J. Pharm. Pharmac., 1972, **24**, 318–323 *Received October* 12, 1971

Responses of blood vessels to various amines applied by microiontophoresis

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Various amines have been applied to arterioles of the rat intestine and mesentery by microiontophoresis. Noradrenaline produced a profound constriction with a long latency and time course. This effect was prevented by phentolamine but not by propranolol. Histamine caused a partial relaxation of noradrenaline-constricted vessels, but had no apparent action on "normal" vessels. Acetylcholine and methacholine had no apparent effect on the vessels. The results are discussed in relation to previous findings. It is suggested that the effects of microiontophoretically applied amines on blood vessels may be of importance in studies of the actions of these substances in the central nervous system using this technique.

The technique of microiontophoresis (Curtis, 1964) allows the controlled ejection of ionized drugs from micropipettes having tip diameters of approximately 1 μm. It is therefore possible to study the effects and interactions of drugs on a very restricted area of tissue, and the technique has been applied to the neuromuscular junction, the peripheral and central nervous systems, and some smooth muscle preparations (Phillis, 1970). It has not, however, been used to study vascular smooth muscle.

Preliminary results, using some agonist and common antagonist agents, obtained by applying the technique to intestinal arterioles of the rat are now presented.

METHODS

Arterioles, less than approximately 500 μm in diameter, of the intestine and mesentery of the rat were used.

Hooded rats, 250–300 g, were anaesthetized with allobarbitone and urethane mixture (Dial, Ciba) 1 ml/kg intraperitoneally. A small incision was made in the abdominal wall and a segment of intestine eased out and carefully placed over a block of Perspex so as to permit visualization of blood vessels with the minimum amount of traction on the tissue. The temperature of the animal was maintained at 37–38° by means of a heating pad under the body. The exposed tissue was kept moist by the frequent application of warm (38°) saline solution, and a small tray was placed beneath the Perspex block carrying the intestine, to allow removal of excess saline (Fig. 1). Incident illumination from an artificial light source was used for observation of the vessels and observation was routinely at $\times 20$ or $\times 50$ diameters magnification on a binocular light microscope. Changes in vessel diameter in response to drug application were often simply observed to determine the direction of change, if any. A quantitative approach was made in some cases, however, by the use of a micrometer eyepiece in the microscope. In these latter experiments measurements were taken of vessel size every 5–10 s, and the results plotted graphically. Estimation of diameter to 20 or 25 μm was convenient at the magnifications

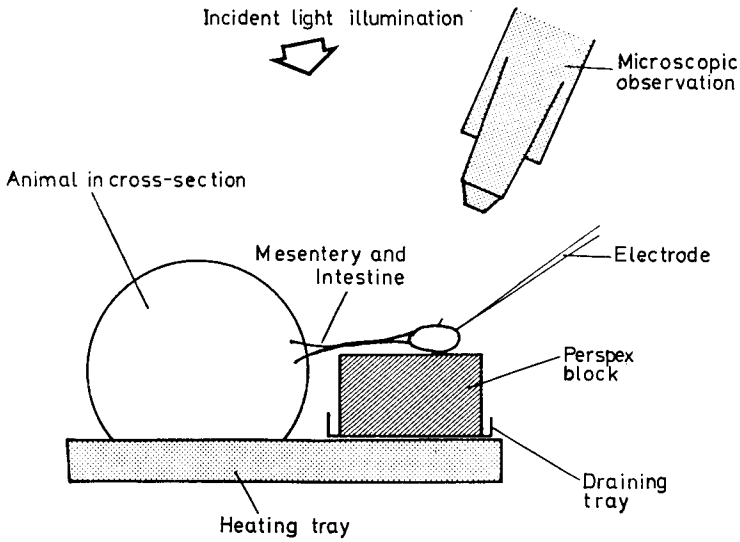

FIG. 1. A diagrammatic representation of the experimental set-up. The blood vessels of a segment of mesentery and intestine are illuminated by incident light and viewed microscopically at $\times 20 - \times 50$ diameters. The draining tray is used to remove excess saline solution which is used to keep the tissue moist.

used ($\times 20$ or $\times 50$). A change of diameter was considered meaningful only when it was reversible by stopping the drug application, and could be repeated.

Microiontophoresis has been described by Curtis (1964). The 5-barrelled micropipettes used here had overall tip diameters of approximately 5 μm and resistances of 2–12 Mohm for each barrel when filled with M potassium chloride.

The drug solutions were made in distilled water and the barrel tips were filled by allowing diffusion of the drug to take place into the tip for 24 h before an experiment. The drugs and solutions used were: Acetylcholine chloride (0·2M; Halewood Chemicals). Acetyl β-methylcholine bromide (0·2M; Koch-Light). Noradrenaline bitartrate (0·2M; Koch-Light). Histamine acid phosphate (0·2M; BDH). Phentolamine, methanesulphonate (0·2M; CIBA). Propranolol hydrochloride (0·2M; ICI).

The pH of the amine solutions was 3·5–5·0, to increase the stability of the solutions.

The currents used for drug ejection varied from 20–300 nA. Braking currents (Curtis, 1964) of 20 nA were applied to the barrels when a drug was not being ejected, so as to reduce spontaneous diffusion from the barrel. The procedure adopted with each blood vessel examined was to manoeuvre the electrode into apparent contact with the vessel, and then to wait for at least 5 min before applying a drug to ensure that the presence of the electrode was not by itself affecting the vessel diameter, for example because of excessive spontaneous drug diffusion, or compression of surrounding tissue. After this control period the applications of drugs were made and changes of vessel diameter noted as above.

RESULTS

Effects of noradrenaline

When applied to vessels over 50 μm in diameter (the smallest arterioles) a dose of noradrenaline almost invariably produced a localized constriction of the vessel. The

effect was observed on 60 of 72 vessels tested in 10 animals. Doses as small as 20 nA would often produce visible changes of diameter, and increasing the dose increased the degree of constriction. A maximal constriction could usually be obtained at doses of approximately 100 nA. The degree of constriction varied somewhat between vessels, but generally, once constriction had been initiated, the larger vessels contracted down very rapidly to approximately 10% of their original size. Indeed, the lumen of many vessels studied seemed to be completely obliterated by maximal doses of the amine. This generalization of the degree of contraction cannot, however, be extended to the few smaller vessels studied (less than 50 μm), since in these cases the end result of constriction was usually of a much smaller size than could conveniently be estimated at the magnifications used.

If the amine ejection were discontinued at the first sign of a response, then the resulting constriction was seen to be restricted to the immediate vicinity of the micropipette tip, no distortion of the vessel being apparent more than approximately 200 μm from this point. However, if the ejection was prolonged, then the constriction would tend to spread along the length of the vessel, both proximally and distally to the site of drug application, until several mm were affected. Whether this was due to diffusion of the amine along the vessel or to the altered haemodynamics within the vessel needs further investigation though the former would seem more likely in view of the similar effect on both sides of the electrode tip.

These effects could be obtained from any point along the observable length of a vessel, and could be repeated several times at any one point.

The latency from the commencement of noradrenaline application to the first sign of constriction of a vessel was quite long, varying from 20 s to 3 min in different vessels. The time interval between ceasing to apply the amine and the first sign of recovery was also of a similar order, but the actual recovery process was also quite slow, so that the vessels often did not regain their original size until some 1–5 min after stopping the drug ejection.

Fig. 2 illustrates the changes in diameter of a large arteriole in response to noradrenaline (80 nA). Several of the characteristics discussed above including the long latency and slow recovery are apparent.

Control experiments were made to ensure that the ejecting current itself, or the

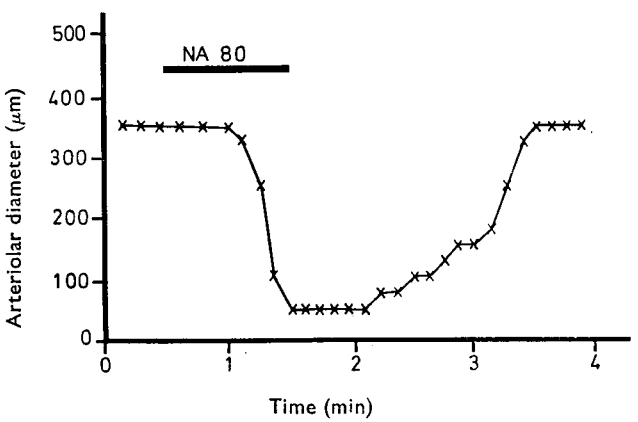

FIG. 2. A graph of the diameter of an intestinal arteriole (ordinate) against time. A microiontophoretic application of noradrenaline with a current of 80 nA (NA 80) causes a pronounced constriction of the vessel, this effect being slow in both onset and recovery.

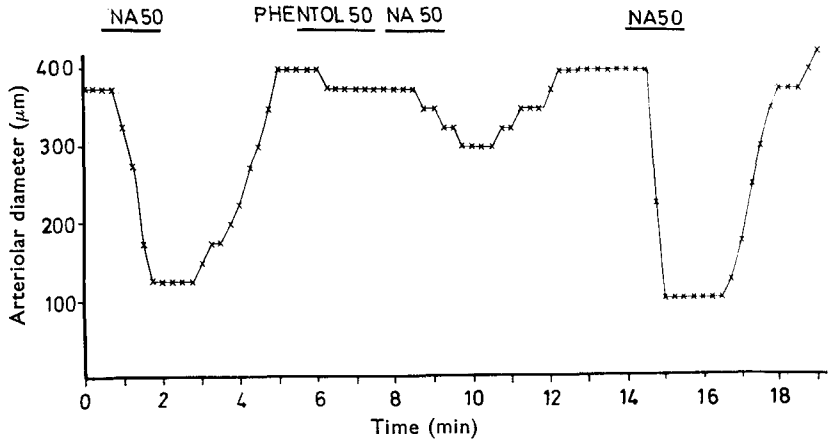

FIG. 3. As for Fig. 2. A 50 nA application of phentolamine methanesulphonate (PHENTOL 50) substantially reduces the response to noradrenaline 50 nA (NA 50).

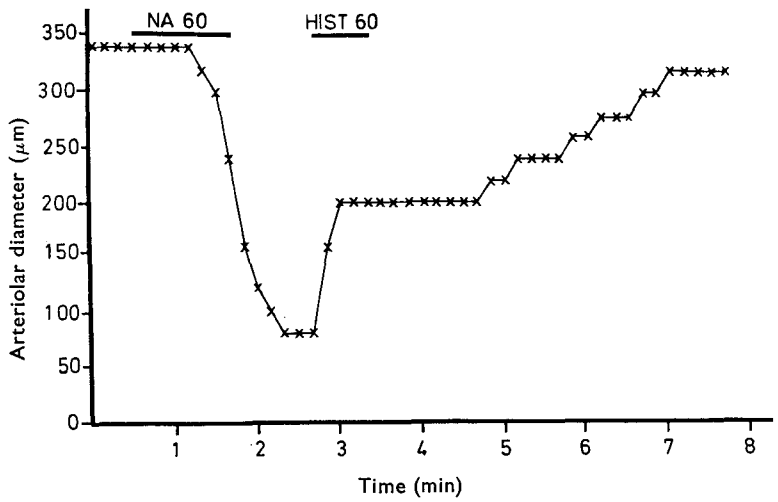

FIG. 4. As for Fig. 2. A 60 nA application of histamine (HIST 60) causes a partial relaxation of the noradrenaline-constricted vessel (NA 60). This partial antagonism was independent of the duration of histamine application.

pH of the ejected solution was not responsible for the effects described above, by passing cationic currents (up to 300 nA) through a barrel containing sodium chloride (0·2M), acidified to pH 3·5 with HCl. No changes in vessel diameter were seen.

Phentolamine

Iontophoresis of phentolamine methanesulphonate normally caused no change in vessel diameter itself, but on 15 of 19 vessels tested there was a resulting marked reduction in the vessel response to noradrenaline (Fig. 3).

Propranolol

On 12 tested vessels propranolol was incapable of affecting the constrictor response to noradrenaline. No diameter changes were produced by propranolol itself.

Histamine

Ejections of histamine acid phosphate with currents of up to 200 nA normally

produced no observable changes of vessel diameter. However, if a vessel was constricted by means of iontophoretic noradrenaline (see above), the noradrenaline ejection stopped, and then histamine application begun, then the vessel would often begin recovering from the effect of noradrenaline within 15 s (Fig. 4). This is in marked contrast to the observation above that several minutes were often needed for recovery from noradrenaline alone. This partial antagonism was clearly seen on 35 of 47 vessels tested. The vessel, once recovery had apparently been initiated by histamine, however, would usually remain partially constricted for some time before recovering further. The time to complete recovery was therefore strictly comparable to that seen with noradrenaline alone. This partial action of histamine was independent of the duration of application.

Acetylcholine and acetyl-β-methylcholine

These compounds were applied with iontophoretic currents of up to 300 nA on 28 and 16 vessels respectively with no observable changes in vessel diameter resulting. Vessels of diameters ranging from 40 to 500 μm were tested, and the drugs applied to several points along each vessel. There did not seem to be any interaction between any of these substances and noradrenaline, histamine, or with each other.

DISCUSSION

A brief report of the effects of microiontophoretic applications of noradrenaline on the mesenteric vascular bed of the frog has recently appeared (Gore & Johnson, 1970). Few practical details are given in that report, but the authors reported a maximal constriction of approximately 65% of the original vessel diameter resulting from noradrenaline applications of up to 200 nA. The results presented here, however, indicate that constriction of vessels in the rat can be provoked to a much greater extent than this, often to such a degree that the vessel disappears from view temporarily. However, the latencies of constriction and recovery noted in the frog were of a similarly long duration to those observed here. The greater potency of noradrenaline in the present experiments compared to those in frog may be related to the suggestion that the sympathetic postganglionic transmitter in the frog is almost entirely adrenaline, contrasting with primarily noradrenaline in the rat (Angelakos, Glossman & others, 1965). The clear antagonism to noradrenaline by phentolamine but not propranolol confirms the α-adrenoceptor property of noradrenaline vasoconstriction.

Microiontophoretic histamine does not, as reported above, seem to affect normal intestinal arterioles. According to Dale (1929), however, the action of histamine generally in rodents is to cause arteriolar vasoconstriction. The discrepancy may lie simply in the different techniques used. Perhaps the tissue concentration achieved by microiontophoresis is insufficient for an observable effect, or perhaps a critical length or area of vessel wall must be affected, as it would during topical drug application, for a visible change of vessel diameter to occur. The latter hypothesis is more likely to be correct since an adequate histamine concentration could be attained here to partially reverse noradrenaline constriction, as described above.

This partial antagonism itself has been encountered previously. Dale & Richards, for example (1919), noted that adrenaline had to be present in the perfusing fluid, even if this was blood, when studying intestinal vessels, in order that histamine could

manifest a vasodilator action. That is, histamine vasodilatation depended on the tone of the intestinal vessels established by perfusion with adrenaline.

In a preliminary report, Baez (1970) has more recently described effects of noradrenaline and histamine and their interaction on rat arterioles very similar to those reported here, although the somewhat less controllable method of applying solutions topically was used. The suggestion implicit in the report of Baez is that histamine may possess an anticonstrictor action quite distinct from any active vasodilatation or constriction properties. The experiments described above support this suggestion. Such an action could represent a homeostatic mechanism whereby histamine, acting during times of intense vasoconstriction due to a high sympathetic tone or circulating catecholamines, maintains a minimum blood flow through the tissues. The mechanism underlying this effect requires further study.

The lack of effect of acetylcholine and acetyl-β-methylcholine reported above could perhaps be explained by their susceptibility to cholinesterase. Experiments with anticholinesterase agents are needed to test this hypothesis. Some reports, however, have described the effects of acetylcholine on intestinal vessels as being relatively slight (Dale & Richards, 1919), and this, considered with the fact that only a very restricted area of tissue is being studied by microiontophoresis, may mean that any change of vessel diameter is so small as to be undetectable at these magnifications.

The growing volume of literature on the use of microiontophoresis in the study of single cells in the central nervous system (Krnjević, 1964; Bradley, 1968; Phillis, 1970) is based on the largely unsubstantiated assumption that the effects observed are due to the actions of drugs on neurons, and not on glia, non-neural membranes, such as synaptic barriers (Curtis & Eccles, 1958), or blood vessels (Stone, 1971). The present report may be relevant to this problem. Noradrenaline has been shown to have effects on neurons in the cns which have characteristics remarkably similar to those described here on blood vessels with respect to the dosage necessary for effect, and the latencies to onset and recovery from the effect (Boakes, Bradley & others, 1971; Johnson, Roberts & others, 1969). It is apparent, therefore, that great caution must be taken in the interpretation of results obtained in central microiontophoretic studies, and the possible effects of substances such as noradrenaline on blood vessels must be taken into account.

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