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Cyclic GMP mediates neurogenic relaxation in the bovine retractor penis muscle

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1 Field stimulation of the non-adrenergic, non-cholinergic inhibitory nerves to the bovine isolated retractor penis muscle evoked a relaxation that was preceded by a rise in the tissue content of cyclic GMP. There was no change in the content of cyclic AMP.

2 The selective cyclic GMP phosphodiesterase inhibitor, 2-*o*-propoxyphenyl-8-azapurin-6-one (M&B 22948), elevated the tissue's cyclic GMP content, and potentiated both the relaxation and the rise in cyclic GMP produced by inhibitory nerve stimulation.

3 Sodium nitroprusside and an inhibitory factor extracted from the bovine retractor penis muscle mimicked the effects of inhibitory nerve stimulation in that they each produced relaxation associated with a selective rise in cyclic GMP concentration.

4 Haemoglobin (in the form of erythrocyte haemolysate) and N-methylhydroxylamine, which are known to block guanylate cyclase, blocked the relaxation and the rise in cyclic GMP content produced by inhibitory nerve stimulation, inhibitory factor and sodium nitroprusside. Haemoglobin itself caused a rise in muscle tone and at the same time reduced the cyclic GMP content of the tissue.

5 8-Bromocyclic GMP, a permeant derivative of cyclic GMP, produced a relaxation of the muscle that, as expected, was not blocked by haemoglobin.

6 Vasoactive intestinal polypeptide, prostaglandin E_1 and forskolin each produced relaxation associated with a selective rise in cyclic AMP content. Their effects were not blocked by haemoglobin or N-methylhydroxylamine.

7 It is concluded that inhibitory nerve stimulation in the bovine retractor penis muscle produces a relaxation that is mediated by cyclic GMP, although some substances relax the muscle without affecting cyclic GMP levels. The results are also compatible with the view that the extracts of muscle contain the inhibitory neurotransmitter.

Introduction

Certain smooth muscles possess an efferent autonomic innervation that cannot be classified as cholinergic or adrenergic—the nerves are often termed non-adrenergic non-cholinergic (NANC). One of the first clues to their existence came from the work of Langley & Anderson (1895) who described atropine-resistant responses to stimulation of the sacral parasympathetic outflow. The bovine retractor penis muscle is an example of a tissue receiving such an innervation; this tissue receives a motor adrenergic and an inhibitory NANC innervation (Klinge & Sjöstrand, 1974). The nature of the neurotransmitter that mediates the inhibitory response remains unknown. Attempts to identify it have recently centred on the isolation, from tissue extracts, of a factor which mimics the relaxant response (Ambache *et al.*, 1975; Gillespie & Martin, 1978; 1980; Bowman *et al.*, 1979).

Although it appears established that cyclic AMP can mediate the smooth muscle relaxant effects of certain neurotransmitters, e.g. noradrenaline (Kukovetz *et al.*, 1981), the physiological role of cyclic GMP in tissues remains uncertain. Early work indicated that contractions elicited by acetylcholine and other neurotransmitters were associated with a rise in tissue cyclic GMP levels (Lee *et al.*, 1972; Murad & Kimura, 1974) leading to the proposal that the nucleotide might mediate the contraction. Subsequent studies revealed, however, that neither cyclic GMP nor a more permeant analogue, 8-bromocyclic GMP, could mimic this contraction (Katsuki & Murad, 1977; Schultz *et al.*, 1977); moreover, the rise in cyclic GMP was produced only after the contraction had developed (Katsuki & Murad, 1977). Alternatively Kukovetz *et al.*, (1982) have suggested that the acetylcholine-induced rise in smooth muscle

cyclic GMP content that is associated with contraction subserves a compensatory negative feedback function to limit the extent of the contraction. Compounds that activate guanylate cyclase, for example, compounds containing a nitro group, such as sodium nitroprusside, are not spasmogens but are powerful relaxants of smooth muscle (Katsuki *et al.*, 1977a, b; Schultz *et al.*, 1977), including the bovine retractor penis muscle (Bowman & Gillespie, 1982). More recently, evidence has been amassed to implicate the elevation of tissue cyclic GMP content in relaxation of vascular smooth muscle (for a review, see Kukovetz *et al.*, 1981), although elevation of the nucleotide level and relaxation do not appear to be causally related in all types of smooth muscle (Diamond, 1983).

In the experiments described in the present paper we have measured changes in cyclic nucleotide content associated with non-adrenergic, non-cholinergic nerve stimulation of the bovine retractor penis muscle. We suspected that neurogenic relaxation might involve cyclic GMP, because recent work has shown that oxyhaemoglobin or carboxyhaemoglobin inhibits the neurogenic or nitroprusside-elicited relaxation, while methaemoglobin is inactive (Bowman & Gillespie, 1982; Bowman *et al.*, 1982b). This pharmacological specificity is identical to that reported by Mittal *et al.*, (1978) for the inhibition of soluble guanylate cyclase. The results indicate that cyclic GMP mediates the neurogenic relaxation of the bovine retractor penis, and we believe this to be the first demonstration of a nerve-elicited response that is mediated by this nucleotide.

Methods

Bovine retractor penis muscle

Bovine penises, with attached retractor penis muscles, were collected from the abattoir in the early afternoon and stored overnight in the refrigerator. Thin strips of bovine retractor penis muscle (30–50 mg) were mounted on silver-silver chloride stimulating electrodes and suspended in an organ bath of Krebs solution, gassed with 5% CO_2 plus 95% O_2 at 36 ± 0.5 °C. The Krebs solution had the following composition (mM): Na^+ 145; K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 127, HCO_3^- 25, $H_2PO_4^-$ 1.2, dextrose 11, it contained guanethidine (10 μM), phentolamine (5 μM) and atropine (1 μM) to block any contribution of noradrenergic or cholinergic nerves to the responses observed. Each experiment involved 30–42 strips taken from the same retractor penis muscle. Some of the strips were attached to Grass FT03 force transducers and tension was recorded on a Grass (model 7) polygraph. Others, used for cyclic nucleotide assays, were set up on a specially designed electrode assembly that included a spring-loaded resistance to contraction (Grass copper springs: 3 g cm^{-1}). Contraction or relaxation could be observed visually making it possible to check that the strips attached to springs behaved in the same way as companion strips connected to the polygraph. At appropriate times the whole assembly was plunged into liquid nitrogen; the transfer took less than 1 s.

The experiments were started 3–4 h after setting up the muscle strips in the organ bath by which time resting tone was high and stable. Field stimulation at 1 Hz with 0.5 ms pulses and supramaximal voltage was applied from a Grass S88 stimulator.

Cyclic nucleotide assays

The frozen tissues were thawed in 2 ml of 6% (w/v) trichloroacetic acid and, in the initial experiments, homogenized using a ground glass homogenizer. Precipitated proteins were then removed by centrifugation (500 *g*; 10 min; 4 °C). Subsequent experiments indicated that homogenization did not increase the yield of cyclic nucleotides in the acid-soluble fraction and this step was consequently omitted. Portions of the acid-soluble fraction were then extracted 3 times with 4 volumes of water-saturated diethylether, the last traces of which were driven off by placing sample tubes in a boiling water bath for 2 min. The samples were then made 50 mM with respect to sodium acetate buffer (pH 6.2), and cyclic nucleotides were assayed by radioimmunoassay using the acetylation method of Harper & Brooker (1975). The cyclic GMP antiserum used was from Amersham International and the cyclic AMP antiserum was raised in rabbits using the method of Steiner *et al.*, (1972). Both antisera exhibited at least 10,000 fold specificity for the appropriate cyclic nucleotide. Mean values (± s.e.mean) for the cyclic GMP and cyclic AMP contents of the retractor penis muscle from all of our studies were 37.0 ± 4.3 ($n = 17$ separate experiments) and 252 ± 23 ($n = 6$) pmol g^{-1} wet weight respectively.

Inhibitory factor from the bovine retractor penis

The method of extraction was as previously described (Gillespie *et al.*, 1981; Bowman & Gillespie, 1982). The inhibitory factor was freeze-dried and stored in sealed ampoules at −20 °C. Immediately before use, the lyophilized powder was reconstituted with a volume of distilled water such that the material extracted from 2 g of original muscle was present in 1 ml of solution. Acid activation was carried out as previously described and the active extract was kept on ice.

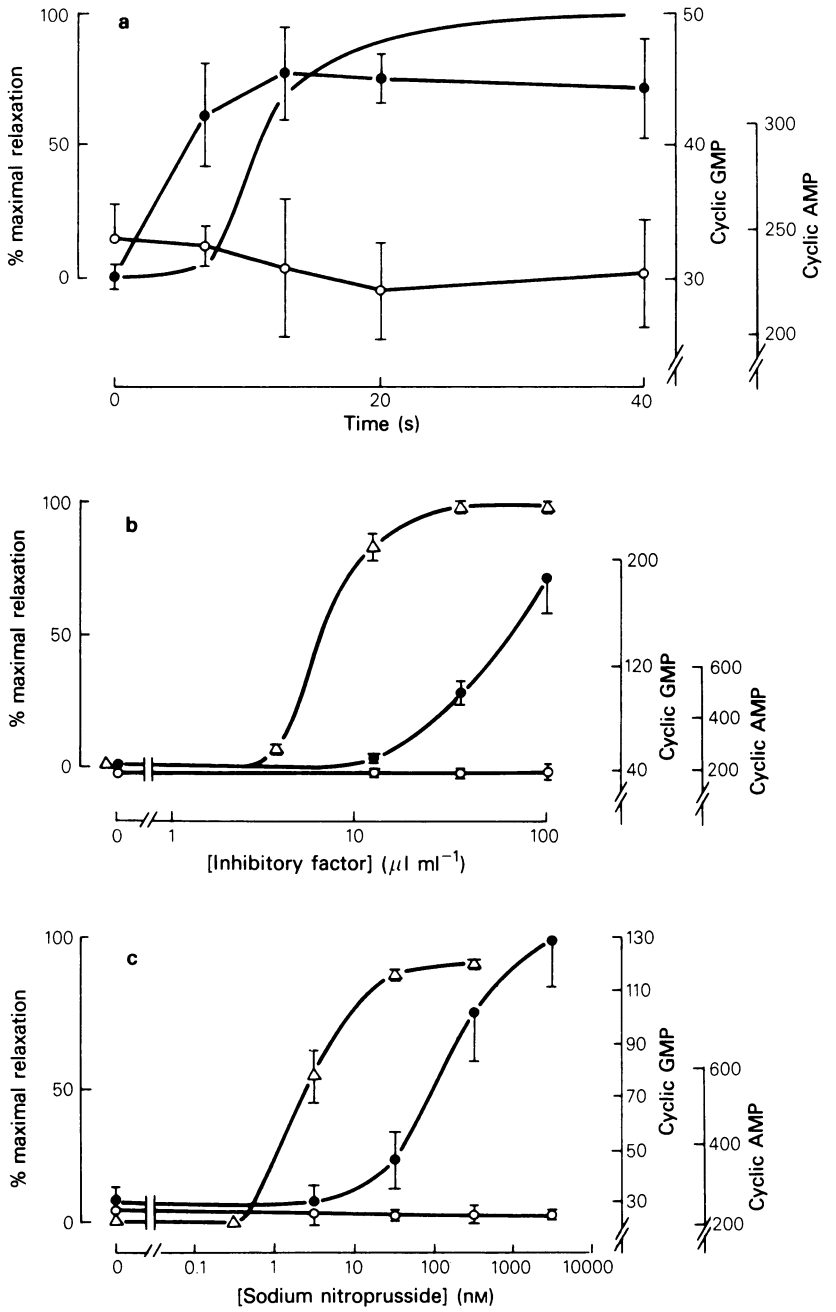

Figure 1 Effects of (a) field stimulation (1 Hz) of inhibitory nerves; (b) inhibitory factor; and (c) sodium nitroprusside on tension and cyclic nucleotide content of bovine retractor penis muscle. All three produced relaxation and increased cyclic GMP content; none affected cyclic AMP content. The rise in cyclic GMP content produced by inhibitory factor or sodium nitroprusside was detectable only with concentrations that produced substantial relaxations. Tissues were exposed to inhibitory factor or sodium nitroprusside for 2 min before freezing. (a) Solid line, relaxation (% maximum); (●——●) cyclic GMP (pmol g^{-1} wet weight), (○——○) cyclic AMP (pmol g^{-1} wet wt.). Values shown are means ± s.e.mean ($n = 6$). (b and c) (△——△) Relaxation (% maximum), otherwise as for (a).

Haemolysate

A washed erythrocyte suspension from guinea-pig blood was haemolysed by addition of 19 volumes of hypotonic phosphate buffer (20 mosmol l^{-1}, 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 100 μM. The method of preparation has been described before (Bowman & Gillespie, 1982).

Drugs and solutions

Drugs used were sodium nitroprusside (B.D.H.), 8-bromoguanosine 3′5′-cyclic monophosphate (Sigma), forskolin (Calbiochem), 2-*o*-propoxyphenyl-8-azapurin-6-one (M & B 22948, May & Baker), N-methylhydroxylamine (Aldrich), vasoactive intestinal polypeptide (VIP, Sigma), prostaglandin E_1 (PGE_1, Upjohn). M & B 22948 was dissolved (4 mM) in dilute sodium hydroxide (pH adjusted to 9.5). The small volume of this vehicle added to the organ bath did not affect the pH. Forskolin was dissolved (2.5 mM) in absolute ethanol.

Results

Nerve stimulation, inhibitory factor and nitroprusside

When strips of bovine retractor penis muscle were suspended, under tension, in Krebs solution, they gradually shortened, or developed tone, during the first 1–2 h. The spontaneous development of tone was not associated with a change in the cyclic GMP or cyclic AMP contents of the muscle. After tone had developed, field stimulation (1 Hz, 0.5 ms pulse width, supramaximal voltage) produced relaxation, which was associated with a rapid rise in tissue content of cyclic GMP (Figure 1a). The relaxation began 6–8 s after the onset of stimulation, by which time the cylic GMP content was elevated significantly. Cyclic AMP levels were unchanged by nerve stimulation (Figure 1a). Stimulation at 1 Hz caused only a 50% increase in the cyclic GMP content; stimulation at 5 Hz produced about 100% increase (see, for instance, Table 1) and higher frequencies caused a still greater elevation (data not shown).

Relaxation elicited by the inhibitory factor was likewise associated with a rise in the tissue content of cyclic GMP (Figure 1b). Again, cyclic AMP levels were unaffected. The dose-response curve for the inhibitory factor-induced cyclic GMP changes was to the right of that for relaxation (Figure 1b). Inhibitory factor has previously been shown to exist in an inactive form which requires acid activation before manifesting its relaxant effect (Gillespie & Martin, 1980). Only the acid-activated form elicited an elevation of cyclic GMP levels.

Sodium nitroprusside, which has already been shown to relax the bovine retractor penis muscle (Bowman & Gillespie, 1982), caused a 4–5 fold rise in the cyclic GMP content of the tissue, without changing the cyclic AMP content (Figure 1c). As with the relaxation induced by inhibitory factor, the cyclic GMP curve was considerably to the right of that for relaxation; approximately 10–30 fold higher concentrations of sodium nitroprusside were necessary to elicit maximal changes in cyclic GMP levels than to elicit maximal relaxation.

Blockade by haemolysate (haemoglobin)

In the presence of haemolysate (haemoglobin, 10 μM), the relaxations in response to field stimulation were abolished and there was no significant change in the cyclic GMP content of the tissue during the period of stimulation (Table 1). Similarly, in the presence of haemolysate, inhibitory factor failed to cause relaxation or to elevate cyclic GMP (Table 1). Haemolysate itself caused a reproducible and statistically significant fall in the resting cyclic GMP content of the bovine retractor penis muscle (Table 1). Interestingly, the initial response of the bovine retractor penis muscle to the addition of haemoglobin or haemolysate is a slow rise in tone (Bowman & Gillespie, 1982; Bowman *et al.*, 1982b).

8-Bromocyclic GMP

8-Bromocyclic GMP is 2–5 times more active than cyclic GMP itself as an activator of cyclic GMP-dependent protein kinase (Kuo *et al.*, 1974; 1976),

Table 1 The effect of haemoglobin on the cyclic nucleotide content of the bovine retractor penis muscle and its response to nerve stimulation and inhibitory factor

	Cyclic GMP (pmol g^{-1}) *untreated*	*Cyclic AMP* (pmol g^{-1}) *untreated*	*Cyclic GMP* (pmol g^{-1}) *+ haemoglobin* (10 μM)	*Cyclic AMP* (pmol g^{-1}) *+ haemoglobin* (10 μM)
Control	41 ± 2	167 ± 6	30 ± 1†	198 ± 40
Nerve stimulation (5 Hz for 20 s)	82 ± 12*	167 ± 9	28 ± 2†‡	156 ± 31
Inhibitory factor (50 μl ml^{-1})	63 ± 5*	141 ± 40	28 ± 3†‡	205 ± 12

*Significantly higher than control values ($P < 0.05$)
†Significantly lower than control values ($P < 0.01$)
‡Not significantly different from haemoglobin-treated control values ($P < 0.05$).
Values shown are means ± s.e.mean ($n = 5$).

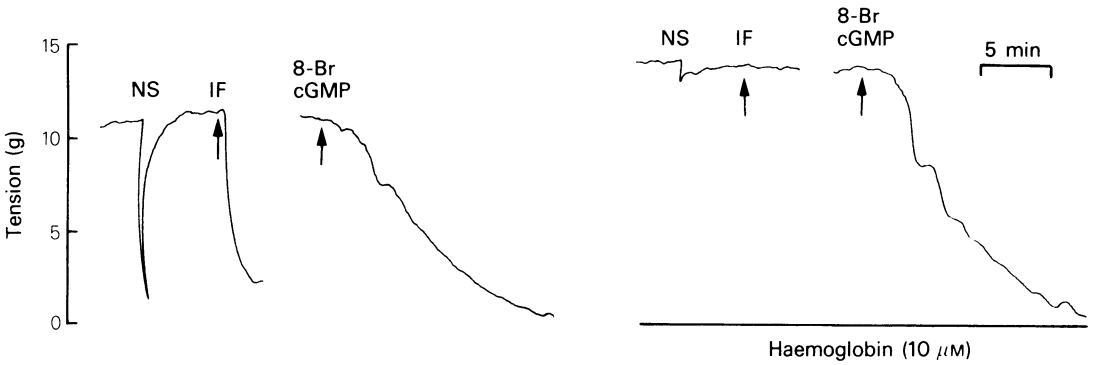

Figure 2 Relaxations of bovine isolated retractor penis muscle elicited by field stimulation (NS, 2 Hz, 0.25 ms pulse width, for 10 s), inhibitory factor (IF, 20 μl ml^{-1}) and by 8-bromocyclic GMP (8-Br cGMP) (0.3 mM). In the presence of haemolysate (10 μM haemoglobin), there is an increase in tone and blockade of the responses to nerve stimulation and inhibitory factor, but 8-bromocyclic GMP still elicits relaxation.

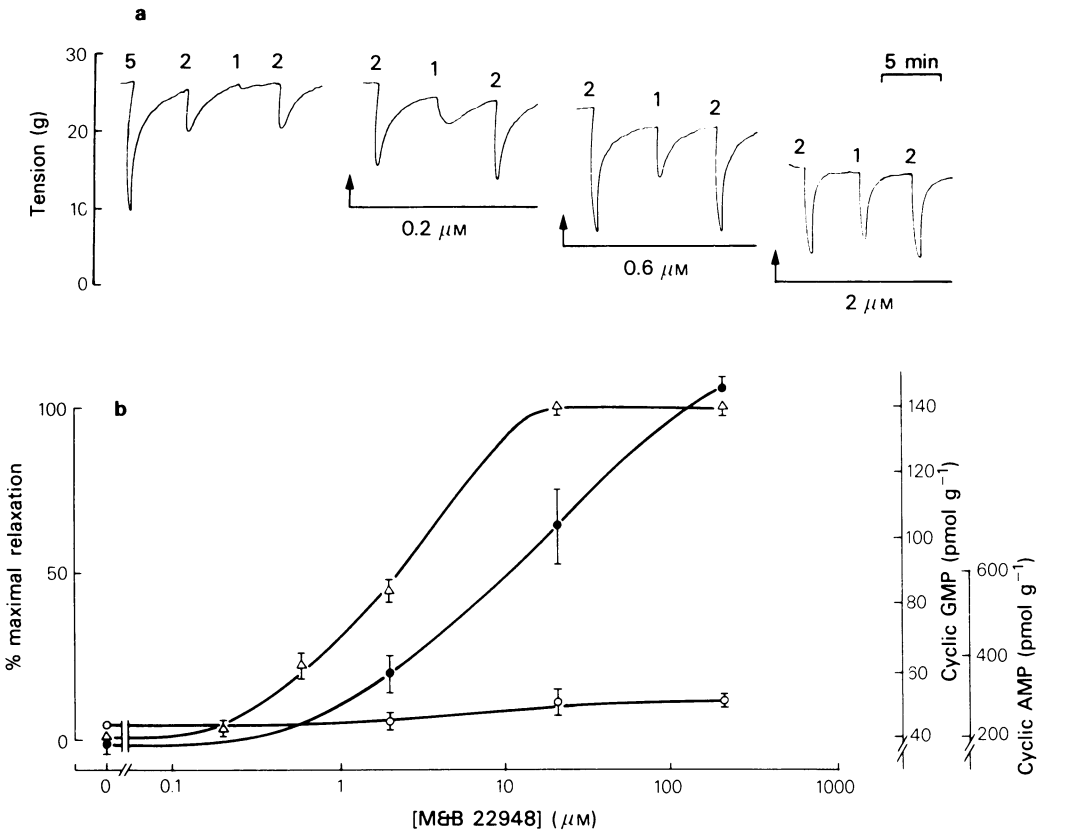

Figure 3 Effects of the cyclic GMP-phosphodiesterase inhibitor M & B 22948 on resting tone, nerve-evoked relaxations and cyclic nucleotide content of bovine isolated retractor penis muscle. (a) Relaxations evoked by field stimulation with single pulses or with 2 or 5 pulses at 1 Hz. In the presence of M & B 22948 0.2 or 0.6 μM the responses to single pulses and 2 Hz were increased and resting tone began to fall. At 2 μM M & B 22948, resting tone had fallen too far to allow further augmentation of the response to 2 Hz to be seen but the response to a single pulse was further increased. (b) M & B 22948 induced relaxation (△——△) and increased the content of cyclic GMP (●——●) but not cyclic AMP (○——○) in the bovine retractor penis muscle. Tissues were exposed to the drug for 5 min before they were plunged into liquid nitrogen. The values shown are means ± s.e.mean of five experiments.

and it is resistant to degradation by phosphodiesterase (Revanker & Robins, 1982). This makes the compound a useful probe for investigating the cellular effects of cyclic GMP. 8-Bromocyclic GMP (0.1–0.5 mM) caused a slow relaxation of the bovine retractor penis muscle (Figure 2). This response was not affected by haemolysate under conditions in which the responses to nerve stimulation and to inhibitory factor were abolished (Figure 2).

M & B 22948, a selective phosphodiesterase inhibitor

Most drugs that inhibit cyclic nucleotide phosphodiesterase, inhibit the breakdown of both cyclic AMP and cyclic GMP. However the realization that multiple forms of the enzyme exist (Thompson & Appleman, 1971) has led to the development of selective phosphodiesterase inhibitors. The anti-allergic drug, M & B 22948 (2-*o*-propoxyphenyl-8-azapurin-6-one, Broughton *et al.*, 1974), has previously been shown to cause a selective inhibition of cyclic GMP-phosphodiesterase in human lung (Bergstrand *et al.*, 1977), rat mast cells (Bergstrand *et al.*, 1978) and bovine coronary smooth muscle (Kukovetz *et al.*, 1979). Although we did not directly measure the ability of M &B 22948 to inhibit cyclic GMP-phosphodiesterase in the bovine retractor penis muscle, we did find that it caused a selective 3–4 fold elevation of the tissue's cyclic GMP content which was associated with relaxation of the muscle (Figure 3b). Cyclic AMP levels, although showing a trend towards an increase, were not significantly affected by M & B 22948 at concentrations below 200 μM (Figure 3b). Low concentrations of M & B 22948 (0.2–2 μM),that did not cause complete loss of muscle tone, potentiated the relaxation response of the muscle to submaximal nerve stimulation (Figure 3a) and also augmented the rise in cyclic GMP in the response to field stimulation (data not shown).

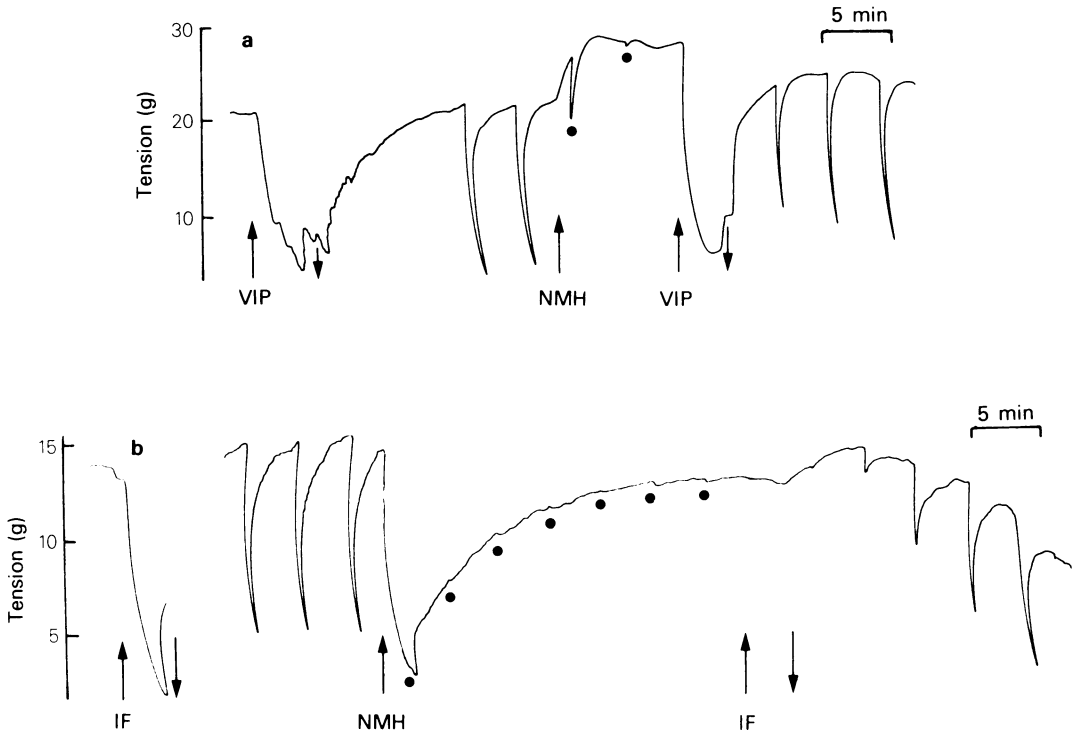

Figure 4 Relaxations of bovine isolated retractor penis muscle elicited by field stimulation (1 Hz for 10 s), vasoactive intestinal peptide (VIP) (30 nM) or inhibitory factor (IF, 10 μl ml^{-1}). N-methylhydroxylamine (NMH, 2 mM) blocked the nerve-evoked relaxations (a and b), and the response to inhibitory factor (20 μl ml^{-1}) (b), but not the response to VIP (a). Attenuated responses to nerve stimulation in the presence of N-methylhydroxylamine are indicated by (●). N-methylhydroxylamine itself sometimes caused a rise (a) and sometimes a fall (b) in tone. The fall was usually transient, muscle tone returning towards the control level while the N-methylhydroxylamine was still present in the bath. At ↓, bath fluid replaced.

N-methylhydroxylamine

N-methylhydroxylamine has been shown to block guanylate cyclase and to decrease hormone-induced elevation of cyclic GMP levels in a number of tissues (Deguchi *et al.*, 1978). Inhibitory responses of the bovine retractor penis to nerve stimulation and to inhibitory factor were reversibly blocked by N-methylhydroxylamine (2 mM) (Figure 4).

A rise in cyclic GMP is not obligatory for relaxation

The bovine retractor penis relaxes in response to other agents, including VIP (Bowman *et al.*, 1982a) and PGE_1 (Bowman & Gillespie, 1982). This action of these agents was confirmed and it was also shown that forskolin (50–500 nM) produced a pronounced relaxation. The relaxations produced by VIP, PGE_1 or forskolin were not blocked by haemolysate or by N-methylhydroxylamine. These observations with VIP, PGE_1 and haemolysate have been described previously (Bowman & Gillespie, 1982; Bowman *et al.*, 1982a). Figure 4a illustrates the inability of N-methylhydroxylamine to impair the response to VIP.

To determine whether the relaxation of the bovine retractor penis muscle is invariably associated with an elevation of cyclic GMP, we studied the effect of these agents on the tissue's cyclic nucleotide content. The concentrations used were in excess of those causing maximal muscle relaxation. VIP, PGE_1 and forskolin did not affect the content of cyclic GMP in the bovine retractor penis muscle, but all three caused a significant elevation of the cyclic AMP content (Table 2). This was particularly prominent in the case of forskolin which has been shown to activate the enzyme adenylate cyclase specifically (Seamon *et al.*, 1981). Thus a rise in cyclic GMP levels does not necessarily accompany relaxation.

Table 2 The effect of forskolin, vasoactive intestinal peptide (VIP) and prostaglandin E_1 (PGE_1) on cyclic nucleotide levels in the bovine retractor penis muscle

Treatment	*Cyclic nucleotide levels* (pmol g^{-1}) *Cyclic AMP*	*Cyclic GMP*
Expt. 1		
Control	301 ± 12	20 ± 3
VIP (0.1 μM)	442 ± 40*	15 ± 2
VIP (1 μM)	495 ± 24**	16 ± 4
Expt. 2		
Control	280 ± 15	53 ± 4
Forskolin (0.5 μM)	600 ± 60**	48 ± 3
Forskolin (5 μM)	1710 ± 190**	68 ± 9
PGE_1 (1.5 μM)	365 ± 42	59 ± 4
PGE_1 (15 μM)	420 ± 19*	59 ± 5

Significantly higher than control values $^*P < 0.02$, $^{**}P < 0.01$.
In Expt 1, tissues were exposed to VIP (Sigma) for 2 min. In Expt. 2, exposure to drug was for 5 min. Values shown are means ± s.e.mean ($n = 5$).

Discussion

The experiments described here demonstrate that while relaxation of the bovine retractor penis muscle elicited by various agents may be associated with increases in the tissue content of either cyclic AMP or cyclic GMP, the relaxant response to non-adrenergic non-cholinergic nerve stimulation is associated with a selective rise in cyclic GMP. The crucial point is whether the rise in cyclic GMP causes the relaxation. The available evidence suggests that it does: (1) the elevation of the tissue cyclic GMP levels occurs prior to measurable relaxation; (2) agents that block guanylate cyclase (haemoglobin and N-methylhydroxylamine) also block the relaxant responses to field stimulation, and to other agonists that cause a rise in cyclic GMP (sodium nitroprusside, inhibitory factor); (3) the 8-bromo derivative of cyclic GMP elicits relaxation by a mechanism that is resistant to blockers of guanylate cyclase; (4) M & B 22948, a selective cyclic GMP-phosphodiesterase inhibitor, relaxes the muscle, potentiates the inhibitory response to nerve stimulation and causes a rise in the cyclic GMP content of the tissue. Also compatible with the hypothesis is the finding that the relaxant action of agonists which do not cause a rise in cyclic GMP (e.g. VIP, forskolin, PGE_1) is not blocked by haemoglobin or N-methylhydroxylamine.

It should be noted that for the three agents that elicited a rise in cyclic GMP (inhibitory factor, nitroprusside, M & B 22948), the dose-response curve for cyclic GMP accumulation was 6–30 fold to the right of the corresponding curve for tissue relaxation. As a result, drug concentrations that gave rise to small relaxation responses ($< 50\%$) did not produce measurable cyclic GMP responses. While this might be construed as evidence against a mediatory role of cyclic GMP in the relaxation responses of the retractor penis muscle to these agents, it seems more likely, in view of the evidence presented above, that this anomaly occurs because of our inability to measure small changes in cyclic GMP against a large background which, at least in part, will be located in physiologically irrelevant cell types or compartments. There are numerous accounts of experiments demonstrating that dose-response curves for neurotransmitter-induced cyclic nucleotide accumulation lie to the right of those for production of the physiological tissue response (see, for example, Drummond *et al.*, 1977).

It is well established that field stimulation of the bovine retractor penis muscle elicits a relaxation that is neurogenic (Klinge & Sjöstrand, 1974); however, the neurotransmitter which mediates this effect is not known. The results presented here are useful in that they limit the number of putative transmitters that are worth considering as candidates for the inhibitory transmitter in the retractor penis and related muscles: VIP and prostaglandin E_1 can clearly be eliminated entirely, not just because haemoglobin and N-methylhydroxylamine do not block their actions, but also because they do not produce the rise in cyclic GMP that is elicited by nerve stimulation. Since these results appear to be the first that delineate a nerve-induced rise in cyclic GMP as a necessary step in producing relaxation, many of the known neurotransmitters can probably be eliminated from the list of candidates, a conclusion that has already been reached by conventional pharmacological studies. The results are also consistent with the possibility that the extract of the bovine retractor penis contains the inhibitory neurotransmitter itself. It is interesting, in this respect, that Furchgott and his colleagues have demonstrated the existence of a factor which is released from endothelial cells and which mediates the vascular relaxant effects of acetylcholine (Furchgott & Zawadzki, 1980). This factor shares a number of properties in common with the inhibitory factor isolated from the bovine retractor penis muscle, among which appears to be the fact that its relaxant effects are mediated by cyclic GMP (Rapoport & Murad, 1983). Furthermore, it has been shown that the relaxant effect of acetylcholine on spiral strips of rabbit aorta is, like that of the inhibitory factor, blocked by haemoglobin (Bowman, A., unpublished observations).

A key finding which has emerged from these studies is the usefulness of the guanylate cyclase inhibitors N-methylhydroxylamine and oxyhaemoglobin in investigations of the physiological role of cyclic GMP. The mechanisms underlying hormonal, or even non-hormonal, activation of guanylate cyclase are still largely unknown or controversial (Mittal & Murad, 1982). Guanylate cyclase exists predominantly, but not exclusively, in the cytosol of mammalian cells (Mittal & Murad, 1982). While a neurotransmitter may be envisaged to activate particulate guanylate cyclase in a manner analogous to adenylate cyclase stimulation, there is little evidence that this occurs physiologically. If the rise in cyclic GMP occurs by a stimulation of cytosolic guanylate cyclase, then one either has to postulate the existence of a further intermediate which transmits the signal from a plasma membrane neurotransmitter receptor to the enzyme, or that the receptor itself exists on the cytosolic guanylate cyclase. There is little available evidence to distinguish between these possibilities, although it is relevant here to consider how haemoglobin, a large and presumably impermeant protein, is able to inhibit guanylate cyclase in an intact cell system. Inhibition of guanylate cyclase does indeed appear to be its mechanism of action since, apart from the pharmacological specificity of the oxy- and carboxy-forms mentioned above, the protein does both lower basal tissue levels of cyclic GMP and block the increase elicited by nerve stimulation, inhibitory factor or sodium nitroprusside. Moreover, the relaxation induced by 8-bromo-cyclic GMP which bypasses the guanylate cyclase step is, as one would expect, insensitive to haemoglobin.

If it is assumed that haemoglobin exerts its effect from outside the cell, then three possible modes of action are (a) that haemoglobin inactivates the neurotransmitter by binding directly to it, (b) that the extracellular haemoglobin can act as a trap for reactive oxygen species, e.g., hydroxyl radicals, which may be necessary for the activation of guanylate cyclase (Mittal & Murad, 1977), or (c) that a membrane-bound guanylate cyclase is involved in this particular mechanism.

The results obtained with haemoglobin may also have therapeutic relevance. There is currently a great deal of interest in the role of haemoglobin in the modulation of vascular tone, stemming from the observation that it might be responsible for the prolonged constriction of cerebral blood vessels that occurs after cerebral haemorrhage (Echlin, 1971; Osaka, 1977; Bouillin, 1980). Certain blood vessels (cerebral, coronary, mesenteric and femoral) appear to be particularly sensitive to haemoglobin (Tanishima, 1980); it has recently been demonstrated that isolated bovine and canine penile arteries are powerfully constricted by haemoglobin, which also blocks NANC nerve-induced vasodilatation and the dilatation produced by the inhibitory factor extracted from the bovine retractor penis muscle (Bowman & Gillespie, 1983). It seems possible that these effects of haemoglobin are mediated by its ability to inhibit guanylate cyclase, and that cyclic GMP in these vessels may play a role in the determination of vascular tone. In the retractor penis muscle, the rise in tone following the addition of haemoglobin was associated with a decline in the resting cyclic GMP content. The possibility that guanylate cyclase activity is important in maintaining cerebral vessel patency should be taken into account when therapeutic measures to control cerebral vasospasm are considered. Drugs such as M & B 22948 might be particularly useful in this condition.

In conclusion, this is the first time that cyclic GMP has been shown to be both produced in response to nerve stimulation and mediate the response of the effector organ. Further studies are necessary to elucidate the mechanism by which nerve stimulation in-

creases the cyclic GMP content of the bovine retractor penis muscle and how cyclic GMP mediates muscle relaxation.

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