Br. J. Pharmac. (1970), **38**, 20–36.

Vascular resistance in the perfused isolated rat tail

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Summary

1. A constant flow perfusion system using the isolated rat tail has been developed to facilitate the study of resistance vessel behaviour and the action of vasoactive drugs.

2. Baseline resistance remains stable for several hours and dose response curves to bolus injections of pressor agents are reproducible when dialysed bovine serum albumen is used in the perfusion medium to maintain osmotic pressure.

3. Noradrenaline, adrenaline, serotonin, vasopressin, angiotensin II, high potassium concentrations and sympathetic nerve stimulation constricted the vascular bed.

4. Angiotensin I, bradykinin, histamine, acetylcholine and isoprenaline did not alter vascular resistance under baseline conditions.

5. Maximal sensitivity to noradrenaline occurred at 32° to 34° C. Below 30° C, resting tone increased and the pressor effect of noradrenaline was prolonged.

6. Low concentrations of (±)-propranolol in the perfusate enhanced adrenaline and noradrenaline vasoconstriction, high concentration of (±)-propranolol had a direct pressor effect and did not affect catecholamine responses.

7. The preparation is a simple and relatively inexpensive adjunct to established methods of studying resistance vessel behaviour under varying experimental conditions.

Introduction

A variety of vascular beds have been used to study the interaction of vasoactive drugs and hormones with the smooth muscle of resistance vessels. Among these preparations the perfused limbs, mesenteric vessels and kidneys of several species and the central ear artery of the rabbit are well established.

Some of these preparations are difficult to set up and maintain. The perfused hind limb requires careful occlusion of collateral blood supply (Green, Cosby & Radzow, 1944) and blood is distributed to several types of tissue in which the resistance vessels differ in their response to vasoactive substances. Most preparations tend to develop increasing resistance to flow during prolonged arterial perfusion (Whittaker & Winton, 1933 ; Hinshaw & Worthen, 1961).

A vascular resistance model which is functionally and structurally more simple would be a valuable adjunct to these preparations. Ideally, such a preparation should be easy to set up, show stable resistance during long periods of perfusion, and should be sensitive to vasoactive substances with reproducible dose response curves.

The perfused rat tail has been used to study resistance vessels (Friedman, Nakashima & Friedman, 1963), but as with most perfusion methods, increasing vascular resistance has limited its use. The properties of the isolated preparation have not been characterized.

This paper describes the preparation and pharmacological properties of the perfused isolated rat tail, a preparation which has proved to be a simple method of studying vascular resistance vessels, and one which appears to fulfil the above requirements.

Methods

Perfusion technique

Male Sprague Dawley rats fed on a standard small animal diet (Spillers Ltd., Banbury) and tap water and weighing between 350 and 500 g were anaesthetized with pentobarbitone sodium B.P. given as an intraperitoneal injection (6 mg/100 g body weight). Heparin, 250 i.u. (Weddel Pharmaceuticals, London), was injected into a femoral vein and the ileo-lumbar artery then exposed through a 15 mm longitudinal incision on the ventral surface at the proximal end of the tail. A nylon cannula with a bevelled tip and an outside diameter of 1 mm was lubricated with sterile liquid paraffin and inserted into the artery through a small incision. The cannula was carefully advanced until the tip was about 5 mm distal to the opening in the roof of the fibrous tunnel in which the artery lies. The cannula was then firmly tied into the artery and the proximal end of the fibrous tunnel opened widely to prevent obstruction to the venous outflow. The tail was then amputated by a clean incision passing through an intervertebral space. The tail and cannula were then rapidly weighed and the tail carefully placed in the water-tight tail-holder, the cannula being adjusted and firmly fixed to avoid kinking or twisting. The tail-holder was then placed in the covered constant temperature water bath (Fig. 1), and the amputated tail perfused at a constant rate with a protein and electrolyte solution.

The perfusion was maintained with a peristaltic roller pump (Watson-Marlow type M.H.R.E. 72) acting on a short segment of silicone rubber tubing (I.D.= 1·0 mm, O.D.=4·0 mm) joined to 76 cm of rigid nylon tubing (I.D.=1·0 mm, O.D.=1·34 mm) immersed in the covered constant temperature water bath. The nylon tubing was joined to the tail cannula by a 3 cm length of rubber pressure tubing (I.D.=3·0 mm, O.D.=9·3 mm) through which test substances were injected. The resistance to flow through the tail was monitored at a " y " junction proximal to the rubber segment using a strain gauge pressure transducer (Bell & Howell type 4-327-L221) recording on a " Grass " model 5D polygraph. The rubber segment was the only part of the circuit distal to the pump not enclosed within the constant temperature bath. The temperature of the bath was maintained at 34·0° ±0·2° C except when the effect of temperature was specifically studied. The apparatus is shown diagrammatically in Fig. 1.

Perfusion was continued at 0·5 ml./min for about 30 min before the experiments were started. During the experiments the tail cannula was disconnected every 30 min and the circuit flushed through to remove any trapped gas bubbles.

Perfusion medium

The medium found to be most suitable for the extended perfusion of the rat tail preparation was a modification of that used by Keatinge (1968) and had the follow-

ing final composition: sodium chloride, 132·82 mM ; sodium bicarbonate, 15·0 mM ; potassium chloride, 4·832 mM ; calcium chloride, 2·50 mM ; magnesium chloride, 0·1050 mM ; dextrose, 0·694 mM ; bovine serum albumen, 40·0 g/l.

Bovine serum albumen was found to be the most suitable agent to prevent excessive water uptake by the tail. The albumen was obtained in the form of a dried powder prepared by Cohn fractionation of bovine plasma (Armour Pharmaceutical Co. Ltd., Eastbourne). Some batches of albumen were associated with excessive weight gain of the tail during the perfusion and a corresponding progressive rise in baseline resistance. Suppression of the response to pressor drugs was also noted. These problems were not encountered when the albumen was first dialysed.

The albumen was made up as a 12 g% solution dissolved in the above glucose and electrolyte solution without the albumen, and then dialysed at 4° C for 36 hr against 20 volumes of the same glucose and electrolyte solution, the dialysate being changed after 18 hr. The dialysed albumen solution was added to fresh glucose and electrolyte solution to give a final protein concentration of 4 g%. A mixture of oxygen (95%) and carbon dioxide (5%) was bubbled through the perfusion solution during the experiments.

Test substances

The effect of various drugs and hormones on the vascular resistance in the tail was investigated by measuring the increment of pressure proximal to the artery resulting from the infusion or direct injection of test substances into the perfusion circuit. Flow within the circuit was pulsatile but damped by a 10 cm segment of silicone rubber tubing between the "y" junction and the pressure transducer. Pressure was recorded as the mean pressure during the pulsatile cycle. Direct injection of test substances was made from a 50 μl. syringe through a 26 gauge needle into the segment of rubber pressure tubing. The concentration of the test

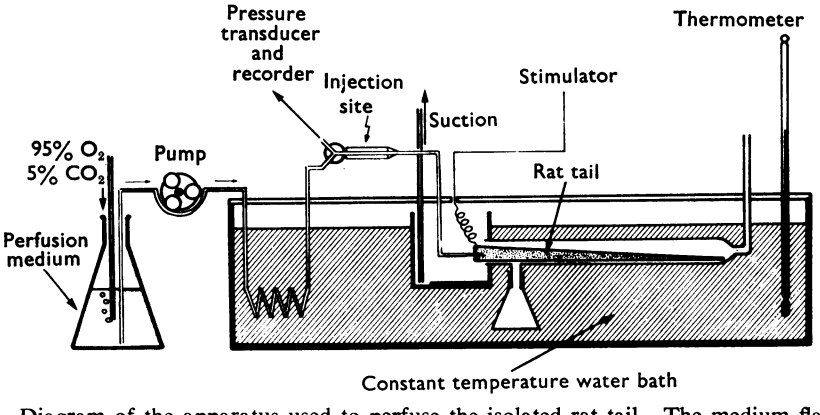

FIG. 1. Diagram of the apparatus used to perfuse the isolated rat tail. The medium flask was constantly gassed with a mixture of oxygen (95%) and carbon dioxide (5%). Perfusion at a known constant rate was maintained by the roller pump acting on a short segment of silicone rubber tubing joined in turn to a coil of nylon tubing enclosed in the covered constant temperature water bath. Vasoactive substances were injected into the short segment of rubber pressure tubing proximal to the tail cannula. Pressure was measured with a pressure transducer connected to the circuit at the "y" junction. Venous outflow from the tail was removed by a suction lead. (Reproduced by permission of the *Journal of Physiology*.)

substances was chosen to ensure that the volume injected was usually less than 40 μl. In some experiments test substances were slowly infused through a "y" junction into the circuit proximal to the roller pump, the infusion being maintained by a constant speed infusion pump (Harvard Apparatus Co. Inc., Dover, Massachusetts).

Test solutions

Solutions of drugs and hormones were made up in silicone-coated volumetric glassware (Siliclad ; Clay-Adams, New York) to limit glass adsorption. The following solutions were used for injection:

1. Aqueous solutions containing 1 μg/ml. or 10 μg/ml. of the test substance and 0·9 g% sodium chloride. Adrenaline (adrenaline injection B.P., Samoore Ltd., London) ; (−)-noradrenaline (Koch-Light Laboratories, Colnbrook, Buckinghamshire, or as Levophed, Parke-Davis & Co., Hounslow, Middlesex) ; acetylcholine (acetylcholine chloride, Sigma, London) ; histamine (histamine acid phosphate, B.D.H., London) ; serotonin (5-hydroxytryptamine, creatinine sulphate complex, Sigma).

2. Solutions containing 10 μg/ml., or 100 μg/ml., of the test substance in 0·1 M sodium phosphate buffer, pH 6·0. [Val^5]-angiotensin I (gift from Dr. F. M. Bumpus, Cleveland, Ohio) ; [Ileu^5]-angiotensin I monoacetate (gift from Dr. S. Wilkinson, Wellcome Research Laboratories, Beckenham, Kent) ; [Ileu^5]-angiotensin I (gift from Dr. L. T. Skeggs, Jr., Cleveland Veterans Hospital, Cleveland, Ohio) ; [Asp^{1}Val^5]-angiotensin II (obtained through the courtesy of Ciba Pharmaceutical Co., Basle) ; [Asn^{1}Val^5]-angiotensin II (Hypertensin, Ciba).

3. Aqueous solutions with 0·9 g% sodium chloride. Vasopressin (Pitressin, Parke-Davis) containing 2 or 4 pressor units/ml. Tyramine (tyrosamine hydrochloride, Sigma) containing 0·18 μmol/ml. ; isoprenaline (isoprenaline sulphate B.P., Martindale, London) containing 40 μg/ml. (±)-Propranolol hydrochloride (Propranolol ; I.C.I. Ltd., Cheshire) containing 100 μg/ml.

Purity of the angiotensin preparations

The three preparations of angiotensin I were equally pressor in the anaesthetized, ganglion blocked rat and were investigated by countercurrent-distribution and gel filtration to test for homogeneity and the absence of angiotensin II.

Countercurrent-distribution

The countercurrent-distribution system used was the method of Skeggs, Marsh, Kahn & Shumway (1954) as modified by Ryan & McKenzie (1968). [Val^5]-angiotensin I and [Ileu^5]-angiotensin I (from Dr. L. T. Skeggs) distributed as a single peak but the preparation of [Ileu^5]-angiotensin I (Wellcome Research Laboratories) distributed as two incompletely resolved peaks, one with a similar partition coefficient to [Val^5]-angiotensin I and the other with a partition coefficient between this and [Asn^{1}Val^5]-angiotensin II.

Gel filtration

The various preparations of angiotensin were eluted from a 40 cm × 2·8 cm bed of Sephadex G-15 with 0·1 M sodium phosphate buffer, pH 6·0, containing 0·1% (w/v) chlorhexidine gluconate 1,6-di-(4-chlorophenyldiguanido)-hexane gluconate,

Hibitane ; Imperial Chemical Industries Ltd., Pharmaceuticals Division, Wilmslow, Cheshire. Flow rates were fixed at 16 ml./hr. 15 min fractions were collected and tested for pressor activity in the anaesthetized, ganglion blocked rat (Peart, Lloyd, Thatcher, Lever, Payne & Stone, 1966). [Val^5]-angiotensin I and [Ileu^5]-angiotensin I (from Dr. L. T. Skeggs) again appeared to be homogenous and were eluted as single sharp peaks. [Ileu^5]-angiotensin I (Wellcome) was eluted as two peaks, neither of which appeared to be angiotensin II.

Sympathetic stimulation

The sympathetic nerve supply to the tail was stimulated through two fine platinum electrodes 1·5 mm apart placed under the central tail artery distal to the tie holding the tail cannula. Stimulation was applied with a " Grass " stimulator using D.C. square-wave pulses of up to 50 ms duration.

Temperature measurement in the rat

Temperature recordings were obtained in the anaesthetized intact rat from various positions along the length of the tail using a Copper/Eureka thermocouple contained within an 18G needle. The thermocouple was inserted through small incisions in the skin of the tail and then advanced into the lateral bundle of tendon sheaths. Deep body temperature was measured with the same thermocouple inserted into the peritoneal cavity. These measurements were first made with the animal maintained at room temperature (21·6° C), and then with radiant heat applied to the body only until the temperature around the trunk was 37° C.

Results

Baseline resistance

Dialysed bovine serum albumen was used to maintain the osmotic pressure of the perfusion medium, as preliminary experiments showed that the use of undialysed albumen or undialysed polyvinylpyrollidone of mean molecular weight 40,000 (Sigma Chemical Co., St. Louis, Missouri) resulted in a progressive rise in tail resistance and decreased sensitivity to noradrenaline.

Flow rates of either 0·5 or 1·0 ml./min were used in most studies. A flow rate of 0·5 ml./min was associated with an initial baseline pressure of 17 to 28 mm Hg, at 1·0 ml./min the corresponding pressures ranged from 32 to 40 mm Hg. The resistance was high when the tail was first connected to the perfusion circuit but subsided rapidly as blood was flushed from the tail. A steady level was reached in about 10 to 20 min. Table 1 shows the mean initial resistance once the preparation had stabilized, and the subsequent hourly rise in baseline resistance in seven normal

TABLE 1. *Changes in baseline resistance with time during the perfusion of tails from seven normal rats*

Duration of perfusion (min)	Baseline resistance (mm Hg)	
	Mean	S.D.
30	22·6	3·6
90	24·1	3·8
150	25·4	3·1
210	27·3	2·2

The perfusion rate in each experiment was constant at 0·5 ml./min, and during the experiments serial dose response curves to noradrenaline were measured.

rat tails which were perfused for 3–4 hr. Three dose response curves to noradrenaline were obtained during each of these perfusion experiments.

Resistance and flow

The relationship between the peripheral resistance and stepwise increases in flow in a typical preparation is shown in Fig. 2. The peripheral resistance (pressure/flow) fell with increasing flow rates up to 3·0 ml./min. The pressure/flow relationship of the perfusion circuit without the tail is also shown. Artificial resistance resulting in pressures up to 200 mm Hg did not reduce flow rates by more than 2%.

Response to sympathomimetic amines

At least half an hour of perfusion was allowed before (−)-noradrenaline injections were tested. Dose response curves to bolus injections of (−)-noradrenaline were then obtained using doses ranging from those giving no detectable pressure rise to those giving increments of pressure up to about 200 mm Hg. The first series of injections was used to ensure that the preparation worked properly; if the pressure peaks were unduly slurred or the pressure failed to return to baseline, the tail position was gently adjusted to eliminate twisting of the artery, and the injections then repeated. Malalignment of the cannula with the tail artery when mounting the preparation in the tail holder was the commonest cause of such artefacts. The tail was rejected if this adjustment was ineffective. Typical responses to increasing doses of noradrenaline are shown in Fig. 3. The reproducibility of the response to (−)-noradrenaline is indicated by comparison of the mean of the response at the beginning and end of 2–3 hr perfusions in tails from seven normal rats (Fig. 4). Serial dose response curves to (−)-noradrenaline obtained during two 6 hr perfusions provided a further indication of the stability of the preparation (Fig. 5).

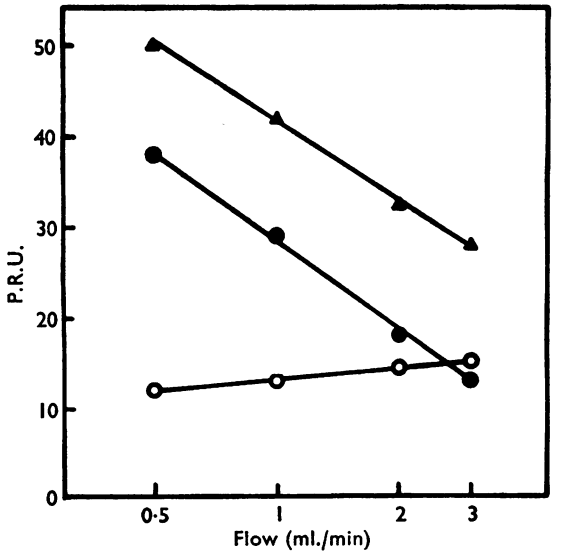

FIG. 2. Relationship between flow rate and peripheral resistance in the isolated rat tail. Flow rates in ml./min are shown on the abscissa, and peripheral resistance units (P.R.U.=pressure increment in mm Hg/flow in ml./min) on the ordinate. ▲, Complete preparation; ○, perfused cannula; ●, obtained by subtraction, represents the peripheral resistance of the tail itself.

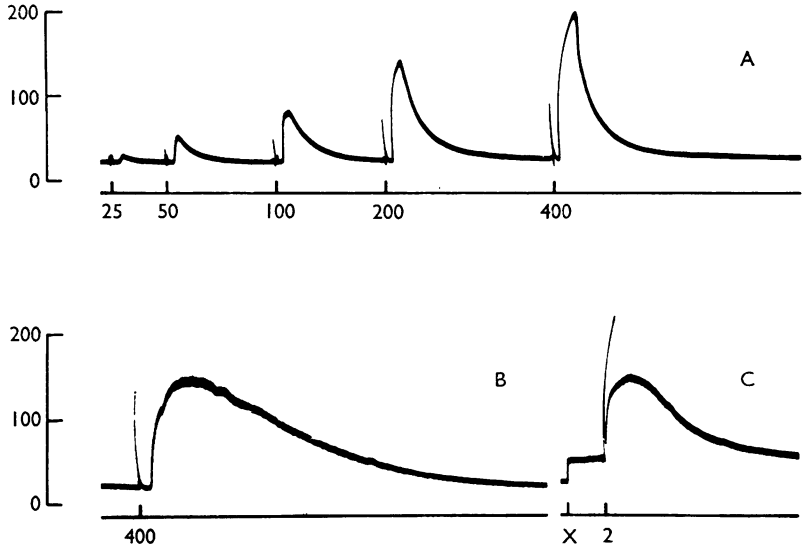

FIG. 3. Typical pressor responses to (−)-noradrenaline (A), vasopressin (B), and [Asn[1]Val[5]]-angiotensin II (C). Pressure in mm Hg is shown on the ordinate. Record A, perfusion rate 0·5 ml./min, noradrenaline doses in ng on the abscissa. Record B, perfusion rate 0·5 ml./min, pressor response to 400 m-u. of vasopressin. Record C, perfusion rate increased to 1·0 ml./min at point X, pressor response to 2 μg of angiotensin II.

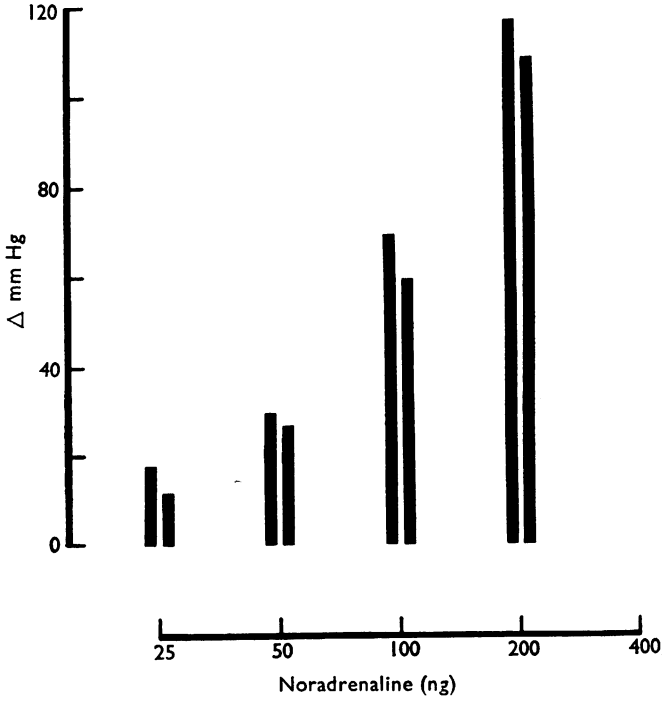

FIG. 4. Dose response curves to noradrenaline from seven isolated rat tails perfused at 0·5 ml./min. The first value of each pair is the mean of the first response measured in each tail. The second value is the mean of the third response measured in each case.

The effect of flow rates varying from 0·5 to 3·0 ml./min on the dose response curves to (−)-noradrenaline is shown in Fig. 6. The response to (−)-noradrenaline increased with flow rates up to 2·0 ml./min and thereafter decreased. The lower perfusion rate was chosen for most studies as adequate dose response curves could be obtained without the risk of excessive tail weight gain. If flow rates of 2·0 and 3·0 ml./min were sustained baseline resistance tended to rise more rapidly.

Adrenoceptors

β-adrenoceptor blockade

Dose response curves to adrenaline and noradrenaline were measured before, during and after constant-rate infusion of medium containing varying concentrations of (±)-propranolol hydrochloride. Constant rate perfusion of medium containing (±)-propranolol hydrochloride resulted in a reversible increase in baseline resistance when the rate of propranolol infusion was greater than 0·4 μg/min. Propranolol infusion at 0·1 and 0·2 μg/min reversibly increased the pressor response to adrenaline and noradrenaline, the effect being more marked with adrenaline. The phenomenon was reversed when the propranolol infusion was stopped. Propranolol infusion at rates above 0·4 μg/min did not increase the pressor response to catecholamines, although the baseline resistance was increased. A typical response is shown in Fig. 7.

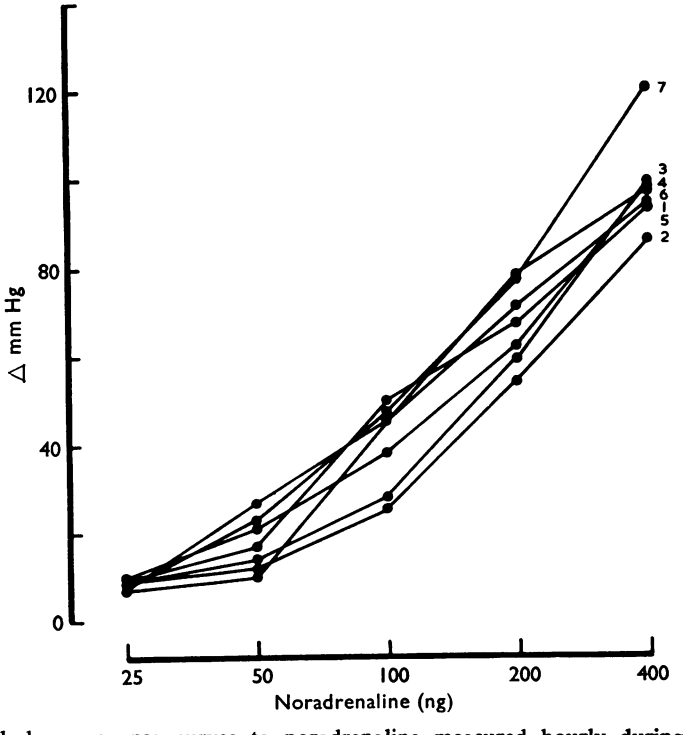

FIG. 5. Serial dose response curves to noradrenaline measured hourly during a prolonged perfusion of an isolated rat tail. The numbers beside each curve indicate the order in which they were measured.

α-adrenoceptor blockade

Direct injection of phenoxybenzamine (1 μg) into the circuit completely abolished the pressor response to a subsequent injection of adrenaline or noradrenaline. The baseline resistance did not fall in response to catecholamine injection after a preceding injection of phenoxybenzamine.

β-adrenoceptor stimulation

Direct injection of isoprenaline in doses up to 2·0 μg did not produce any measurable change in the resistance of the vascular bed.

Effect of temperature

Dose response curves to noradrenaline were measured in tails from normal rats at temperatures from 16° to 42° C. Experiments were performed with several initial bath temperatures. After measuring responses at various temperature increments the bath was returned to the starting temperature and the dose response again measured to check for any systematic change of sensitivity independent of temperature. A 10 min period was allowed to ensure equilibration of the tail temperature after each change in bath temperature.

Maximal sensitivity in the dose response curve to (−)-noradrenaline was seen between 32° and 34° C in five experiments. At 37° C decreased sensitivity was

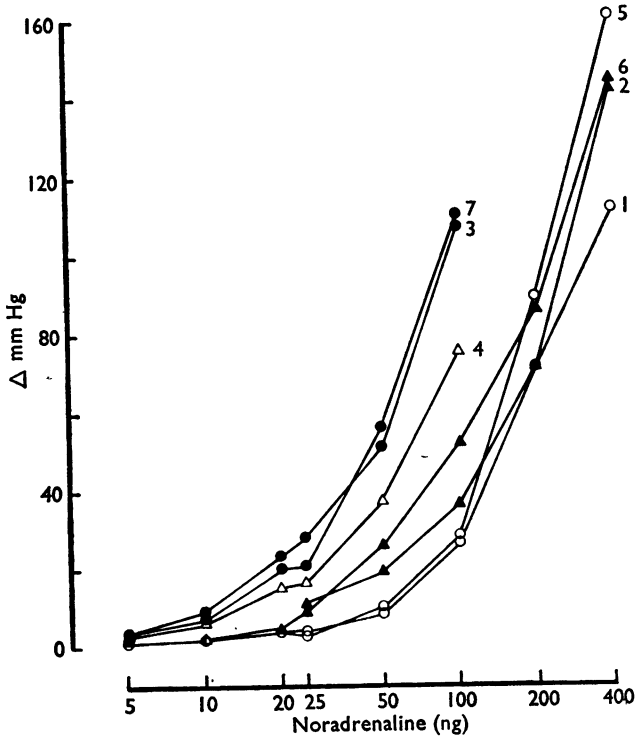

FIG. 6. Effect of perfusion rate on the pressor response to noradrenaline in the isolated rat tail. Serial dose response curves to noradrenaline were measured at perfusion rates of 0·5 ml./min (○——○), 1·0 ml./min (▲——▲), 2·0 ml./min (●——●) and 3·0 ml./min (△——△). The order in which the responses were measured is indicated beside each curve.

usually observed but there was no change in baseline resistance. Sensitivity was further reduced at 40° and 42° C and after a period at 42° C normal sensitivity at 34° C did not always return.

Temperatures below 30° C were associated with an increase in baseline resistance and a decreased pressor response to noradrenaline. Contractions were more sustained and often biphasic. Baseline resistance often remained high after exposure of the preparation to temperatures below 28° C. At temperatures below 30° C each pressor injection of noradrenaline led to a further sustained rise in baseline resistance.

The temperature within the tail of the intact animal was considerably less than deep body temperature. A rat maintained at an environmental temperature of 21 to 22° C had tail temperatures ranging from about 21° C near the distal end of the

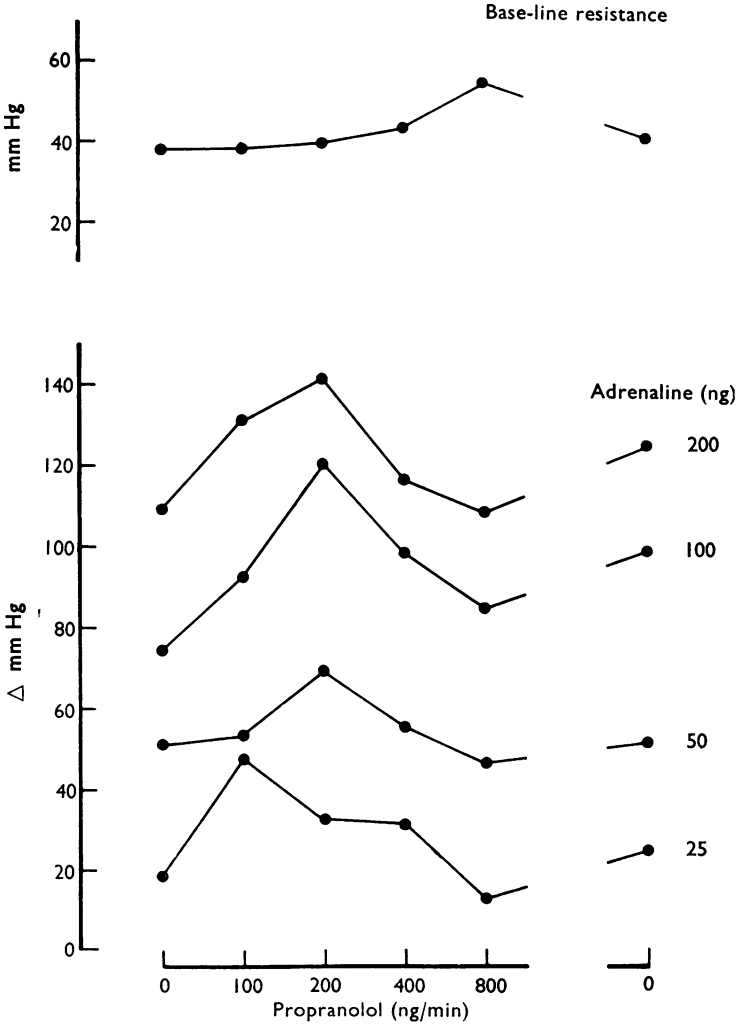

FIG. 7. Effect of (±)-propranolol hydrochloride on baseline resistance and the response to adrenaline in the isolated rat tail. Propranolol was infused into the circuit proximal to the roller pump, and the total perfusion rate (propranolol plus medium) was maintained constant at 1·0 ml./min.

tail to 26° C measured 4 cm from the proximal end. Deep body temperature was close to 37° C. Warming the whole animal, or the body excluding the tail, in a box maintained at 37° C produced marked vasodilatation of the tail and a corresponding rise in the tail temperature.

Response to pressor peptides

Angiotensin

[Asp^{1}Val^5]-angiotensin II and [Asn^{1}Val^5]-angiotensin II were both pressor and produced similar increases in vascular resistance in equimolar quantities. The increased vascular resistance produced was slower in onset and more prolonged than an equivalent pressure rise resulting from adrenaline or noradrenaline. A typical response is shown in Fig. 3. Doses as low as 25 ng of either peptide were regularly pressor, and the shape of the dose response curve was similar to that seen with adrenaline and noradrenaline, equimolar quantities of either angiotensin II, adrenaline and noradrenaline producing similar increases in vascular resistance (Figs. 4 and 8).

Tachyphylaxis to [Asn^{1}Val^5]-angiotensin II and [Asp^{1}Val^5]-angiotensin II was prolonged (Table 2). About 30 minutes between test doses was necessary to restore full sensitivity. Prior injection of [Val^5]-angiotensin I or [Ileu^5]-angiotensin I (from Dr. Skeggs) did not induce tachyphylaxis to angiotensin II.

At all doses tested (up to 2·0 μg) [Val^5]-angiotensin I and [Ileu^5]-angiotensin I (from Dr. Skeggs) failed to alter the resistance of the tail vascular bed. Both these preparations were eluted as a single sharp peak from Sephadex G-15, and were each distributed as a single peak in a 20 transfer countercurrent distribution between butan-1-ol (2 vol.) and 0·05 M sodium phosphate buffer pH 7·0 (1 vol.). The preparation of [Ileu^5]-angiotensin I (Wellcome Research Laboratories) was pressor in the

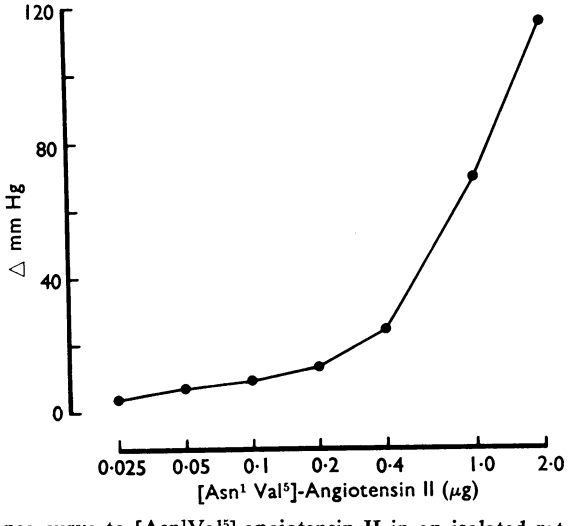

FIG. 8. Dose response curve to [Asn^{1}Val^5]-angiotensin II in an isolated rat tail perfused at 1·0 ml./min. Injections were given every 45 min to allow recovery from the period of tachyphylaxis.

intact rat, producing about half the pressor response of an equivalent weight of [Asn^{1}Val^5]-angiotensin II. This preparation of angiotensin I was not homogeneous on gel filtration of countercurrent distribution.

Vasopressin

Vasopressin was pressor in doses above 5×10^{-3} pressor units (1×10^{-8} mM). The pressor response was much more prolonged than an equivalent pressor response from adrenaline or noradrenaline (Fig. 3), and frequently slower in onset. Tachyphylaxis to vasopressin was not encountered. A typical dose response curve for vasopressin doses between 5×10^{-3} and 20×10^{-1} pressor units is shown in Fig. 9.

Bradykinin

Direct injection of up to $5{\cdot}9 \times 10^{-9}$M of bradykinin did not alter the vascular resistance of the preparation.

Other vasoactive substances

Injections of potassium chloride in doses ranging from 8 to 65 μmoles resulted in a pressor response (Fig. 10) with pressure peaks similar to those of noradrenaline and serotonin. Potassium had no effect on baseline resistance unless this had failed to return to normal following supramaximal doses of noradrenaline, in which case the resistance usually fell to normal subsequent to the constriction induced by potassium. Dose response curves to potassium chloride were usually steeper than those obtained with the other drugs.

Direct injections of histamine (up to 3×10^{-9}M) and acetylcholine (up to 3×10^{-9}M) did not alter the vascular resistance of the preparation.

Serotonin injections were pressor in doses above $0{\cdot}1 \times 10^{-9}$M, producing responses similar in shape to those seen with adrenaline and noradrenaline. Equimolar quantities were about half as pressor as noradrenaline.

TABLE 2. *Tachyphylaxis to [Asn^{1}Val^5]-angiotensin II in an isolated rat tail preparation perfused at 1·0 ml./min*

Time between injections of angiotensin II (min)	Pressor response (mm Hg)
0	61
30	58
30	58
30	56
5	29
5	14
5	10
20	36
30	54
30	59
30	55
30	52

Repeated injections of 0·2 μg of angiotensin II into the perfusion circuit at the time intervals shown clearly demonstrated marked tachyphylaxis which persisted for up to 30 minutes. During the period in which 0·1 μg [Asn^{1}Val^5]-angiotensin II was injected every 30 min, injection of up to 2 μg of [Val^5]-angiotensin I or [Ileu^5]-angiotensin I immediately before the angiotensin II did not induce tachyphylaxis to angiotensin II, and the decapeptide itself was not pressor.

Tyramine was pressor in doses above 6×10^{-9}M, but the response to this agent was variable, and tended to decrease with repeated injections or after several hours' perfusion. The resultant pressor responses were slower in onset than those following direct injections of noradrenaline.

A vasoconstrictor effect of plasma injections or infusions similar to that noted by Uchida, Bohr & Hoobler (1967) was also observed.

Sympathetic nerve stimulation

Square wave D.C. pulses of up to 50 ms duration produced prompt and reproducible increases in resistance when the stimulus was above a threshold level. Voltages of 15 to 40 V were necessary to produce measurable increases in resistance in most tails, and at these voltages a current in excess of 30 μA was flowing between

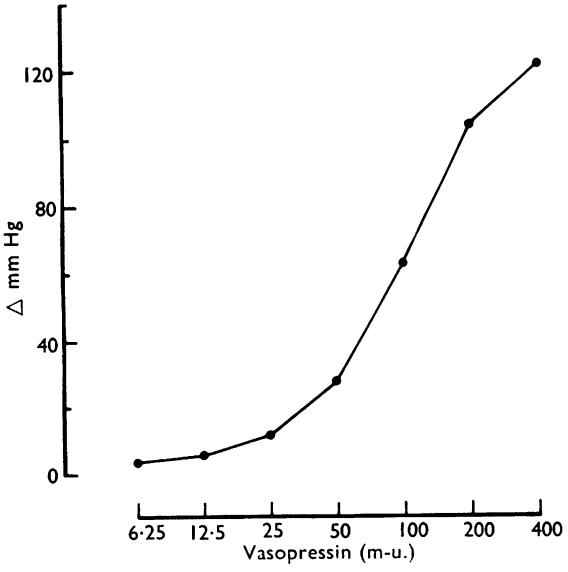

FIG. 9. Typical dose response curve to vasopressin in the isolated rat tail preparation perfused at 0·5 ml./min. Test injections were given immediately the resistance had returned to baseline after the preceding pressor response.

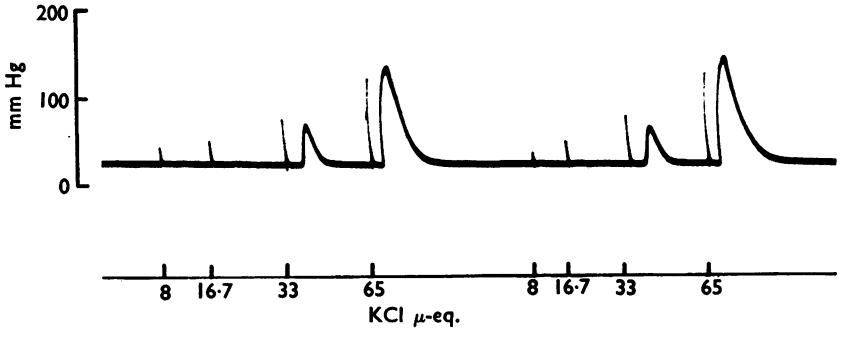

FIG. 10. Typical pressor response to potassium chloride (KCl) injections. Doses of KCl are shown on the abscissa. Pressure in mm Hg is recorded on the ordinate. The vertical lines preceding each peak are injection artefacts. Perfusion rate 0·5 ml./min.

the electrodes. There was considerable variation in the applied voltage necessary to produce any given pressure rise in different tails.

The maximal response at any given voltage was observed when the frequency of stimuli was between 6 and 14 pulses/s. Constant stimulation at voltages above threshold produced a biphasic rise in resistance, with an initial peak followed by a lower plateau which was sustained during the period of stimulation. Both components of the pressor response were blocked following direct injection of phenoxybenzamine (2 μg) into the perfusion circuit.

Discussion

The progressive increase in vascular resistance which occurs in most isolated perfused organs can be largely overcome in the rat tail by perfusing the preparation at relatively low pressures with a medium containing dialysed albumen to maintain the intravascular osmotic pressure. Reproducible dose response curves to various pressor agents were obtained under these conditions during experiments lasting several hours. Undialysed albumen or polyvinylpyrollidone in the perfusion medium was associated with a more rapid rise in baseline resistance and a decreasing pressor response to noradrenaline. These changes are presumably due to the passage of small molecules into the arteriolar walls resulting in swelling and interference with smooth muscle contraction.

Bolus injections of test substances were used in most experiments as they resulted in reproducible pressor responses which could be rapidly repeated or interspersed with injections of other test compounds. It was impractical to use sustained infusions of drugs to study pressor responses, as it was difficult to obtain a plateau response except by prolonged infusion. Consequently few dose levels of any one drug could be studied in a single experiment.

Perfusion rates of 0·5 or 1·0 ml./min resulted in baseline perfusion pressures around 20 and 36 mm Hg respectively, under which conditions pressure peaks in response to noradrenaline were easily measured. Although the response to noradrenaline increased with flow rates up to 2·0 ml./min, the increment was not large (Fig. 6). The maximal pressor response at 2·0 ml./min was associated with perfusion pressures of around 60 mm Hg and may have been due to either the increased concentration of drug reaching the smooth muscle at this flow rate or to the increased tension in the vessel wall. Uchida *et al.* (1967) also noted a maximal pressor response to noradrenaline in isolated segments of arteriole when the distending pressure was 60 mm Hg. There are several possible explanations for the decreased response to noradrenaline at flow rates above 2·0 ml./min. Rapid transit of drug through the arterioles allowing insufficient time for maximal contact with the adrenoceptors is one possibility, but most probably the major cause was an increase in the diameter of resistance vessels at the higher flow rates.

There was no evidence that changes in flow induced any active alteration of myogenic tone in the resistance vessels. Increasing the rate of the perfusion led to a progressive fall in the peripheral resistance, suggesting passive distension of the vascular bed (Fig. 2). This might lead to a decreased pressor response either because the peripheral resistance would change less (assuming a constant reduction of circumference with a given dose of pressor agent) (Peterson, 1966) or because of direct inhibition of vascular smooth musc'e contraction with high resting tensions (Uchida *et al.*, 1967 ; Speden, 1960 ; Sparks & Bohr, 1962). The absence of any

fall in resistance following the injection of bradykinin or isoprenaline further suggests that the resistance vessels are without any active myogenic tone in the resting state, and this is in keeping with the low perfusion pressures at the high flow rates achieved.

Noradrenaline, adrenaline, vasopressin, serotonin, [Asp^{1}Val^5]-angiotensin II, [Asn^{1}Val^5]-angiotensin II and potassium had significant pressor activity, whilst isoprenaline, acetylcholine, bradykinin, histamine, [Val^5]-angiotensin I, and [Ileu^5]-angiotensin I did not affect the vascular resistance.

Infusion of the β-adrenoceptor blocking agent ($\pm$)-propranolol caused only a modest increase in the pressor response to adrenaline and to a lesser extent noradrenaline, indicating that the smooth muscle adrenoceptors in these vessels are predominantly "α" in nature. This is probably related to the absence of voluntary muscle in the isolated tail preparation, which consists predominantly of bone, tendon and skin.

The direct pressor effect and reduced response to adrenaline and noradrenaline seen with higher concentrations of ($\pm$)-propranolol may be the result of weak affinity of the drug (or one of the isomers) for "α-adrenoceptors", perhaps after saturation of all the "β-adrenoceptors". Study of the individual isomers under similar conditions may answer some of these questions.

The nature of the pressor response to angiotensin II and the prolonged tachyphylaxis was similar to that seen in the isolated perfused hind limb and kidney of the rat (Tetreault, Beauhies & Pausset, 1963). However, the dose required to produce a given response was considerably greater than in the anaesthetized ganglion-blocked rat. Angiotensin I did not alter the vascular resistance of the perfused tail, suggesting that its pressor activity in the intact rat depends on conversion to angiotensin II, and that there is no converting enzyme activity in the resistance vessels themselves. It is unlikely that angiotensin I combines with the angiotensin "receptor" site, as the decapeptide did not affect the response to subsequent injections of angiotensin II.

Equimolar quantities of noradrenaline, adrenaline and angiotensin II had similar pressor activity, whereas serotonin was about half as potent and vasopressin between one-fifth and one-tenth as pressor. Angiotensin II and vasopressin produced much more sustained pressor responses than the other agents. Tachyphylaxis to vasopressin could not be demonstrated, although it has been noted in mesenteric and cerebral resistance vessels (Peterson, 1966). The pressor effects of injections of potassium chloride were presumably due to depolarization of the smooth muscle membrane by the relatively high concentrations of potassium reaching it.

Smooth muscle tone and sensitivity in several vascular beds are known to change with temperature. Smith (1952) showed that arteries from swine and dogs constricted when cooled to between 4° and 6° C, and that the arteries were most sensitive to adrenaline at 17° C. Glover, Strangeways & Wallace (1968) noted an increase in tone when femoral and ear arteries of the rabbit were cooled from 37° C to 3° C. The ear artery was most sensitive to noradrenaline at 24° C, whereas the sensitivity of the femoral artery decreased progressively below 37° C.

The vascular resistance of the isolated rat tail preparation increased at temperatures below 30° C, and sensitivity to noradrenaline was maximal between 32° C and 34° C; for these reasons 34° C was chosen as the most suitable operating temperature. This temperature is certainly closer to the true tail temperature of rats maintained at an environmental temperature of 22° C. Maximal sensitivity of the tail

vascular bed at a temperature below that of the body may be related to the possible role of the tail as a temperature regulating organ, for the tail arteries in the intact animal are constricted at normal environmental temperatures.

Keatinge (1964) described a decrease in the smooth muscle resting membrane potential in isolated aortic strips as the temperature was reduced. A similar phenomenon in the rat tail may be related to the increased sensitivity to noradrenaline at 32 to 34° C, as a moderate reduction of the resting membrane potential might be expected to reduce the depolarization necessary to reach threshold levels. The reduced sensitivity to noradrenaline, delayed relaxation and increased resting tone at still lower temperatures may be due to inhibition of energy dependent processes concerned with transmembrane fluxes of ions and smooth muscle contraction.

Sympathetic nerve stimulation resulted in sustained increases in resistance after an initial peak response. Responses were reproducible in any one tail but varied considerably from tail to tail, variation in the position of the electrodes being a major factor. The maximum response was seen when the rate of stimulation was between 6 and 14 Hz, which is similar to that found in skeletal muscle, and faster than the presumed rate of sympathetic discharge in the intact animal (Folkow, 1952). Phenoxybenzamine blocked completely the response to this type of stimulation, indicating that direct stimulation of the arterial smooth muscle was not involved.

We wish to thank Professor Sir George Pickering for his encouragement, Dr. R. Keatinge for his advice and Dr. A. J. Honour for help with the studies of sympathetic nerve stimulation. Mr. E. Bown and Mr. B. Cobern provided skilled technical assistance. This work was supported by a grant from the Wellcome Trust, and by a travel grant from the Postgraduate Committee in Medicine of the University of Sydney.

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(*Received September* 2, 1969)