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MEDIATION BY NITRIC OXIDE OF NEUROGENIC RELAXATION OF THE URINARY BLADDER NECK MUSCLE IN SHEEP

BY K. D. THORNBURY, M. A. HOLLYWOOD AND N. G. McHALE
From the Department of Physiology, School of Biomedical Science, the Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland

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SUMMARY

1. Mechanical recordings were made *in vitro* from circularly oriented strips of the bladder neck muscle of sheep. In the absence of drugs, electrical field stimulation at frequencies of 0·2–1 Hz evoked clear-cut relaxations throughout 1 min stimulation periods, while higher stimulus frequencies (2–8 Hz) evoked variable responses consisting of relaxation, contraction or a mixture of both. All of the responses were abolished by tetrodotoxin (10^{-6} M).

2. The contractions were reduced by guanethidine (10^{-6} M) and atropine (10^{-6} M), so that in the presence of these drugs clear-cut relaxations were obtained at 0·2–8 Hz stimulation, indicating that the relaxations were mediated by non-adrenergic, non-cholinergic (NANC) nerves.

3. The NANC relaxations were blocked by L-N^{G} nitro arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis. The antagonism by L-NAME was reversed by L-arginine.

4. Another feature of the NANC relaxation was 'rebound contraction' which occurred when the stimulus was switched off. The rebound contraction was also blocked by L-NAME and restored by L-arginine.

5. The relaxations and rebound contractions were unaffected by either α,β-methylene ATP (10^{-5} M) or 2-methylthio ATP (10^{-5} M).

6. *S*-Nitroso-L-cysteine, a substance which spontaneously releases NO at physiological pH, mimicked the relaxation and rebound contraction produced by nerve stimulation.

7. It is concluded that nerve-evoked relaxation of the bladder neck is mediated by NO, or a closely related substance such as *S*-nitroso-L-cysteine.

INTRODUCTION

There has been considerable debate as to how the internal smooth muscle sphincter at the outlet of the urinary bladder opens during voiding. One proposal is that fibres of the sphincter continue into the base of the detrusor muscle in such a way that when the latter contracts it pulls the sphincter open (Denny-Brown & Robertson, 1933*a*, *b*; Kuru, 1965). Other studies, however, have shown that the sphincter has an inhibitory non-adrenergic, non-cholinergic (NANC) innervation which causes relaxation both of the intrinsic tone inherent in this muscle, and of agonist-induced

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contractions (Andersson, Mattiasson & Sjogren, 1983; Klarskov, Gerstenberg, Ramirez & Hald, 1983; Hills, Meldrum, Klarskov & Burnstock, 1984; Klarskov, 1987; Mattiasson, Andersson, Andersson, Larsson, Sjogren & Uvelius, 1990). Although several putative neurotransmitters including vasoactive intestinal polypeptide (VIP), adenosine triphosphate (ATP), adenosine and 5-hydroxytryptamine (5-HT) cause relaxation of the internal sphincter, antagonizing these substances has no effect on the response to nerve stimulation (Hills *et al.* 1984; Klarskov, 1987). Another potential neurotransmitter which has not previously been considered is nitric oxide (NO). Some central and peripheral neurones contain an enzyme apparently identical to endothelial NO-synthase (Bredt, Hwang & Snyder, 1990), which forms NO from the terminal guanidino nitrogens of L-arginine (Palmer, Ashton & Moncada, 1988). A number of recent studies have concluded that NO mediates NANC inhibition in a variety of visceral organs including the anococcygeus muscle (Gillespie, Xiarong & Martin, 1989; Gibson, Mirzazadeh, Hobbs & Moore, 1990) the gastrointestinal tract (Bult, Boeckxstaens, Pelckmans, Jordaens, Van Maercke & Herman, 1990; Boeckxstaens, Pelckmans, Bult, De Man, Herman & Van Maercke, 1991; Dalziel, Thornbury, Ward & Sanders, 1991; Desai, Sessa & Vane, 1991; Thornbury, Ward, Dalziel, Carl, Westfall & Sanders, 1991), and corpus cavernosum muscle (Ignarro, Bush, Buga, Wood, Fukuto & Rajfer, 1990). Such conclusions are supported by the following observations: (1) specific inhibitors of NO-synthase block the effects of nerve stimulation; (2) L-arginine, the substrate for NO-synthase, competitively reverses the effect of NO-synthase inhibition, but D-arginine is without effect; (3) NO and NO-releasing compounds mimic the inhibitory effects of nerve stimulation. The aim of the present study was to determine whether NO mediates NANC relaxation of the internal smooth muscle sphincter at the bladder neck.

METHODS

Urinary bladders of sheep of either sex were obtained from an abattoir approximately 15 min after slaughter and transported to the laboratory for dissection in oxygenated Krebs solution. Circularly oriented rings were cut from the region of the bladder just below the trigone and these were opened and the mucosa removed by sharp dissection to give strips with approximate dimensions of 10 mm × 4 mm × 4 mm. The strips were mounted in organ baths (volume 5 ml), with initial tension adjusted to 6 mN, and perfused with Krebs solution of composition (mM): NaCl, 120; $NaHCO_3$, 25; KCl, 5·9; NaH_2PO_4, 1·2; $CaCl_2$, 2·5; $MgCl_2$, 1·2; glucose, 11; gassed with O_2 (95%) and CO_2 (5%). Tension changes were measured with Statham UC3 and Dynamometer UF1 isometric transducers the outputs of which were written on Gould 2400S and Lectromed MC 216 chart recorders. Field stimulation was applied via platinum ring electrodes mounted at either end of the tissue strip. Pulses of 0·3 ms duration were delivered in trains at constant frequencies of 0·2, 0·5, 1· 2, 4 and 8 Hz from Grass S88 and S11 stimulators set at nominal output voltages of 50 V. Stimulation periods always lasted 1 min and the recovery time between stimuli was at least 5 min. In nerve stimulation experiments the preparations were perfused continuously with Krebs solution and drugs were delivered in the perfusing solution. However, when testing the effect of *S*-nitroso-L-cysteine a different approach was used since this compound has a half-life of less than 30 s (Myers, Minor, Guerra, Bates & Harrison, 1990; Bates, Aldape & Baker, 1991). In these experiments the tissue was mounted in 20 ml organ baths which were continuously bubbled with O_2 (95%) and CO_2 (5%). These baths were also perfused, but when cumulative additions of *S*-nitroso-L-cysteine were made the perfusion was switched off. The *S*-nitroso-L-cysteine was then washed out by recommencing the perfusion.

S-Nitroso-L-cysteine was synthesized by reacting L-cysteine with sodium nitrite under acidic conditions as described previously (Field, Dilts, Ravichandran, Lenhert & Carnahan, 1978;

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Kowaluk & Fung, 1990). Reactant solution contained in 1 N-HCl (5 ml), MeOH (5 ml), concentrated H_2SO_4 (0·5 ml), sodium nitrite (10 mM) and L-cysteine (5 mM). In control experiments, dilutions of the reactant solution (containing all of the reactants except L-cysteine) equivalent to *S*-nitroso-L-cysteine (10^{-5} M) had no effect on the preparation. Other drugs were used as follows: tetrodotoxin (TTX; Sigma); guanethidine sulphate (Sigma); atropine sulphate (Sigma); L-N^G-nitro arginine methyl ester (L-NAME; Sigma); L-arginine (Sigma; D-arginine (Sigma); α,β-methylene adenosine triphosphate (α,β-methylene ATP; Sigma); 2-methylthio adenosine triphosphate (2 methylthio ATP; Research Biochemicals Inc., USA), noradrenaline bitartrate (Sigma). All of these were made up to their final concentration in the Krebs solution; all drugs present in each type of experiment are stated in the figure legends. Summarized results are expressed throughout as mean responses ± S.E.M, and statistical comparisons were made using ANOVA and Fischer's PLSD test, taking the $P < 0·05$ level as significant.

RESULTS

Responses to electrical field stimulation

The effect of electrical field stimulation (0·2–8 Hz) was studied on an initial sample of thirty-one bladder neck strips before addition of drugs. Clear-cut relaxations were always apparent at the lower stimulus frequencies tested (0·2–1 Hz). At 0·2 Hz the response consisted of individual relaxations coinciding with each stimulus pulse and partially summated to give a tonic relaxation (Fig. 1, upper trace). At 0·5 and 1 Hz summation was complete, giving tonic relaxations which reached their peak within 20 s. These were well maintained throughout the stimulus period and were followed by a contraction which occurred after termination of the stimulus. This will henceforth be referred to as the 'rebound contraction' (see below). The maximum relaxation was obtained at 1 Hz and averaged 0·61 ± 0·10 mN ($n = 31$). Relaxations were also observed during stimulation at higher frequencies (2–8 Hz), although they were partly or completely offset by contractions occurring during the stimulus period. This contraction appeared either as a partial escape from the relaxation during stimulation (e.g. response to 2 Hz, Fig. 1) or crossed the baseline to produce an increase in tone above the resting level (e.g. response to 4 Hz, Fig. 1). The latter contractions were reduced by a combination of guanethidine (10^{-6} M) and atropine (10^{-6} M), so that the responses to the higher frequencies of stimulation also became predominantly relaxant (e.g. Fig. 2). Relaxations, in contrast, were either unaffected by guanethidine and atropine, or were further unmasked by these drugs, confirming previous observations that they are NANC in character (Klarskov *et al.* 1983; Hills *et al.* 1984; Klarskov, 1987). All of the responses to stimulation at frequencies of 0·2–8 Hz were completely abolished by TTX (10^{-6} M; $n = 8$), indicating that they were neurally mediated (e.g. Fig. 1, lower trace).

Effect of L-NAME and L-arginine

In order to test whether products of the NO synthesis pathway mediate the NANC relaxations, the effects of L-NAME (10^{-4} M), a competitive inhibitor of NO-synthase (Rees, Palmer, Schultz, Hodson & Moncada, 1990), was tested. These experiments were carried out in the presence of atropine (10^{-6} M) and guanethidine (10^{-6} M) in order to reduce adrenergic and cholinergic responses. An example of one such experiment is shown in Fig. 2. Before exposure to L-NAME, relaxations occurred in response to stimulus frequencies of 0·2–4 Hz and prominent rebound contractions occurred at the ends of the stimulus periods (Fig. 2, top trace). L-NAME (10^{-4} M) had no effect on baseline tone, but abolished the relaxations evoked by 0·2 Hz stimulation

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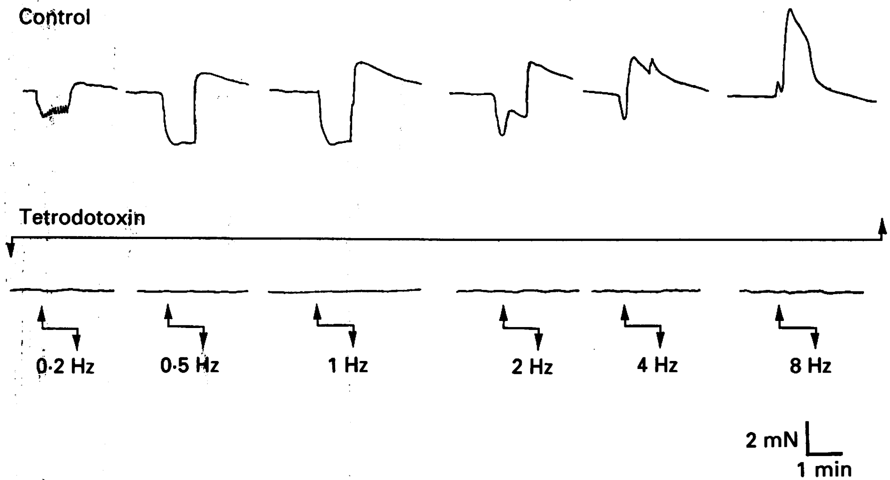

Fig. 1. Responses of the bladder neck muscle to 1 min periods of field stimulation at frequencies of 0·2–8 Hz in the presence of Krebs solution (upper trace), and in tetrodotoxin (10^{-6} M; lower trace).

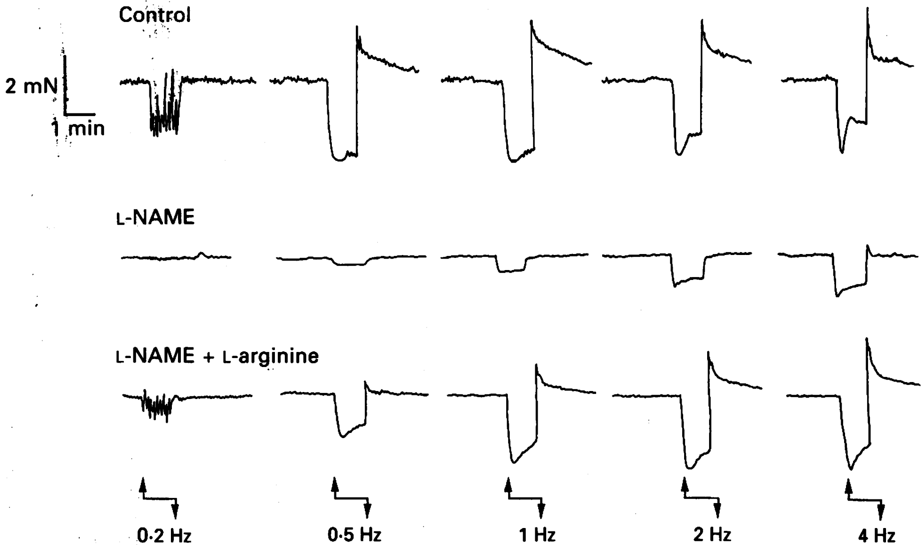

Fig. 2. Relaxations and 'rebound contractions' in response to 1 min periods of field stimulation at 0·2–4 Hz in Krebs solution containing atropine (10^{-6} M) and guanethidine (10^{-6} M) (top trace); after addition of L-NAME (10^{-4} M) (middle trace); and after addition of L-NAME (10^{-4} M) and L-arginine (10^{-3} M) (bottom trace).

and greatly reduced the relaxations and rebound contractions at all other frequencies (Fig. 2, middle trace), thus suggesting that both responses depended on synthesis of NO. In other types of smooth muscle the effects of NO-synthase inhibitors such as L-NAME can be competitively reversed by L-arginine, but not by D-arginine (Gibson *et al.* 1990; Dalziel *et al.* 1991; Desai *et al.* 1991). Figure 2 (bottom trace) shows that, in the bladder neck muscle, L-arginine (10^{-3} M) partly reversed the effects of L-

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NAME, restoring both the relaxations and the rebound contractions throughout the range of stimulus frequencies. In contrast, D-arginine (10^{-3} M) was without effect ($n = 3$; not shown). A summary of the effects of L-NAME (10^{-4} M) and L-arginine (10^{-3} M) in eleven experiments is presented in Fig. 3. The mean effect of L-NAME was

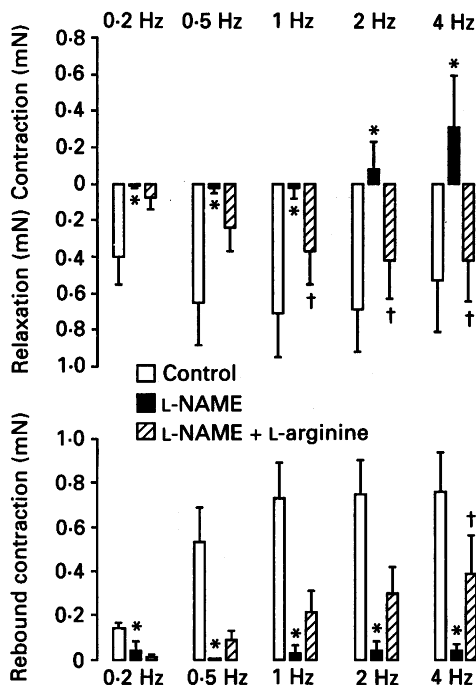

Fig. 3. Summary of the effects of L-NAME (10^{-4} M) and L-NAME (10^{-4} M) + L-arginine (10^{-3} M) on the responses to field stimulation ($n = 11$). Atropine (10^{-6} M) and guanethidine (10^{-6} M) were present throughout. Upper panel indicates responses occurring during the 1 min periods of field stimulation, lower panel indicates rebound contractions occurring after field stimulation. Columns represent mean responses before addition of L-NAME (□), in presence of L-NAME (■), and in presence of L-NAME + L-arginine (▨). Error bars represent 1 S.E.M. * significant depression in presence of L-NAME ($P < 0.05$), † significant recovery in L-NAME and L-arginine ($P < 0.05$).

to significantly reduce the relaxations evoked by stimulus frequencies 0·2–1 Hz, while at 2–4 Hz the relaxations were converted to contractions (Fig. 3, upper panel). These effects were significantly reversed by L-arginine, thus providing further support for the hypothesis that the relaxations depended on synthesis of NO. The rebound contraction was also significantly reduced by L-NAME at all stimulus frequencies, and there was a clear trend for this to be reversed by L-arginine, although the effect was only statistically significant at 4 Hz stimulation (Fig. 3, lower panel).

Effect of S-nitroso-L-cysteine

If the NANC relaxations are mediated by NO, it ought to be possible to mimic the effects of nerve stimulation by applying exogenous NO to the preparation. Therefore the effect of *S*-nitroso-L-cysteine, a NO-releasing compound and putative endogenous

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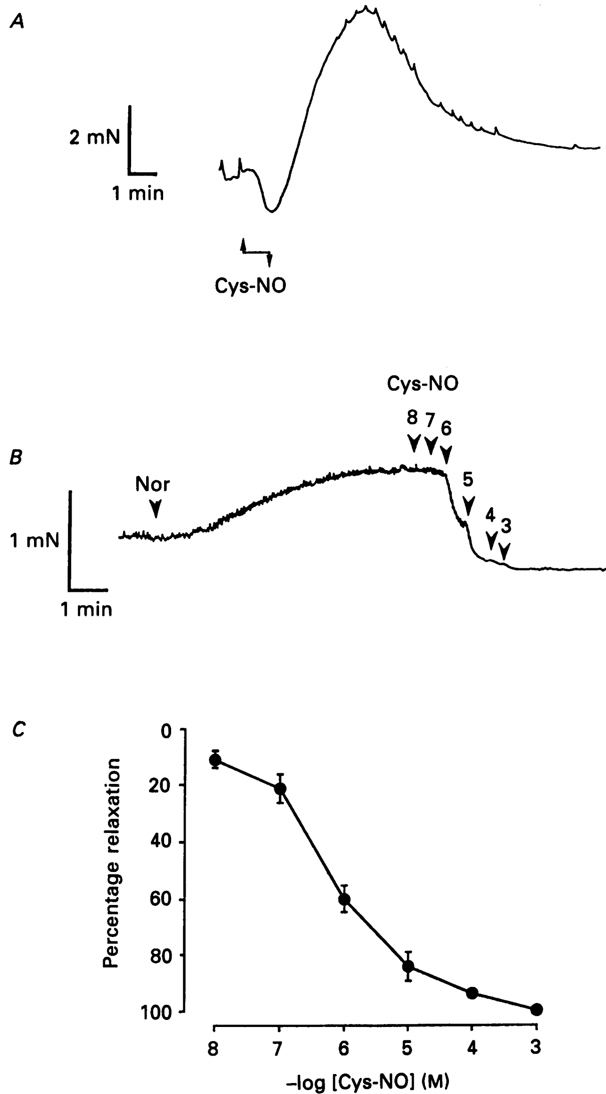

Fig. 4. *A*, response of a bladder neck muscle strip to exposure to *S*-nitroso-L-cysteine (Cys-NO; $1{\cdot}6\times10^{-6}$ M) for 1 min. Strip was precontracted with noradrenaline (10^{-6} M). *B*, strip was precontracted with noradrenaline (Nor; 10^{-5} M) and then exposed to cumulative concentrations of *S*-nitroso-L-cysteine (10^{-8}–10^{-3} M); doses expressed as $-\log$ [*S*-nitroso-L-cysteine]. The bath was continuously aerated with O_2 (95%) and CO_2 (5%) and was not perfused during the additions of *S*-nitroso-L-cysteine. *C*, cumulative dose–response relationship for *S*-nitroso-L-cysteine ($n = 7$; ● indicate means; bars indicate ± S.E.M.). Strips were precontracted with noradrenaline (10^{-5} M). Relaxations are normalized to the maximum relaxation, (obtained at *S*-nitroso-L-cysteine, 10^{-3} M) and plotted against $-\log$ [*S*-nitroso-L-cysteine].

carrier of NO (Ignarro, 1990; Myers *et al.* 1990) was examined to see if it mimicked the effects of nerve stimulation. *S*-Nitroso-L-cysteine caused rapid relaxation of the bladder neck muscle and, when brief additions and rapid washouts were performed,

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there were also rebound contractions (Fig. 4*A*). Thus, *S*-nitroso-L-cysteine could mimic both of the components of NANC nerve stimulation. The ability of *S*-nitroso-L-cysteine to relax the tissue was quantified by examining the effects of cumulative additions to tissue precontracted with noradrenaline (10^{-5} M). Noradrenaline induced

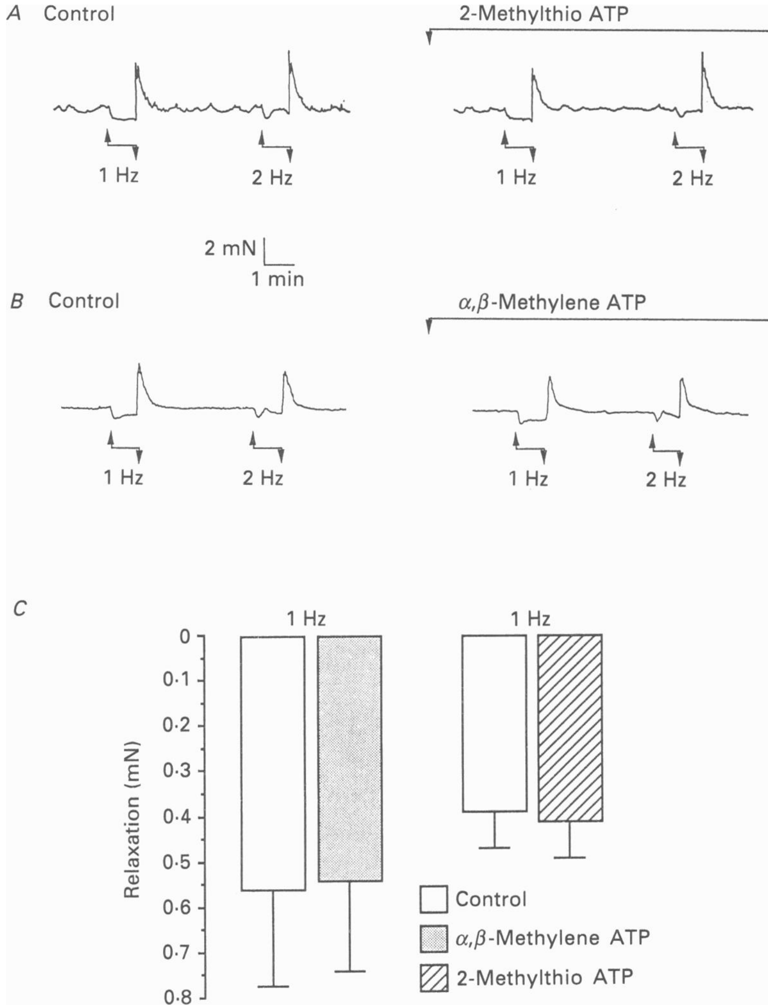

Fig. 5. *A*, effect of 2-methylthio ATP (10^{-5} M) on response to 1 Hz and 2 Hz field stimulation. *B*, effect of α,β-methylene ATP (10^{-5} M) on response to 1 Hz and 2 Hz field stimulation. Control responses were obtained in Krebs solution in each case. *C*, summary of the effect of 2-methylthio ATP (10^{-5} M) and α,β-methylene ATP (10^{-5} M) on relaxations in response to 1 Hz stimulation. Each column refers to the mean response of six preparations. Each preparation was used once only, first to test the control response in Krebs solution (□) and then during perfusion of either 2-methylthio ATP (▩) or α,β-methylene ATP (▨).

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contractures averaging $2{\cdot}72 \pm 0{\cdot}36$ mN and these were dose-dependently reduced by *S*-nitroso-L-cysteine (10^{-7}–10^{-3} M; e.g. Fig. 4*B*). Threshold concentrations necessary to cause relaxation varied from 10^{-8} to 10^{-7} M between tissues, and maximal relaxations occurred at 10^{-4}–10^{-3} M, at which concentrations tone was reduced to below the pre-noradrenaline resting level. These effects were reversible on wash (not shown). A summary of the mean responses to cumulative additions of *S*-nitroso-L-cysteine in seven preparations is shown in Fig. 4*C*.

Effects of 2-methylthio ATP and α,β-methylene ATP

High concentrations of ATP have also been shown to cause relaxation in the bladder internal sphincter (Hills *et al.* 1984; Klarskov, 1987). The possibility that ATP participated in the relaxations evoked by nerve stimulation was tested by exposing the tissues to ATP analogues in order to desensitize ATP receptors. Preparations were first stimulated under control conditions (no drugs present), and then stimulation was repeated after at least 30 min exposure to either the P_{2Y} receptor agonist, 2-methylthio ATP (10^{-5} M), or the P_{2X} receptor agonist, α,β-methylene ATP (10^{-5} M). Typical examples of the effects of these compounds on nerve stimulation are shown in Fig. 5. Neither compound affected the basal tone or the relaxations evoked by nerve stimulation, suggesting that P_2 receptors did not participate in the nerve-evoked responses. Summary data of the effects of 2-methylthio ATP ($n = 6$) and α,β-methylene ATP ($n = 6$) on relaxations induced by 1 Hz stimulation are presented in Fig. 5*C*. Neither drug produced any significant change in the relaxation (Fig. 5*C*), or the rebound contraction (not shown).

DISCUSSION

Despite early reports which postulated the existence of antagonistic inhibitory and excitatory nerves to the internal urethral sphincter (Barrington, 1914, 1933), for most of the last fifty years the accepted view has been that the opening of this muscle was an entirely passive process (Kuru, 1965; Denny-Brown & Robertson, 1933*a*). It was argued that the sphincter could not be opened independently of contraction of the detrusor muscle (Kuru, 1965; Denny-Brown & Robertson, 1933*b*), and hypothesized that the fibres of the sphincter continued into the base of the detrusor in such a manner that when the latter contracted it pulled open the sphincter (Kuru, 1965; Denny-Brown & Robertson, 1933*a*). This view, however, was challenged when it was demonstrated that stimulation of the sacral parasympathetic nerves in the human female could produce a fall in intra-urethral pressure without changing vesicular pressure (Torrens, 1978). It was proposed that active relaxation of the sphincter occurred in response to stimulation of parasympathetic inhibitory nerves. Subsequently, neurogenic relaxation of the bladder neck muscle and proximal urethra has been shown to occur *in vitro* in humans (Andersson *et al.* 1983), rabbits (Mattiasson *et al.* 1990) and pigs (Klarskov *et al.* 1983; Hills *et al.* 1984; Klarskov, 1987). Although several attempts have been made to identify the NANC neurotransmitter(s) mediating these responses, no satisfactory candidate has been established. Vasoactive intestinal polypeptide (VIP) was found to cause relaxation which could be antagonized by chymotrypsin (Hills *et al.* 1984) or VIP antiserum

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(Klarskov, 1987); however, neither treatment altered the relaxations evoked by nerve stimulation. Similarly, high doses of ATP (10^{-5}–10^{-3} M) caused relaxation, but desensitization to ATP or α,β-methylene ATP (Hills *et al.* 1984; Klarskov, 1987) failed to alter the response to nerve stimulation. Adenosine and 5-HT produced relaxations which were inhibited by 8-phenyltheophylline and methysergide, respectively, but again these antagonists failed to modify the nerve-mediated relaxations (Hills *et al.* 1984).

The recent identification of several highly specific inhibitors of NO-synthase (Palmer *et al.* 1988; Rees *et al.* 1990) has lead to the discovery that NO is involved in mediating NANC inhibitory responses in a number of other visceral smooth muscle preparations (Gillespie *et al.* 1989; Bult, *et al.* 1990; Gibson *et al.* 1990; Ignarro *et al.* 1990; Boeckxstaens *et al.* 1991; Dalziel *et al.* 1991; Desai *et al.* 1991; Thornbury *et al.* 1991). Our experiments now strongly suggest that this system is also responsible for a large component of the NANC relaxations of the bladder neck muscle. This view is supported by the following pieces of evidence: (1) the relaxations were reduced or abolished by L-NAME, a competitive inhibitor of Ca^{2+}–calmodulin-dependent NO-synthase; (2) the effect of L-NAME was partially reversed by L-arginine, the endogenous substrate for NO synthase, but was not affected by D-arginine; (3) exogenously applied *S*-nitroso-L-cysteine, a substance which spontaneously releases NO (Ignarro, 1990; Kowaluk & Fung, 1990; Myers *et al.* 1990; Bates *et al.* 1991) mimicked the relaxations caused by nerve stimulation. In this respect, the three criteria which are most commonly applied to establish NO as an important link in the process of inhibitory neurotransmission in other tissues (Gillespie *et al.* 1989; Gibson *et al.* 1990; Ignarro *et al* 1990; Boeckxstaens *et al.* 1991; Dalziel *et al.* 1991; Desai *et al.* 1991) have now been satisfied in the bladder. In the gastrointestinal tract two other points have strengthened this argument: (1) bioassay experiments have directly demonstrated TTX-sensitive release of NO-like substance upon field stimulation (Bult *et al.* 1990), and (2) Ca^{2+}–calmodulin-dependent NO-synthase has been shown to be present in the nerves of the myenteric plexus (Bredt *et al.* 1990). These experiments provide circumstantial evidence that NO may be released from nerves and have led to the proposal that NO is the actual inhibitory neurotransmitter (Bult *et al.* 1990). However, since NO-synthase is also present in endothelial cells (and possibly other cells), release of NO from a non-neural cell in response to release of another neurotransmitter cannot be excluded. In the present study we re-examined the role to ATP as a potential neurotransmitter which might perform this function. We felt that this was necessary since this substance is now known to release NO from vascular endothelial cells via an action on P_{2Y} receptors (Mathie, Ralevic, Alexander & Burnstock, 1991; Ralevic, Mathie, Alexander & Burnstock, 1991), a fact not known to previous workers who investigated the innervation of the bladder internal sphincter (Hills *et al.* 1984; Klarskov, 1987). Our results showed that the P_{2Y} receptor agonist, 2-methylthio ATP, failed to cause relaxation or interfere with the inhibitory responses to nerve stimulation, and confirmed the lack of effect of the P_{2X} receptor agonist, α,β-methylene ATP, described previously (Hills *et al.* 1984). These results suggest that it is unlikely that the final neurotransmitter is ATP. They do not, however, exclude the possibility that ATP may be a neurotransmitter in the inhibitory pathway, which then causes NO release from the final neurone in the path.

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Such a mechanism has been proposed to explain ATP-mediated relaxations in the dog ileocolonic junction, which are TTX- and L-NAME-sensitive (Boeckxstaens *et al.* 1991). Some of the other putative neurotransmitters referred to above could operate in this way since blocking their action with antagonists or by desensitization of receptors would not be expected to block the effects of field stimulation on the final nerve in the pathway. The question of which putative neurotransmitters cause TTX-sensitive release of NO in the bladder neck muscle warrants further investigation, but this was beyond the scope of the present study.

Even if it was proven that NO is released from nerves it might never be regarded as a neurotransmitter in the classical sense. Firstly, it is thought to exert most of its effects by acting intracellularly rather than via surface receptors (for review see Ignarro, 1990). Secondly, NO is an extremely lipophilic, labile substance which could not be stored easily in nerve vesicles. This necessitates proposing non-classical models to explain release in response to nerve stimulation. One such model proposes that NO is synthesized *de novo* as a result of an influx of Ca^{2+} into the nerve terminal, which then activates the Ca^{2+}–calmodulin-dependent synthase enzyme (Garthwaite, 1991). Alternatively, NO might be stored in vesicles if combined with another molecule to produce a more stable complex. *S*-Nitroso-L-cysteine has been proposed as the naturally occurring carrier for NO in vascular endothelial cells (Ignarro, 1990; Myers *et al.* 1990). Direct chemiluminescent measurements have demonstrated that NO is spontaneously liberated into the headspace of solutions of *S*-nitroso-L-cysteine (and similar *S*-nitrosothiols) in oxygenated Krebs buffer at 37 °C (Kowaluk & Fung, 1990). *S*-Nitroso-L-cysteine is relatively stable under acidic conditions but at pH 7·4 spontaneously liberates NO with a half-life of 15–30 s (Myers *et al.* 1990; Bates *et al.* 1991). It has been suggested that *S*-nitroso-L-cysteine is stored intracellularly in acidified vesicles and spontaneously degrades to release NO when the contents of the vesicle are expelled into the extracellular space (Ignarro, 1990). Electrophysiological studies in the canine colon have demonstrated that *S*-nitroso-L-cysteine is indistinguishable from NO in its ability to cause smooth muscle hyperpolarization (Thornbury *et al.* 1991). This suggests that this substance degrades to produce NO quickly enough (at least under experimental conditions) to account for changes attributed to endogenous release of NO. In the present study *S*-nitroso-L-cysteine produced relaxations and rebound contractions similar to the effects of nerve stimulation, making it a possible candidate for the substance released from the inhibitory nerves of the bladder.

The results of the present study demonstrate that NANC relaxation and rebound contraction in the smooth muscle sphincter of the bladder depend on the synthesis and release of NO, or a closely related compound. While it seems attractive to speculate that a response characterized by rapid relaxation followed by abrupt contraction would be ideally suited to participate in the normal voiding cycle of micturition, the previous lack of a specific antagonist for these nerves has made their physiological importance difficult to evaluate (Klarskov *et al.* 1983; Mattiasson *et al.* 1990). The demonstration that relaxation of the internal sphincter is antagonized by the NO-synthase inhibitor, L-NAME, provides an opportunity to assess the role of inhibitory NANC nerves *in vivo*, and may improve our understanding of clinical conditions such as stress incontinence, urge incontinence and urinary retention.

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