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A perfused tail artery preparation from the rat

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The isolated perfused tail artery of the rat responds by constriction to 1·0 ng (−)-noradrenaline, and would be suitable for the assay of sympathomimetic amines. Electrical stimulation of this preparation is shown to activate solely postganglionic adrenergic nerve terminals. Tachyphyllaxis to angiotensin, vasopressin and bradykinin preclude the use of this preparation for their assay.

Initial studies on the hypotensive actions of diuretics (Lockett & Nicholas, 1968) were made in rats. Consequently, arteries from rats were preferred for *in vitro* studies of the direct arterial actions of these drugs. The isolated arteries most used are perfused preparations made from the central ear artery of the rabbit (de la Lande & Rand, 1965) and the mesenteric vessels of the rat (McGregor, 1965). Unfortunately the mesenteric artery of the rat is insensitive to catecholamines *in vitro*. Hence the purpose of the present work was to find a simple, rapidly prepared, reliable, rat isolated arterial preparation with a noradrenaline threshold sensitivity of about 20 ng. The tail artery has satisfied these requirements.

EXPERIMENTAL

Methods

Preparation of the artery. Male Wistar rats (230–260 g) were killed by a blow on the head. Blunt scissors were used to make a ventral incision extending for 7 cm from the base of the tail. The tail artery and vein were together separated from the fascia. Two ligatures were tied around the caudal artery and vein, 5 cm apart. The segment of vessels complete with ligatures was transferred to Krebs solution for cannulation of the proximal end of the artery with a 2 cm length of polyethylene 100 tubing (inner diameter 0·034 inch; outer diameter 0·060 inch), drawn out to a suitable diameter at the tip. The distal end of the artery was similarly cannulated with polyethylene 100 tubing premoulded into a U-tube for delivery of effluent perfusate. The final length of the artery at room temperature in Krebs solution was roughly standardized at 3·5 cm since the length perfused has considerable bearing on sensitivity (de la Lande & Rand, 1965). The artery together with the vein was then transferred to a 25 ml organ bath and was perfused at 37° with Krebs bicarbonate solution (Umbreit, Burris & Stauffer, 1964), gassed with carbon dioxide in oxygen by a Watson Marlow flow inducer. Slight irregularities in the rate of delivery from this pump were effectively damped by running the pump at high speed and limiting the delivery to the artery by means of an adjustable clamp. Perfusion pressure was continuously monitored by an E & M linear transducer coupled to a pen recorder. The fluid was not recirculated.

Electrical stimulation, when used, was periarterial. Platinum electrodes were placed closely adjacent to and on either side of the proximal 0·5 cm of the preparation.

A Grass stimulator (S4K) was used to deliver rectangular pulses of 1 ms duration at a fixed voltage of 15 V at various frequencies.

Drugs. (−)-Noradrenaline (Winthrop Laboratories), vasopressin (Parke Davis & Co. Ltd, Pitressin), phentolamine mesylate and angiotensin II (Ciba Laboratories Ltd.), histamine acid phosphate and tyramine hydrochloride (Koch-Light Laboratories Ltd.), bradykinin triacetate (Sigma Chemical Co.), cocaine hydrochloride (Macfarlan Smith Ltd.), reserpine (Ciba), guanethidine sulphate (Ciba), amphetamine sulphate (May & Baker).

RESULTS

The relation between flow and perfusion pressure

Flow rates of 2 to 3 ml/min through the tail artery initially generated high perfusion pressures which did not exceed 70 mm Hg pressure. During the first 10–20 min the initial pressures invariably decreased steadily and stabilized within the range 15 to 30 mm Hg. Once stabilized at a given flow rate the arteries responded differently to short and to long periods of change in flow. When an increase in flow was maintained for no more than 3 min, the pressure rose during the greater flow but returned to the initial value when the resting flow was restored. If, however, the raised flow was maintained the pressure gradually fell and re-stabilized at a value close to that at which it had first stabilized. Fig. 1 A shows results from an experiment in which the flow rate was increased at 2 min intervals to raise the perfusion pressure step by step from 23 to 58 mm Hg. Thereafter, flow rate was decreased step by step. The results demonstrate that this procedure has decreased the tone of the tail artery. Fig. 1 A also shows that if this procedure is carried out with return to the initial flow rate between each step, tail arterial tone is almost unaffected.

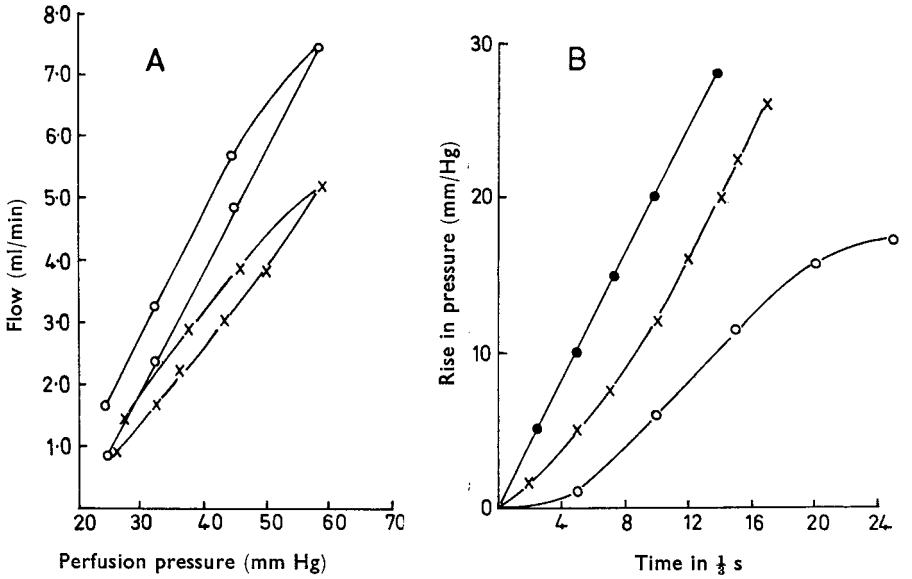

FIG. 1. A: the effects of increased perfusion pressure on flow through a perfused rat tail artery. B: comparison of the rise in pressure caused by 5 ng of noradrenaline in a low toned high flow preparation ○ – ○ and in a high toned low flow preparation × – ×, showing also the effects of distal arterial clamping ● – ●.

The relation between arterial tone and vasoconstrictor response

High initial flows produced high flow low toned preparations whereas low initial flows yielded high toned low flow preparations. All stabilized at pressure within the range of 15 to 30 mm Hg. Low toned high flow preparations usually responded to constrictor agents by reduction and not by cessation of flow (Fig. 1 B, open circles). High toned low flow preparations responded to vasoconstrictors by cessation of flow and the resulting rise in perfusion pressure was directly related to the duration of arrest. Fig. 1 B shows results from a single artery, clamped, and responding to 5 ng of noradrenaline either by occlusion during high tone low flow conditions or reduction the low tone high flow state. Sensitivity to noradrenaline was much enhanced by pre-selection of high tone low flow conditions.

Comparison of the effects of noradrenaline and electrical stimulation on the isolated tail artery of the rat

Electrical excitation caused constriction of the tail artery and hence a rise in perfusion pressure. When the voltage used produced maximal effect this rise of pressure was directly related to the log of the pulse frequency. Log frequency effect curves were found parallel to log dose-effect curves for (−)-noradrenaline. Both curves were similarly shifted to the right by phentolamine mesylate (Fig. 2A) and cocaine potentiated the effects of electrical stimulation and of (−)-noradrenaline similarly (Fig. 2 B, and Fig. 3). Phentolamine antagonism of the response to

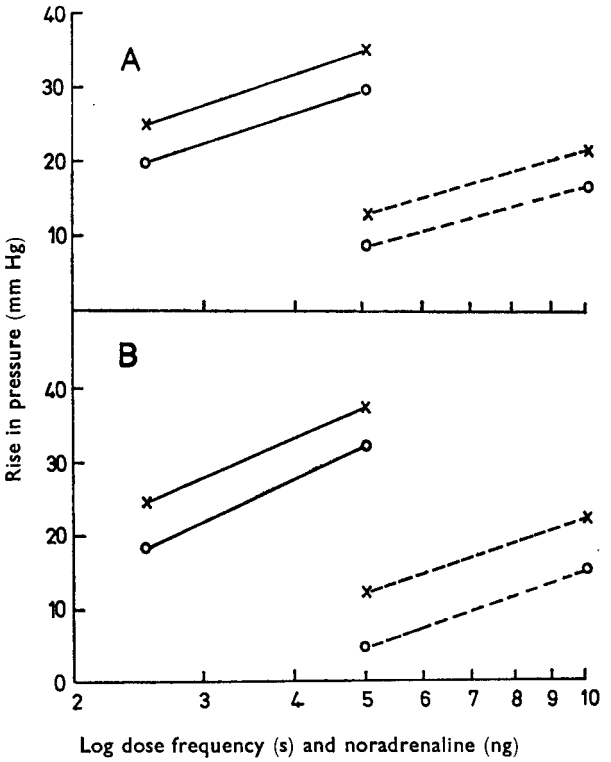

FIG. 2. Effects of the addition of 500 ng of phentolamine to the bath fluid (A) and of cocaine hydrochloride (B) perfused through the isolated rat tail artery, 3 μg/min, on the responses of the artery to noradrenaline (——) and to electrical stimulation (– – –). × Before, ○ after treatment.

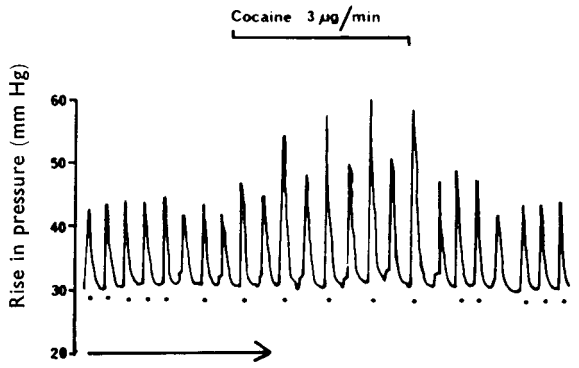

FIG. 3. Typical effects of cocaine hydrochloride, 3 μg/min, on the responses of a rat tail artery to electrical stimulation (●) and to the injection of 5 ng of noradrenaline.

periarterial stimulation was, however, more easily reversed than the responses to noradrenaline. Extraluminal concentrations of guanethidine up to 1·0 μg/ml often potentiated the effects of periarterial stimulation and produced the expected supersensitivity to noradrenaline (Fig. 4b). Concentrations of 1·5 to 2·0 μg/ml guanethidine regularly inhibited arterial responses to electrical stimulation and amphetamine sulphate 200 mg/ml reversed this inhibition (Fig. 4a). Higher concentrations of

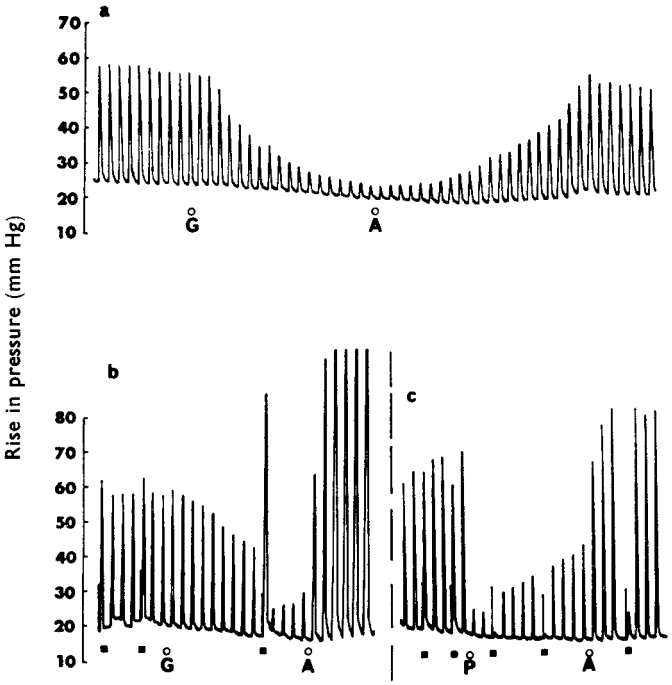

FIG. 4. (a) The effect of extraluminal doses of 1·5 μg/ml of guanethidine (G) followed by amphetamine (20 ng/ml) (A) on the responses of the perfused artery to electrical stimulation.

(b) The effect of extraluminal doses of guanethidine (1·5 μg/ml) (G) followed by 1·5 μg/ml of amphetamine (A) on the response of the perfused artery to electrical stimulation, 10/s, to 10 ng of noradrenaline (■).

(c) The response of a perfused artery to extraluminal doses of 20 ng/ml of phentolamine (P) followed by 600 ng/ml of amphetamine (A) during periarterial stimulation interrupted by injections of 10ng of noradrenaline (■).

amphetamine 400 mg/ml and upward, grossly enhanced the effects of periarterial stimuli in the absence of guanethidine and in the presence of phentolamine 20 mg/ml (Fig. 4b and c). Arteries taken from rats 24 h after intraperitoneal reserpine 5 mg/kg, showed greatly reduced responses to periarterial stimulation. Addition of pentolinium (250 μg) and atropine sulphate (0·5 μg) to the organ bath did not influence the responses of the preparation to electrical stimulation or to noradrenaline.

The threshold dose of noradrenaline, given as a single injection, was usually 0·5 ng at a flow rate of 2·5 ml/min. Responses to 5 ng noradrenaline at intervals of 3 min or to electrical stimulation delivered for 3 s at 5/s, every minute were maintained for 8 or more hours with no more than a 10% reduction in response. Over the whole of this period the base-line perfusion pressure rarely fell by more than 5 mm Hg.

The sensitivity of the isolated tail artery of the rat to various pressor agents

The isolated tail artery of the rat responded to electrical stimulation at intervals of 1 min and to single injections of noradrenaline at 3 min intervals for many hours without appreciable reduction in sensitivity. Mean log dose effect curves for the actions of noradrenaline, tyramine and electrical stimulation are shown in Fig. 5. These curves are calculated regression lines: the broken lines delineate the standard deviation of the slopes. Data for the effects of angiotensin and of vasopressin were less reliable since tachyphyllaxis was produced by both compounds. Arteries were initially very sensitive to angiotensin and usually responded to 2 ng, but tachyphyllaxis developed rapidly even when the doses used were minimally effective. The extent

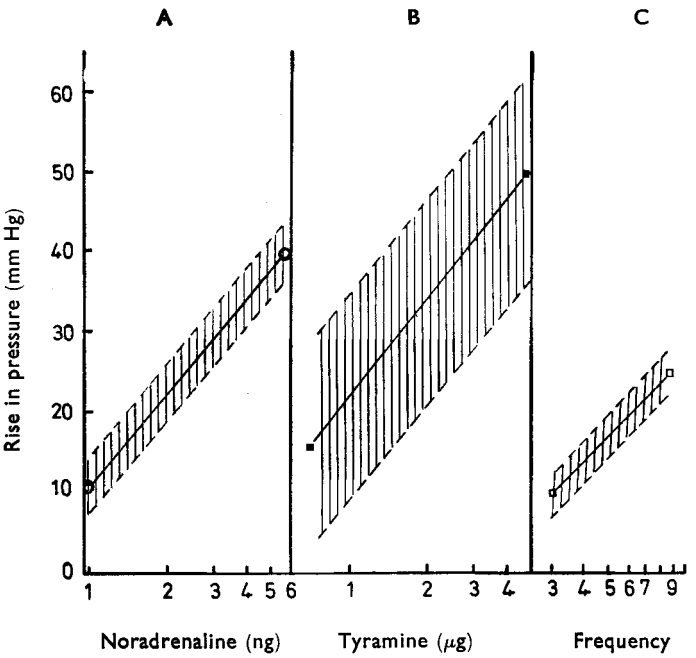

FIG. 5 Log dose effect curves for the actions of (−)-noradrenaline (A) tyramine (B) and periarterial stimulation (C) on the perfused tail artery of the rat. These curves are regression lines calculated from data supplied by 38, 9 and 23 arteries respectively. The shaded areas within broken lines depict the standard deviations of the slopes.

of the reduction in the response to a fixed dose, administered several times, was directly related to dose frequency. Tachyphyllaxis to vasopressin was less marked than to angiotensin but was extreme for bradykinin. The development of tachyphyllaxis to one pressor agent did not affect responses to any other.

The preparation was remarkably insensitive to histamine and regularly failed to respond to 40 μg.

DISCUSSION

An isolated perfused rat tail artery as described is very easy to set up and is exceptionally sensitive to catecholamines. The artery can either be used as a low-tone preparation where the response to vasoconstriction is a function of the extent to which the lumen is narrowed, or a high-tone preparation where the response is dependent on the period the lumen remains closed. Sensitivity is enhanced by preselection of high-tone low flow conditions, and a change in the slope of a log dose response relation can be artificially generated by selection of a flow rate which produces an arterial lumen obliterated by high doses and merely reduced by lower doses of a vasoconstrictor agent.

The perfused tail artery of the rat has been found to be very sensitive to periarterial stimulation. These effects of periarterial stimulation are in very large part mediated by the sympathetic nervous system since they are readily reduced to 10% of control values by phentolamine, guanethidine or prior treatment with reserpine, and are potentiated by cocaine. Postganglionic neurons are alone involved since the response to periarterial stimulation is unaffected by pentolinium and atropine. Amphetamine reversed the effects of guanethidine as would be expected from the work of Day & Rand (1963) who showed amphetamine to be a competitive antagonist of guanethidine. Observations made by these former workers do not, however, explain the gross potentiation caused in the responses of these arteries to periarterial stimulation which resulted from excessive concentrations of amphetamine. Day & Rand found that doses of dexamphetamine larger than those required to antagonize the blocking action of guanethidine abolished and did not potentiate the responses of the nictitating membrane, ileum or vas deferens to nerve stimulation.

Readily reproducible parallel effects of phentolamine on the dose-effect curves for noradrenaline and the frequency-effect curve for periarterial stimulation were found only when phentolamine mesylate was applied to the extra luminal surface of the artery. These observations contrasted with the rapid abolition of responses, even to 500 ng noradrenaline intra-arterially and more gradual reduction of the effects of periarterial stimulation by 10 ng/min phentolamine mesylate intraluminally.

Since the completion of this work, two papers by Hinke & Wilson (1962), have been encountered. These authors have examined the tail artery of the rat for its elastic properties and its responses to changes in the electrolyte composition of its environment.

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