of skeletal muscle caused by nerve stimulation in these curarized preparations also declined as stimulation continued, suggesting failure at another cholinergic junction.

The fall in blood pressure after the end of the infusion of noradrenaline was due to a further, usually fatal, decline in cardiac output from a heart which had become dependent on a continuous supply of noradrenaline. This suggests that the heart, unlike the smooth muscle, cannot accumulate noradrenaline in a way that permits a continuing action. This might be due to the number of spare receptors on vascular smooth muscle exceeding that on cardiac muscle.

SUMMARY

1. The effect of continuous intravenous infusion of noradrenaline and continuous stimulation of sympathetic nerves on the blood pressure and on the perfusion pressure of one hind limb has been measured in the pithed rat. The hind limb was perfused with the animal's own arterial blood *via* a constant output pump and so gave a measure of peripheral resistance independent of cardiac output.

2. The rise in blood pressure caused by infused noradrenaline was not maintained, declining with time to the pre-infusion level. The perfusion pressure in the limb did not decline; on the contrary, after the initial rapid rise there was a further steady increase until the end of the infusion.

3. Replacing air with O_2 as the respiratory gas caused a temporary slight restoration of the blood pressure response to noradrenaline, which then declined at the same rate as before. Respiring with O_2 from the beginning of the infusion did not prevent the decline in blood pressure response.

4. When the infusion of noradrenaline was stopped the blood pressure fell after a short latency ; the pressor response in the limb continued, and with high rates of infusion remained undiminished for periods up to 1 hr. This response was reversible with amyl nitrite and abolished by phentolamine, and was, therefore, due to a continuing action of noradrenaline.

5. The rise in blood pressure with continuous stimulation of the sympathetic nerves was also not maintained. In the limb in four experiments the pressor response was maintained, in a further nine experiments there was some fall. In no experiment was the pressor response in the perfused limb maintained after the end of the stimulation.

6. The results imply that the fall in blood pressure during continuous infusion of noradrenaline or continuous stimulation of sympathetic nerves is not due to densitization of the vascular smooth muscle, whose contraction is undiminished throughout. The fall is, therefore, due to a diminished cardiac output.

We wish to acknowledge the skilled technical assistance of Miss Gladys M. Marren.

REFERENCES

BAINBRIDGE, F. A. & TREVAN, J. W. (1917). M.R.C. memorandum upon surgical shock and some allied conditions. *Br. med. J.*, **i**, 381–383.

BEYTH, J. & GUTMAN, J. (1965). Change of vasomotor response to catechol amines on repeated administration. *Br. J. Pharmac. Chemother.*, **24**, 612–623.

BLACKET, R. B., PICKERING, G. W. & WILSON, G. M. (1950). The effects of prolonged infusions of noradrenaline and adrenaline on the arterial pressure of the rabbit. *Clin. Sci.*, **9**, 247–257.

BYGDEMAN, S. (1963). Vascular reactivity in cats during induced changes in the acid-base balance of the blood. *Acta physiol. scand.*, **61**, supp. No. 222.

CHAPPEL, C. I., RONA, G., BALÁZS, T. & GAUDRY, R. (1959). Comparison of cardiotoxic actions of certain sympathomimetic amines. *Can. J. Biochem. Physiol.*, **37**, 35–42.

CHEN, K. K. & SCHMIDT, C. F. (1925). The action of ephedrine, the active principle of the Chinese drug MA HUANG. *J. Pharmac. exp. Ther.*, **24**, 339–357.

CHEN, K. K. (1928). Variations in blood pressure on repeated administration of *l*- and *dl*-ephedrines *J. Pharmac. exp. Ther.*, **33**, 219–235.

COPPOLA, J. A. & DIPALMA, J. R. (1962). Histamine as a factor in tolerance to levarterenol. *Am J. Physiol.*, **202**, 114–116.

CROXATTO, R. & HUIDOBRO, F. (1956). Fundamental basis of the specificity of pressor and depressor amines in their vascular effects. V. Theoretical fundamentals; drug receptor linkage. *Archs int. pharmacodyn. Ther.*, **106**, 207–243.

DUNER, H. & VON EULER, U. S. (1957). Secondary fall in blood pressure following noradrenaline infusion in the cat. *Acta physiol. scand.*, **38**, 355–363.

ELLIOTT, T. R. (1905). The action of adrenalin. *J. Physiol.*, **32**, 401–467.

ERLANGER, J. & GASSER, H. S. (1919). Studies in secondary traumatic shock. III. Circulatory failure due to adrenalin. *Am. J. Physiol.*, **49**, 345–376.

FREEMAN, N. E., FREEDMAN, H. & MILLER, C. C. (1941). The production of shock by the prolonged continuous injection of adrenalin in unanesthetized dogs. *Am. J. Physiol.*, **131**, 545–553.

GILLESPIE, J. S. & MUIR, T. C. (1967a). A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. *Br. J. Pharmac. Chemother.*, **30**, 78–87.

GILLESPIE, J. S. & MUIR, T. C. (1967c). The effect of infusing noradrenaline and of stimulating the sympathetic nerves on the blood pressure response to indirect sympathomimetics. *Br. J. Pharmac. Chemother.*, **30**, 99–107.

GYÖRGY, L. & DÓDA, M. (1960). Adrenaline tachyphylaxis after dibenamine. *Archs int. Pharmacodyn. Ther.*, **124**, 66–75.

HORITA, A., WEST, T. C. & DILLE, J. M. (1953). Cardiovascular responses during amphetamine tachyphylaxis. *J. Pharmac. exp. Ther.*, **108**, 224–232.

HUIDOBRO, F., CROXATTO, R., ALLENDE, J. & DEL RÍO, J. (1950). Fundamental basis of the specificity of pressor and depressor amines in their vascular effects. 1. Blockade of the hypertensive action of vasopressor amines by the effect of the same substances. *Acta physiol. latinoam.*, **1**, 91–109.

MÜGGE, H. (1932). Ein beitrag zur analyse der wirkung des ephedrins und einiger chemisch verwandter substanzen. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **165**, 230–243.

NICKERSON, M. (1962). Drug therapy of shock. In *Shock*, pp. 356–370, ed. BOCK, K. D. Springer, Berlin.

PATON, W. D. M. (1961). A theory of drug action based on the rate of drug-receptor combination. *Proc. R. Soc. B.*, **154**, 21–69.

PATON, W. D. M. & RANG, H. P. (1966). A kinetic approach to the mechanism of drug action. *Advances in Drug Research*, **3**, 57–80.

PEREZ-REYES, M. & LIPTON, M. A. (1963). Tachyphylaxis to epinephrine and its modification by cocaine. *Proc. Soc. exp. Biol. Med.*, **112**, 181–186.

POHLE, W. & MATTHIES, H. (1963). Die abnahme der Blutdruckwirkung von Noradrenalin bei wiederholter Applikation. *Acta biol. med. germ.*, **11**, 519–529.

ROSENTHALE, M. E. & DIPALMA, J. R. (1963). Norepinephrine storage and acute tolerance. *Archs int. Pharmacodyn. Ther.*, **146**, 529–536.

SEAGER, L. D. (1952). The role of the heart in the phenomenon of tachyphylaxis to vasopressor compounds. *J. Pharmac. exp. Ther.*, **106**, 413–414.

SMALL, H. S., WEITZNER, S. W. & NAHAS, G. G. (1959). Cardiovascular effects of levarterenol, hydrocortisone hemisuccinate and aldosterone in the dog. *Am. J. Physiol.*, **196**, 1025–1028.

STRAUGHAN, D. W. (1958). Assay of acetylcholine on the rat blood pressure. *J. Pharm. Pharmac.*, **10**, 783–784.

SZAKÁCS, J. E. & MEHLMAN, B. (1960). Pathologic changes induced by l-norepinephrine. *Am. J. Cardiol.*, **5**, 619–627.

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