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NEUROGENIC VASODILATATION IN ISOLATED BOVINE AND CANINE PENILE ARTERIES

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SUMMARY

1. Field stimulation of isolated, perfused bovine or canine penile arteries produced dilatation, after the adrenergic motor component of the response had been blocked with guanethidine and the vessels had developed a background tone. The vasodilatation was blocked by tetrodotoxin but not by atropine.

2. The vasodilator responses to field stimulation were compared with those produced by ATP, by vasoactive intestinal peptide (VIP), and by the inhibitory factor extracted from the bovine retractor penis muscle. Of the three putative transmitters, the inhibitory factor produced responses that most closely resembled those to field stimulation.

3. Haemoglobin, which blocks non-adrenergic, non-cholinergic inhibitory transmission in the bovine and canine retractor penis muscles, did not impair the vasodilatations produced by ATP or VIP, but slowly reduced or abolished those produced by field stimulation or by the inhibitory factor. Haemoglobin itself produced a powerful constriction of the isolated penile arteries.

4. The results are compatable with the possibility that the inhibitory factor from the bovine retractor penis muscle (which may be the inhibitory transmitter in that muscle) is, or closely resembles, the transmitter of non-adrenergic, non-cholinergic vasodilator fibres in the penile arteries of dog and ox.

INTRODUCTION

There is growing evidence in the literature indicating that some blood vessels receive a non-adrenergic, non-cholinergic vasodilator innervation (see, for example, Lee, Hume, Su & Bevan, 1978; Bevan, Bevan, Buga, Florence, Jope, Jope & Moritoki, 1981; Toda, 1981; and reviews by Burnstock, 1979, 1980). One example of such blood vessels is the penile artery of the ox (Klinge & Sjöstrand, 1974), which is of particular interest to us because we have recently been investigating the nature of the non-adrenergic, non-cholinergic transmitter to an adjacent tissue, the smooth muscle of the bovine retractor penis. We have, therefore, attempted to determine the extent of any similarities that might exist between the two neuroeffector mechanisms. The penile artery and retractor penis muscle of the dog also receive an inhibitory innervation, considered to be non-cholinergic because of atropine resistance (Langley & Anderson, 1895; Luduena & Grigas, 1966; Dorr & Brody, 1967) and because

acetylcholine fails to mimic the inhibitory response to nerve stimulation (Luduena & Grigas, 1966; Dorr & Brody, 1967). The scope of this study was extended to include these canine tissues.

One putative transmitter of non-adrenergic inhibition of the bovine retractor penis muscle is the as yet unidentified inhibitory factor originally extracted from that muscle by Ambache, Killick & Zar (1975), and further purified in this laboratory (Gillespie & Martin, 1978, 1980; Bowman, Gillespie & Martin, 1979; Gillespie, Hunter & Martin, 1981). This factor, as well as causing relaxation of the retractor penis muscle, is a powerful vasodilator (Bowman, Gillespie & Martin, 1981), and its properties are, therefore, consistent with those of a substance mediating the co-ordinated penile functions of protrusion and erection. Such a substance may of course play a wider role in vasodilator mechanisms for which the penile artery might provide a model.

Of the many other possible mediators of non-adrenergic, non-cholinergic neurogenic vasodilatation, we have selected two, vasoactive intestinal polypeptide (VIP) and ATP, for comparison with the inhibitory factor from the bovine retractor penis. Two antagonists of non-adrenergic, non-cholinergic transmission have been used as tools in the investigation: these are apamin and haemoglobin. Apamin is a polypeptide from bee venom, which has been shown to prevent some responses to ATP and VIP (Banks, Brown, Burgess, Burnstock, Claret, Cocks & Jenkinson, 1979; Sjöqvist, Delbro, Jodal & Lundgren, 1980). Haemoglobin has been shown to antagonize both inhibitory nerve stimulation and the response to the inhibitory factor in the bovine retractor penis muscle (Bowman & Gillespie, 1981, 1982*a*; Bowman, Gillespie & Pollock, 1982).

Preliminary accounts of some of these results were presented to the British Pharmacological Society (Bowman & Gillespie, 1982*b*), and to the Physiological Society (Bowman, Gillespie & Hunter, 1982).

METHODS

Bovine retractor penis muscle and penile artery

Bovine penises with associated tissue were collected from the abattoir. Strips of retractor penis muscles were dissected off and set up in the isolated organ bath as described in a previous paper (Gillespie & Martin, 1980). The dorsal penile artery runs superficially for most of the length of the penis. Pieces up to 50 cm in length could be dissected out, but not all of it was suitable for isolated preparations. The proximal end is surrounded by thick layers of connective tissue wrapped spirally around the artery. The distal end did not respond to field stimulation. Segments about 3 cm long were, therefore, taken from the middle region, near the points of attachment of the retractor penis muscles. These segments were cannulated, immersed in an organ bath containing Krebs solution, and perfused at a constant flow (3 ml./min) with warm (36–37 °C) Krebs solution by means of a Watson Marlow flow inducer. The Krebs solution in the organ bath and in the perfusion reservoir was bubbled with 5% CO_2 in O_2. Perfusion pressure was recorded with a Statham transducer (P23 ID) attached to a Grass recorder. Agonist drugs were injected into the perfusion fluid through thick-walled rubber tubing between the heating coil and the arterial cannula, and antagonist drugs were added to the reservoir of perfusion fluid. Field stimulation was applied through ring electrodes from a Tektronix (series 160) pulse generator. This method was first described for the rabbit isolated ear artery by de la Lande & Rand (1965). In most experiments the smooth muscles were collected and dissected during the early afternoon (the timing was determined by the availability of the tissue), stored overnight at 4 °C in oxygenated Krebs solution, and used the next day. Observations were made on forty-seven segments of arteries isolated from thirty oxen.

Canine rectractor penis muscle and penile artery

Penises were taken from greyhounds that had been used for other experiments. They had been anaesthetized with chloralose, but had received no other long acting drugs. Only the distal portion of the paired retractor penis muscle was used, so as to avoid the striated muscle fibres that are present in the proximal end (Bell & McLean, 1970). There are two penile arteries in the dog, from which four isolated preparations could be made. Isolated canine retractor penis muscles and penile arteries were set up in the same way as the bovine tissues described above, usually after overnight storage in the refrigerator. Observations were made on twenty-eight segments of artery and seven retractor penis muscles taken from sixteen greyhounds.

Preparation of inhibitory factor from the bovine retractor penis muscle

The method has been described before (Bowman & Gillespie, 1982*a*; Gillespie *et al.* 1981) so only a brief resumé is included here. A methanol extract of the minced muscle was applied to an anion-exchange resin (Bio-Rad AG1-X8); the column was washed with distilled water and the inhibitory factor was eluted with 0·5 M-NaCl. The eluate was freeze-dried and stored at −20 °C. Before use, the lyophylized powder was reconstituted with distilled water (1 ml. water per g of original muscle), and the major remaining contaminant, ATP, was removed by adsorption onto alkaline alumina (Bowman *et al.* 1979). The inhibitory factor was then activated by exposure to acid (pH 2) for 10 min, followed by neutralization. It was kept on ice. One or both of two control procedures were routinely used to check that the inhibitory activity of the muscle extract was due to the inhibitory factor: first, that the inhibitory effect was absent in extracts not activated by acid, and secondly, that it was abolished after placing the extract in a sealed tube in a boiling water bath for 2 min.

Preparation of haemolysate and haemoglobin

This method has also been described before (Bowman & Gillespie, 1982*a*). A washed erythrocyte suspension from rat, guinea-pig or human blood was haemolysed by addition of 19 volumes of hypotonic phosphate buffer (20 m-osmole/l., pH 7·4). The supernatant, after centrifugation of the mixture at 20,000 *g* for 30–40 min at 4 °C, constituted the haemolysate. Its haemoglobin concentration was approximately 10^{-4} M. Purified human haemoglobin was prepared from commercially available 'haemoglobin' (which is mostly in the form of methaemoglobin) by reduction with sodium dithionite (Bowman, Gillespie & Pollock, 1982).

Drugs

Drugs used were: adenosine triphosphate disodium salt (Sigma), angiotensin amide (Hypertensin, Ciba), apamin (Serva), atropine sulphate (British Drug Houses), ergotamine tartrate (Femergin, Sandoz), guanethidine sulphate (Ciba), haemoglobin (Sigma, human type IV), histamine acid phosphate (British Drug Houses), 3-isobutyl-l-methylxanthine (Sigma), (−)-noradrenaline bitartrate (Koch-Light), phentolamine mesylate (Regitine, Ciba), phenylephrine hydrochloride (Bayer), propranolol hydrochloride (I.C.I.), tetrodotoxin (Boehringer Mannheim), vasoactive intestinal peptide (VIP, Sigma).

RESULTS

Field stimulation of penile arteries

When first set up, penile arteries from both species responded to field stimulation (10 Hz for 10 sec, pulse width 0·3–0·5 msec) with constriction, causing a rise in perfusion pressure. The vasoconstriction was usually abolished by the addition of guanethidine (10 μM) to the perfusion fluid and this concentration of guanethidine was added routinely at the beginning of all experiments.

In order to demonstrate dilatation in response to field stimulation, it was necessary that some degree of tone in the vessels pre-existed. In about two-thirds of the preparations, tone developed with no further drug treatment, other than the guanethidine, although in some instances a period as long as 2–4 hr was required

before the degree of tone was sufficient. As tone developed, inhibitory responses to nerve stimulation became apparent. In some preparations the rate of rise of perfusion pressure slowed down after 1–2 hr, and pressure then remained constant; reproducible dilator responses to field stimulation could subsequently be obtained for many hours. In other preparations, perfusion pressure continued to rise for the entire duration of the experiment; in such arteries, the dilator response to field stimulation became gradually larger throughout the experiment. The evoked fall in perfusion pressure was apparently enhanced by the rise in basal tone of the artery. The rise in tone by itself never acted to reduce the inhibitor response. In no instance did the dilator response to field stimulation wane (in the absence of drugs that blocked it) even in experiments that lasted for 7–10 hr.

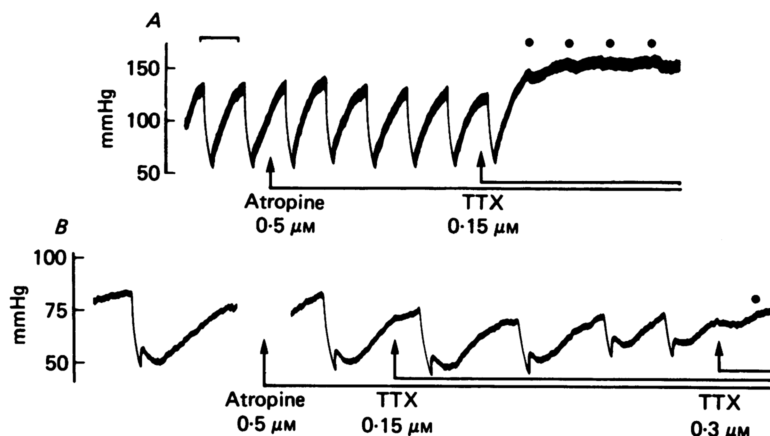

Fig. 1. Dilator responses to periarterial stimulation (10 Hz for 10 sec, pulse width 0·5 msec) in isolated segments of canine penile arteries. Guanethidine (10 μM) was present throughout. *A*, dilator responses were not affected by atropine (0·5 μM) but were abolished by tetrodotoxin (TTX; 0·15 μM). *B*, in some canine arteries, the dilator response to field stimulation consisted of two components, a rapid initial fall in perfusion pressure, whose recovery was interrupted by a slowly developing and longer lasting fall. Neither component of this type of response was blocked by atropine (0·5 μM), but both were reduced by 0·15 μM tetrodotoxin, and abolished by 0·3 μM. Time bar, 5 min.

In a few experiments, a guanethidine-resistant motor response to field stimulation was evident. It occurred more often when pulse widths in the range of 0·7–1 msec were used; it was unaffected by phentolamine (5 μM) and by tetrodotoxin (0·7 μM). This type of response was often seen in preparations that did not develop a steady tone, but which displayed rhythmic oscillations in tone or occasional spontaneous constrictions. It is possible that these constrictions (both spontaneous and stimulation-induced) arose from excitation of a pace-maker in the smooth muscle. In order to avoid this kind of response, pulse widths of 0·5 msec or below were used in the majority of experiments.

Dilatation of the penile arteries of both species in response to field stimulation was abolished by tetrodotoxin (0·1–0·3 μM) and was, therefore, assumed to be neurogenic. Fig. 1 shows abolition by tetrodotoxin of dilatation in the canine isolated penile artery. In both the canine and the bovine penile arteries, this vasodilator response

to field stimulation was not reduced by atropine (0·5–1 μM, Fig. 1), nor by propranolol (5 μM), nor by increasing the concentration of guanethidine to 100 μM. The optimal frequency of stimulation for producing the dilator response was usually between 2 and 5 Hz; the threshold was about 0·1 Hz. In some canine arteries (eighteen out of twenty-eight), the vasodilator response to field stimulation consisted of two components: an initial rapid fall in pressure, whose recovery was interrupted by a second, more slowly developing fall, which recovered only slowly (Fig. 1 *B*). In arteries with this pattern of response, both components were evident at all frequencies of nerve stimulation tested (0·2–10 Hz), and both, though unaffected by atropine (0·5 μM), were equally susceptible to block by tetrodotoxin (Fig. 1 *B*). Responses of this type were not seen in the bovine arteries.

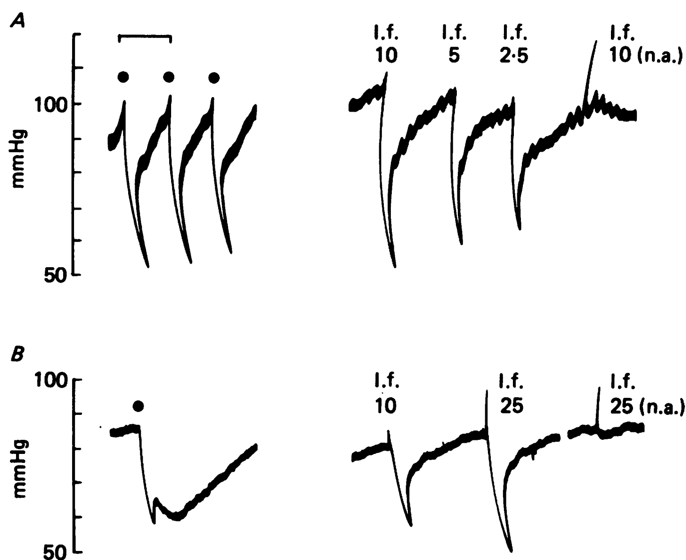

Fig. 2. Changes in perfusion pressure in isolated segments of canine penile arteries elicited by field stimulation or by the inhibitory factor extracted from the bovine retractor penis muscle (guanethidine, 10 μM, was present throughout). *A*, responses to field stimulation (10 Hz for 10 sec at ●) are mimicked by the injection of the inhibitory factor (i.f., 10, 5 and 2·5 μl., the amounts extracted from 10, 5 and 2·5 mg of the muscle). The last injection was 10 μl. inhibitory factor before acid activation, and served as a control, as it contained all the material present in the tissue extract except the inhibitory factor in its active form (n.a., not activated). *B*, in arteries that showed both a rapid and a slow component of the dilator response to field stimulation (10 Hz for 10 sec, at ●), the dilatation produced by the inhibitory factor (i.f., 10 and 25 μl.) mimicked only the rapid component of the stimulation-induced response. Time bar, 5 min.

Inhibitory factor, VIP and ATP

Inhibitory factor. Before acid activation (see Methods), the inhibitory factor from the bovine retractor penis was without effect on the penile arteries of ox or dog. After acid activation, it produced an abrupt and transient vasodilatation in volumes ranging from 2·5–50 μl. injected into the perfusion fluid (these volumes contained the material extracted from 2·5–50 mg of retractor penis muscle). This dilatation was not

blocked by atropine (0·5 μM), nor by tetrodotoxin (0·6 μM). The shape of the response to the inhibitory factor was the same (i.e. spiky and transient) whether the artery exhibited the biphasic type of dilator response to field stimulation or the single component dilator response (Fig. 2).

VIP. VIP was neither as potent nor as consistently effective a vasodilator in penile arteries as it has been reported to be in other vessels. In amounts ranging from 0·3–1 m-mole, VIP was injected into ten bovine arteries, but produced dilatation in only three of them. In five out of seven canine arteries VIP (0·1–0·6 n-mole) produced

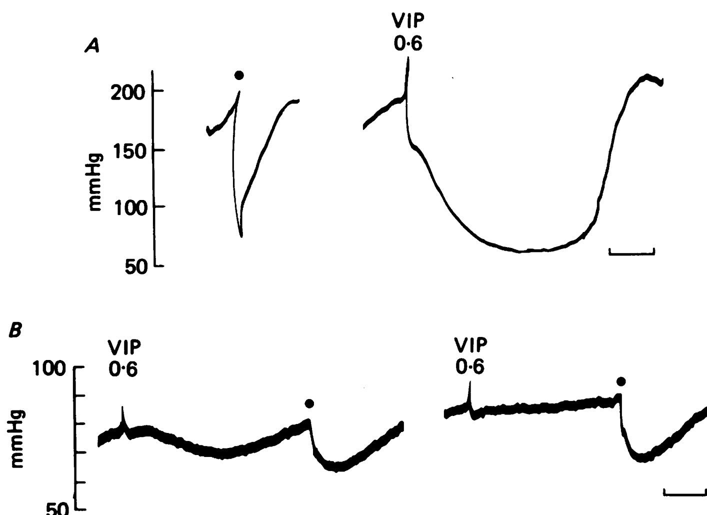

Fig. 3. Changes in perfusion pressure in isolated segments of penile arteries elicited by field stimulation or by VIP (guanethidine, 10 μM, present throughout). *A*, bovine penile artery. Dilatation produced by VIP (0·6 n-mole) was slower to develop and longer in duration than that produced by field stimulation (10 Hz for 10 sec at ●). *B*, canine penile artery. Rapidly developing tachyphylaxis to VIP (first and third injections of 0·6 n-mole are shown) was not accompanied by a diminished response to nerve stimulation (10 Hz for 10 sec at ●). Time bars, 5 min.

dilatations. The fall in pressure produced by VIP was always slow in onset, compared to the response to nerve stimulation, and was long lasting (Fig. 3*A*). In some arteries, responses of one of which are illustrated in Fig. 3*B*, a rapidly developing tachyphylaxis to VIP was apparent. The preparation illustrated in Fig. 3*B* was one in which nerve stimulation evoked a two-component response, with merging rapid and slow phases. However, even when complete tachyphylaxis to VIP had developed, the responses to nerve stimulation remained unchanged, suggesting that neither the fast nor the slow component of the neurally evoked response involved VIP.

ATP. The effect of ATP was tested in twelve bovine arteries and six canine arteries that had developed tone; all of them responded to ATP. In four out of the twelve bovine arteries and five out of the six canine arteries, the response to ATP consisted simply of dose-related dilatations, resembling the responses to nerve stimulation (Fig. 4*A*); the threshold dose of ATP for this effect was 1·5 n-mole. In two of the bovine arteries, responses of one of which are illustrated in Fig. 4*B*,

ATP (2·5–25 n-mole) produced only vasoconstriction, although field stimulation produced the usual dilator response. In six bovine and one canine artery, low doses of ATP produced dilatation, but higher doses (25–50 n-mole) produced either constriction or biphasic responses consisting of constriction followed by dilatation. However, again nerve stimulation at all frequencies produced only dilatation in these

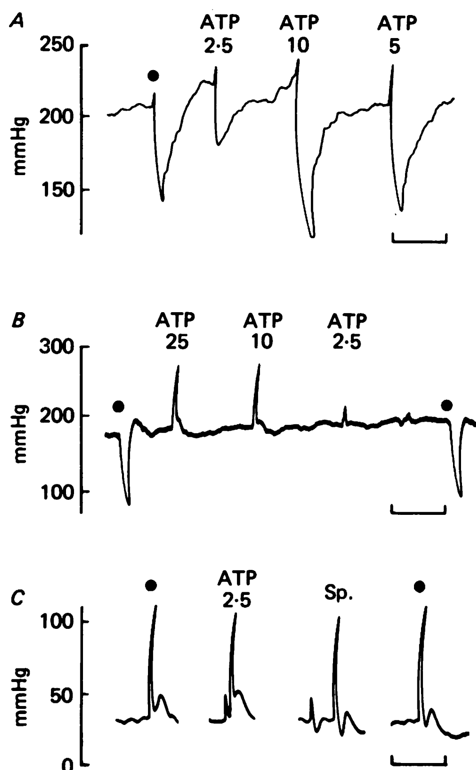

Fig. 4. Changes in perfusion pressure in isolated segments of penile arteries elicited by field stimulation (10 Hz for 10 sec) or by ATP (guanethidine, 10 μM, present throughout). *A*, canine penile artery. In this preparation, ATP (2·5–10 n-mole) produced a dilator response that closely mimicked the response to field stimulation (●). *B*, bovine penile artery. ATP (2·5–25 n-mole) produced constriction, whereas field stimulation (●) produced dilatation. *C*, bovine penile artery, before development of tone. (This was one of the preparations that displayed guanethidine-resistant motor responses to field stimulation.) Note the similarity between the shapes of the constrictor responses to ATP (2·5 n-mole) and field stimulation (●) and those occurring spontaneously (Sp.). Time bars, 5 min.

same artery preparations. In a few bovine preparations that did not develop tone, and that responded to field stimulation by contractions that were apparently not neurogenic, ATP produced contractions in all effective doses (2·5–25 n-mole). Such an experiment is illustrated in Fig. 4*C*, which shows the similarity in the shape of the contractions elicited by ATP and by field stimulation, and those that occasionally occurred spontaneously.

Apamin

Apamin was tested in four bovine and five canine arteries, in concentrations ranging from 0·05 to 0·5 μM, perfused for 20 min to 1 hr. It was without effect on the vasodilator responses elicited by field stimulation, by inhibitory factor, or by ATP. VIP was also tested in four of these experiments. In three of them in the presence of apamin, there was a diminution in response that was not greater than that attributable to tachyphylaxis as judged from other experiments. In one experiment, there was a clear augmentation of the response to VIP in the presence of apamin, associated with, and possibly a consequence of, a rise in background tone. Over-all, there was no evidence that apamin reduced responses to VIP.

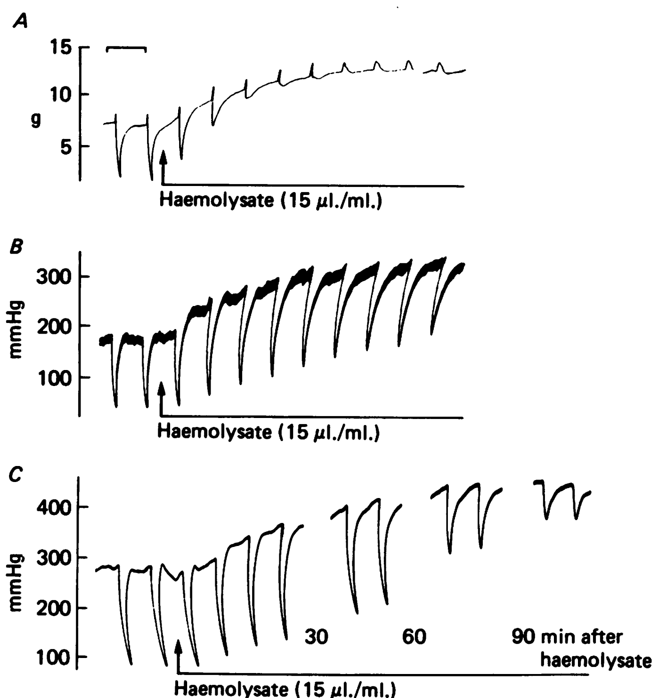

Fig. 5. *A* and *B*, a comparison of the effect of haemoglobin (in the form of haemolysate of guinea-pig erythrocytes, 15 μl./ml. ≡ $1·5 \times 10^{-6}$ M-haemoglobin) on inhibitory responses to field stimulation of bovine isolated retractor penis and penile artery. Records made simultaneously. Block of inhibition in the retractor penis is rapid in onset and complete within 15 min. However, the predominant effect in the penile artery, at the commencement of perfusion, is an augmentation of the dilator response. In both preparations, haemoglobin causes a rise in tone. *C*, after more prolonged perfusion with haemolysate (15 μl./ml.), dilator responses to nerve stimulation are considerably reduced. Time bar, 5 min.

Haemolysate and haemoglobin

When haemolysate (10–30 μl./ml.) was added to the fluid perfusing the penile arteries, it produced an immediate vasoconstriction, as shown by the prompt and progressive rise in perfusion pressure. At the same time, the dilator response to nerve stimulation appeared to be enhanced (Fig. 5*B*). Augmentation of dilator responses

by an increase in background tone in the vessel is referred to above. Therefore, it is probable that the initial increase in size of the dilator responses that was produced by the haemolysate was a consequence of the vasoconstriction that it produced. Fig. 5*A* and *B* illustrates a comparison of the effects of haemolysate (10 µl./ml. in each case) made simultaneously on the retractor penis muscle and penile artery from the same animal, and shows that while haemolysate also causes a rise in tone in the

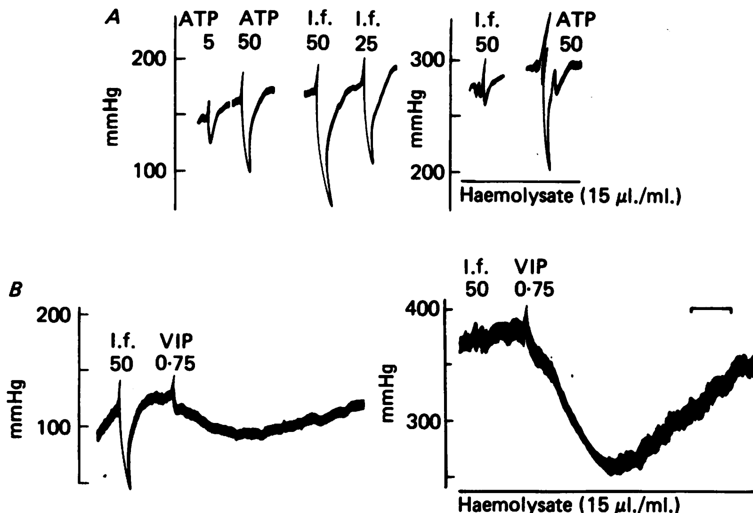

Fig. 6. *A*, bovine isolated penile artery. Left-hand side, control dilator responses to ATP (5 and 50 n-mole) and inhibitory factor (i.f., 50 and 25 µl.); right-hand side, in the presence of haemolysate of guinea-pig erythrocytes (15 µl./ml. ≡ $1{\cdot}5 \times 10^{-6}$ M-haemoglobin), the dilator response to inhibitory factor is practically abolished, while that to ATP is not. Note the increase in perfusion pressure, caused by haemoglobin-induced vasoconstriction. *B*, canine isolated penile artery. Control dilator responses to inhibitory factor (i.f., 25 µl.) and VIP (0·75 n-mole). In the presence of haemolysate of guinea-pig erythrocytes (15 µl./ml. ≡ $1{\cdot}5 \times 10^{-6}$ M-haemoglobin), dilator response to inhibitory factor is abolished, while that to VIP is considerably augmented (probably as a consequence of the haemoglobin-induced vasoconstriction). Time bar, 5 min.

retractor penis muscle, this does not obscure its blocking action on the nerve-evoked relaxations. However, while the responses to stimulation in the retractor penis muscle are diminishing, those in the penile artery are increasing in size. If the experiment were allowed to continue, as for example, in Fig. 5*C*, it became apparent that, despite the continual rise in perfusion pressure, the nerve-evoked dilatations begin to grow smaller and are finally blocked. The lowest effective concentrations of haemolysate (2–5 µl./ml.) produced only vasoconstriction with no reduction in the absolute size of the dilator response, so it was not possible to achieve a selective blocking action of haemolysate by manipulation of the dose. After changing back to haemoglobin-free Krebs solution, the tone of the preparation returned towards the control level. More prolonged washing with haemoglobin-free Krebs was required before the dilator responses began to recover.

No difference was found between the sensitivities of the bovine and canine arteries to haemolysate. In those canine arteries that exhibited a biphasic dilator response

to nerve stimulation, haemolysate blocked both phases equally. No differences were seen between the effects of purified human haemoglobin or haemolysate made from rat, guinea-pig or human erythrocytes, on either bovine or canine arteries.

Haemolysate consistently blocked the dilator responses to the inhibitory factor in the bovine and canine arteries. In contrast, dilator responses to ATP or VIP were not reduced by haemolysate, but in fact were potentiated, probably as a consequence of the increase in background tone. Fig. 6 illustrates the effect of haemolysate on responses to inhibitory factor, ATP and VIP.

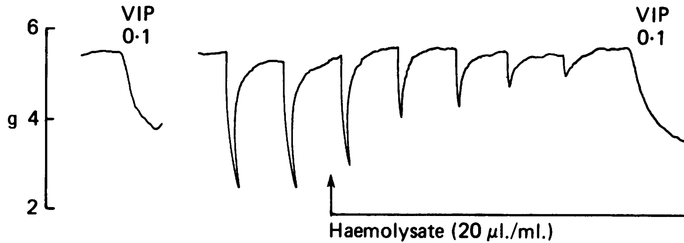

Fig. 7. Relaxations of canine isolated retractor penis muscle in response to VIP (0·1 μM) and field stimulation (5 Hz for 10 sec, 1 msec pulses, at 4·5 min intervals). Haemolysate of guinea-pig erythrocytes (20 μl./ml. ≡ 2×10^{-6} M-haemoglobin) greatly reduces the response to field stimulation, but not that to VIP.

Retractor penis muscles

The effects of haemolysate and apamin on the responses of the bovine retractor penis to inhibitory factor, to VIP and to field stimulation have been described previously (Bowman & Gillespie, 1982*a*; Bowman, Gillespie & Hunter, 1982). The opportunity was taken here to study the responses of the canine retractor penis, in order to complete the comparison with the arterial preparations from the same species. Like the bovine muscle, the canine retractor penis responded to the inhibitory factor with relaxation. The only difference between the canine and the bovine retractor penis muscles was that spontaneous tone never developed in the canine muscle, although it usually did in the bovine muscle. Guanethidine was, therefore, necessarily always used for the dual purpose of blocking adrenergic motor responses and of inducing background tone in the canine muscle.

Briefly, in the canine retractor penis the following results were obtained. Haemolysate blocked the relaxations produced by field stimulation and by inhibitory factor, but not those produced by VIP (Fig. 7). Apamin was without effect on all inhibitory responses. The canine retractor penis was insensitive to ATP, which, in a high dose (10^{-4} M), produced at most a small contraction in the untreated preparation, but had no effect after tone was raised with guanethidine (this confirms earlier reports by Luduena & Grigas, 1972, and Klinge & Sjöstrand, 1977).

DISCUSSION

In species, such as the dog, that possess a large amount of penile erectile tissue, dilatation of the penile artery is the main mechanism causing erection. In contrast,

in species, such as the ox, with fibro-elastic penises that contain little erectile tissue, other factors (mainly contraction of adjacent striated muscles) are of greater importance (for review see Bell, 1972). Despite this difference in the importance of dilatation in the ox and in the dog, the isolated penile artery segments from both species were found to possess powerful vasodilator innervations with similar characteristics. Klinge & Sjöstrand (1974), who studied isolated, spirally cut strips of the bovine penile artery, concluded that non-adrenergic, non-cholinergic vasodilator fibres were present. The results described in this paper confirm their conclusion and demonstrate the presence of similar fibres in the penile artery of the dog.

Three putative transmitters were chosen for study, viz, VIP, ATP and the inhibitory factor from the bovine retractor penis. All three are vasodilators and have been suggested to be involved in neurogenic vasodilatation; all are known to be present in penile blood vessels or related structures, at least of some species. VIPergic nerves are densely concentrated around the pudendal arteries and in the erectile tissue of the corpus cavernosum of the human penis (Polak, Gu, Mina & Bloom, 1981). ATP has frequently featured as a putative transmitter of non-adrenergic non-cholinergic transmission, particularly in the arguments put forward by Burnstock (see, for example, Burnstock, 1972, 1980). Finally, what little evidence is so far available, is at least consistent with the possibility that the inhibitory factor from the bovine retractor penis muscle may be the transmitter of the non-adrenergic non-cholinergic relaxations in that tissue, although as yet it is far from proven that this material does fulfil a transmitter role.

All three putative transmitters were capable of producing vasodilatation in the penile artery, although not consistently in the cases of ATP and VIP. None of the three exactly mimicked the shapes of the responses to transmural stimulation in all preparations. Discrepancies of this type, depending on degree, should not be taken as conclusive negative evidence, since exogenous application of a transmitter cannot be expected to mimic exactly the more rapid and precise application that occurs from nerve endings. However, the rate of development of and recovery from the vasodilator response to VIP was so slow that it is inconceivable that it could be the main transmitter. Moreover, in many bovine preparations, dilator responses to VIP were not demonstrable at all, even though transmural stimulation was normally effective. Sjöstrand, Klinge & Himberg (1981) found VIP to be completely ineffective in isolated spiral strips of bovine penile artery. Finally, tachyphylaxis was a prominent feature of responses to VIP in penile arteries of both species, but again responses to nerve stimulation continued unabated after responses to VIP had disappeared completely. In some canine arteries, the dilator response to nerve stimulation consisted of a fast and a slow component, and in these instances the possibility was considered that VIP, released as a co-transmitter, might be responsible for the secondary slow component. However, even here evidence from the use of haemolysate (referred to again below) was against the possibility of a role for VIP.

ATP sometimes closely mimicked the dilator response to nerve stimulation, but it behaved inconsistently, sometimes producing constriction, and its effect frequently failed to match the response to nerve stimulation in the same preparation. Dilatation was the only response produced by the inhibitory factor from the bovine retractor penis muscle, and when nerve stimulation also produced pure dilatation, the two

responses were not dissimilar. At its most potent, the amount of inhibitory factor extracted from 2·5 mg (wet weight) original bovine retractor penis muscle was sufficient to produce almost maximal vasodilatation.

Apamin blocks the inhibitory effect of ATP, of inhibitory nerve stimulation, and of noradrenaline in the taenia caecum of the guinea-pig (Banks *et al.* 1979 and see review by Jenkinson, 1981). It also blocks VIP-induced and neurogenic vasodilatation in the cat small intestine (Sjöqvist *et al.* 1980). However, the failure of apamin to block the vasodilator responses to nerve stimulation, ATP, VIP or the inhibitory factor in the penile arteries suggests that the mechanism underlying relaxation of the smooth muscle in these vessels differs from that of the guinea-pig taenia caecum and cat intestinal blood vessels. Likewise, apamin failed to block the inhibitory responses of the retractor penis muscles to nerve stimulation or to inhibitory factor (Bowman & Gillespie, 1982*a*).

Neither ATP nor VIP were blocked by haemolysate or haemoglobin, whereas the dilator responses to inhibitory factor and to nerve stimulation were reduced or abolished. This observation provides further evidence against a transmitter role for ATP or VIP. The blocking action of haemoglobin was not as clear in the penile artery as it was in the bovine retractor penis muscle (Bowman & Gillespie, 1982*a*). In the penile artery, it was necessary to perfuse the haemoglobin for many minutes before the blocking action became evident. Two factors that might contribute to the slow onset of the block are as follows. The very pronounced vasoconstrictor action of haemoglobin tends to enhance dilator responses and, therefore, to mask any simultaneously developing block of the responses occurring through other mechanisms. When the results were calculated as percentage decrease in perfusion pressure, rather than as absolute decreases, the reduction in the vasodilatation was seen to develop more rapidly. A second factor could be the large size of the haemoglobin molecule which might retard access to the sites at which it acts to impair the dilator responses to nerve stimulation and to inhibitory factor. If this is so, it implies that the sites at which it causes vasoconstriction are more accessible to the haemoglobin molecule, for constriction was evident within a few seconds of the haemoglobin reaching the artery.

The intensity of the constrictor response of the penile arteries to haemoglobin is worthy of note. Rises in perfusion pressure of 100–200 mmHg were usual. Some, but not all, isolated blood vessels constrict in response to haemoglobin: the coronary and cerebral vessels have been reported to be the most sensitive and responsive, whereas others are less so (Tanishima, 1980). It would seem that the penile arteries resemble the coronary and cerebral vessels in this respect. Because of its instability and its constrictor action, haemoglobin is far from the ideal blocking drug for non-cholinergic, non-adrenergic nerves in the retractor penis muscle and penile artery. However, once its mechanisms of action are determined, it may be possible to prepare more selective derivatives.

In summary, we have considered the possibility that the same transmitter mediates neurogenic vasodilatation in isolated bovine and canine penile arteries and relaxation of the bovine and canine retractor penis muscles. VIP relaxes both the bovine (Sjöstrand *et al.* 1981) and the canine retractor penis muscles, but is less effective in dilating penile arteries; in none of these preparations is its action blocked by

haemoglobin (Bowman, Gillespie & Hunter, 1982, and this paper). ATP produces either contraction or relaxation of the smooth muscle of the retractor penis or arteries; it does not closely mimic the response to nerve stimulation, and it is not blocked by haemoglobin. The inhibitory factor from the bovine retractor penis causes relaxation of the retractor penis muscle and of the penile arteries from both species, and all these effects are blocked by haemoglobin, as is neurogenic inhibition in these tissues. These results are thus consistent with the hypothesis that the inhibitory factor from the bovine retractor penis could be the transmitter of all these inhibitory nerves.

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