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Partial mediation by nitric oxide of the relaxation of human isolated detrusor strips in response to electrical field stimulation

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1 A method for reproducing relaxation of human isolated detrusor smooth muscle *in vitro* in response to electrical field stimulation is described.
2 The parameters of stimulation associated with relaxation were those which would be expected to give a largely nerve-mediated response: the relaxations were not reduced by tetrodotoxin (3×10^{-7} M) and were therefore not dependent on voltage sensitive sodium channels.
3 The relaxations were decreased (mean 74.1%) by nitro L-arginine (NOARG, 10^{-5} M).
4 Methylene blue (10^{-5} M), an inhibitor of soluble guanylate cyclase, abolished the relaxations.
5 These results indicate that there may be a relaxation mechanism in the human bladder which is at least partly mediated via the production of nitric oxide.

Keywords relaxation smooth muscle nitric oxide human bladder

Introduction

A noradrenergic innervation of the bladder neck produces the nerve-mediated relaxation that can be demonstrated in smooth muscle from this area (Kluck, 1980), but no similar relaxation has been reported in the body of the bladder.

Nerve-mediated relaxations have been demonstrated in a variety of smooth muscle preparations from a number of species (including man) which are non-adrenergic, non-cholinergic (NANC) responses. In vascular preparations these relaxations have been shown to be dependent on the presence of an intact endothelium, leading to the demonstration of an endothelium-derived relaxant factor (EDRF) which mediates relaxation. It is now generally accepted that a major EDRF in blood vessels is nitric oxide (NO) (Palmer *et al.*, 1987). In addition, it has been suggested that at least part of the NANC relaxation in some non-vascular smooth muscle preparations is mediated by nitric oxide (mouse anococcygeus (Gibson *et al.*, 1990), guinea-pig trachea (Tucker *et al.*, 1990), rabbit and guinea-pig taenia coli (Shikano *et al.*, 1988) and human corpus cavernosum (Pickard *et al.*, 1991)).

The present study investigated the possibility that there might be a relaxant mechanism present in human detrusor. A method has been developed for reproducing relaxations in human isolated detrusor *in vitro* in response to electrical field stimulation. Some mechanisms underlying these relaxations have been investigated, and in particular the role of NO.

A preliminary account of some of the findings was given at a meeting of the Clinical Section of the British Pharmacological Society (Lyon, April 1991) and at a meeting of the American Urological Association (Toronto, June 1991).

Methods

Electrical field stimulation of human isolated detrusor strips

Biopsies were obtained from patients undergoing a variety of endoscopic bladder procedures. The patients were a heterogenous clinical group and no attempt was made to distinguish differences in the responses according to diagnosis. The biopsies were taken from areas of the bladder away from the trigone using the cold-cup biopsy forceps employed routinely to obtain pinch biopsies of bladder mucosa for histological diagnosis. These forceps do not use diathermy and so cause minimal tissue damage. Biopsies were not taken from patients known to have urinary tract infection, patients with urinary catheters, or patients in whom the bladder

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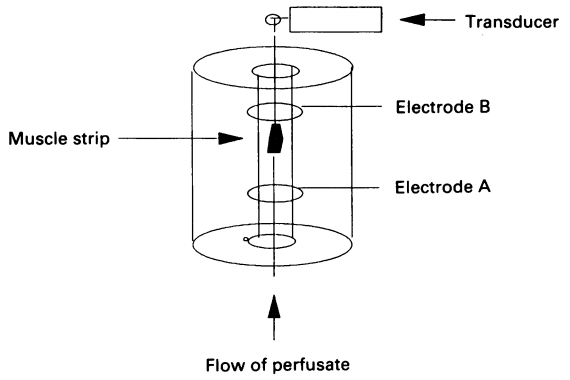

Figure 1 Diagram of the organ bath.

appeared to be inflamed at cystoscopy. Biopsies were taken only when there was a clinical indication to do so for histological diagnosis. The biopsies were transported in chilled, pre-oxygenated Krebs' solution, but were used fresh (within 1 h). Muscle strips (approximately 2 mm × 1 mm) were dissected from the biopsies and mounted in 0.2 ml organ baths (Brading & Sibley, 1983) (Figure 1). As many strips as possible were prepared from each patient up to a maximum of six. They were perfused with gassed (95% O_2, 5% CO_2) Krebs' solution (composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHPO_4$ 1.2, $NaHCO_3$ 25, glucose 5.5, pH 7.4) at a rate of 2 ml min^{-1}, and at a temperature of 37° C (± 0.5°). The tissue was placed under 200 mg initial tension. Electrical field stimulation was then applied for 15 s and any strips which did not contract were replaced with fresh strips if sufficient biopsy material were available. The strips were then allowed 1 h to equilibrate.

Changes in isometric tension were recorded using Grass FTO3 transducers and a Grass six-channel polygraph.

In most experiments atropine (10^{-6} M) was added to the perfusate to abolish any muscarinic contractile response, and the tone was raised using KCl (20 mM above basal, substituted for equivalent NaCl in the perfusate). In some experiments contraction was abolished by using tetrodotoxin (TTX, 3×10^{-7} M); tone was then raised with carbachol (10^{-7} M to 10^{-5} M), KCl (as above), or allowed to develop spontaneously.

After the equilibration period, electrical field stimulation was applied for periods of 15 s at intervals of 5 min. Rectangular wave pulses were delivered from a stimulator (Scientific and Research Instruments) through platinum ring electrodes built into the organ bath. Unless otherwise stated, the standard conditions used were: raised tone (achieved by a raised concentration of KCl, 20 mM above basal), atropine (10^{-6} M) present, and parameters of stimulation of a frequency of 10 Hz, a pulse-width of 90 μs, and a voltage of 50 v. Eighty-five percent of strips which had originally been shown to contract in response to the original test stimulation subsequently displayed relaxation under the experimental conditions used. The other 15% either did not react to stimulation or displayed a response which continued to be contractile, and these strips were excluded from the remainder of each experiment.

Drugs

Drugs were added to the perfusate and delivered to the tissue for a minimum of 20 min. Drugs used were atropine sulphate, propranolol hydrochloride, nitro L-arginine, L-arginine, methylene blue, indomethacin and tetrodotoxin (all obtained from Sigma).

Data analysis

The responses obtained showed variability, both between patients and between individual strips from each patient. In order to compare the responses they were therefore expressed as a percentage of the maximum response attained by each individual strip. The precise point at which the maximum was achieved could vary between strips, and so the mean data do not always reach 100%. Data were analysed using the programme Statmode. The data were not assumed to be normally distributed and so were analysed using the Wilcoxon sign-rank test. In order to indicate the scatter of results standard errors (s.e.) are included. The differences were assumed to be significant at values of $P < 0.05$. Unless otherwise stated, n refers to numbers of patients. The results for each patient were the means of measurements on 3 to 6 strips.

Results

Contraction: effect of TTX

A pulse-width contraction curve was made over a range of pulse-widths from 20–60 μs. TTX (3×10^{-7} M) was then added to the perfusate and a second pulse-width response curve performed, with additional stimuli delivered at 80, 100 and 200 μs (50 v, 10 Hz). TTX abolished all contraction up to 40 μs; at higher pulse widths there was a small but gradually increasing contraction.

Establishing relaxation in response to field-stimulation

To study relaxations the normal contractile response to electrical field stimulation was prevented (using atropine, 10^{-6} M or TTX, 3×10^{-7} M) and tone was raised (using KCl, 20 mM, or carbachol, 10^{-7} M to 10^{-5} M, or by allowing tone to develop spontaneously). Examples of these responses are shown in Figure 2.

Under the conditions of raised tone, and in the presence of atropine or TTX, electrical field stimulation resulted in relaxation, and when stimulation ended there was typically an after-contraction. This biphasic response is seen in Figure 2.

Parameter response curves

Voltage, frequency, and pulse-width responses were investigated (Figure 3). A voltage of 50 v, a frequency of 10–20 Hz, and a pulse-width of 90–100 μs were found to give the largest magnitude relaxations. These standard parameters (50 v, 10 Hz, 90 μs) were used in all subsequent experiments unless otherwise stated. We

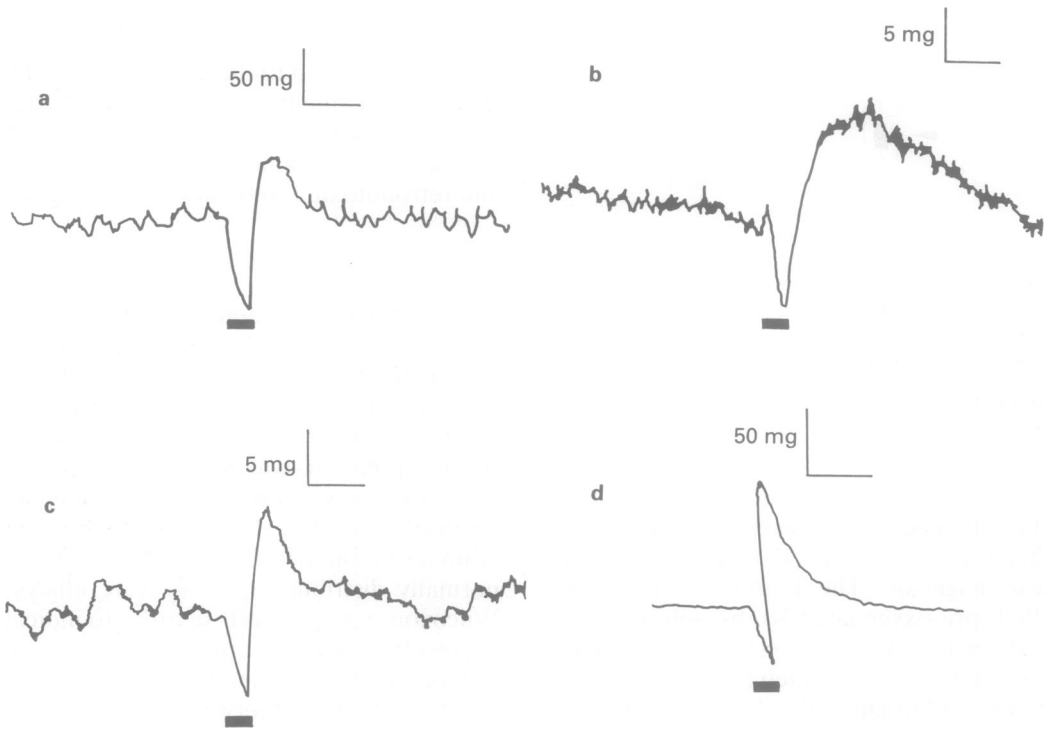

Figure 2 Examples of responses using a variety of agents to raise tone and block the contractile response. Scale bars represent time (30 s, x-axis) and tension (as labelled, y-axis). The horizontal bar represents the period of stimulation (15 s). Typical biphasic responses were seen with relaxation (downward deflection) during stimulation, and contraction after stimulation ceased. a) KCl (+20 mM)-induced tone with atropine (10^{-6} M) present; b) carbachol (10^{-6} M)-induced tone with TTX (3×10^{-7} M) present; c) spontaneous tone with atropine (10^{-6} M) present; d) KCl (+20 mM)-induced tone with atropine (10^{-6} M) and indomethacin (10^{-5} M) present.

report here the data pertaining to the relaxant phase of the response only, although we are also studying the after-contractions.

Effect of TTX on relaxation

Muscle strips were stimulated at 80 μs, 10 Hz and 50 v ($n = 6$). They were then exposed to TTX (3×10^{-7} M) for 20 min and the stimulus repeated. TTX did not abolish the relaxations. Their magnitude was increased by a mean of 90% (±39.1) after exposure to TTX.

Effect of propranolol

Muscle strips ($n = 5$) were stimulated under standard conditions. Propranolol (10^{-6} M) was then added to the perfusate, and stimulation repeated. There was no significant change in relaxant response in the presence of propranolol.

Effect of indomethacin

In a pilot study of eight muscle strips from two patients stimulated under standard conditions, addition of indomethacin (10^{-5} M) caused a marked decrease in tone and in the relaxant response to electrical stimulation. In a further 18 strips from another five patients indomethacin was added at the outset and did not prevent tone from developing in response to KCl, although the rise in tone was less well sustained. In 15 of these 18 strips relaxations were developed in the presence of indomethacin, but the relaxations were generally of small size and took longer to develop (Figure 2d).

Effect of nitro L-arginine (NOARG) on relaxation

Muscle strips were stimulated at a range of frequencies from 2–20 Hz (50 v, 90 μs). NOARG (10^{-5} M) was then added to the perfusate, and after 30 min the stimulation sequence was repeated. L-arginine (10^{-4} M) was then added to the perfusate and after a further 30 min the stimulation sequence was repeated a third time (Figure 4). The relaxations were inhibited by NOARG by up to 74.1% at 20 Hz. There was no statistically significant reversal of this inhibition when L-arginine was added to the perfusate.

In order to investigate the effect of time on the responses, a second series of experiments was performed. The strips were repeatedly stimulated at 90 μs (10 Hz, 50 v). Half the strips were exposed to NOARG (10^{-5} M), and the other half acted as time-matched controls (Figure 5). There was an increase in magnitude of the relaxations up to 30 min after the start of stimulation in the control strips. The relaxations in the strips treated with NOARG were again significantly diminished, both with respect to their initial magnitude, and with respect to the time matched controls.

A third series of experiments was performed in which strips from nine patients were studied allowing a longer time (90 min) for equilibration to minimise the effect of any change in the responses with time. Control relaxations were obtained to standard stimulation in all strips. Half the strips were exposed to L-arginine (10^{-4} M) for 30 min and the stimulation repeated. All strips were then exposed to NOARG (10^{-4} M) for 30 min and the stimulation repeated a third time (Figure 6). After exposure to L-arginine there was a 60% increase in the

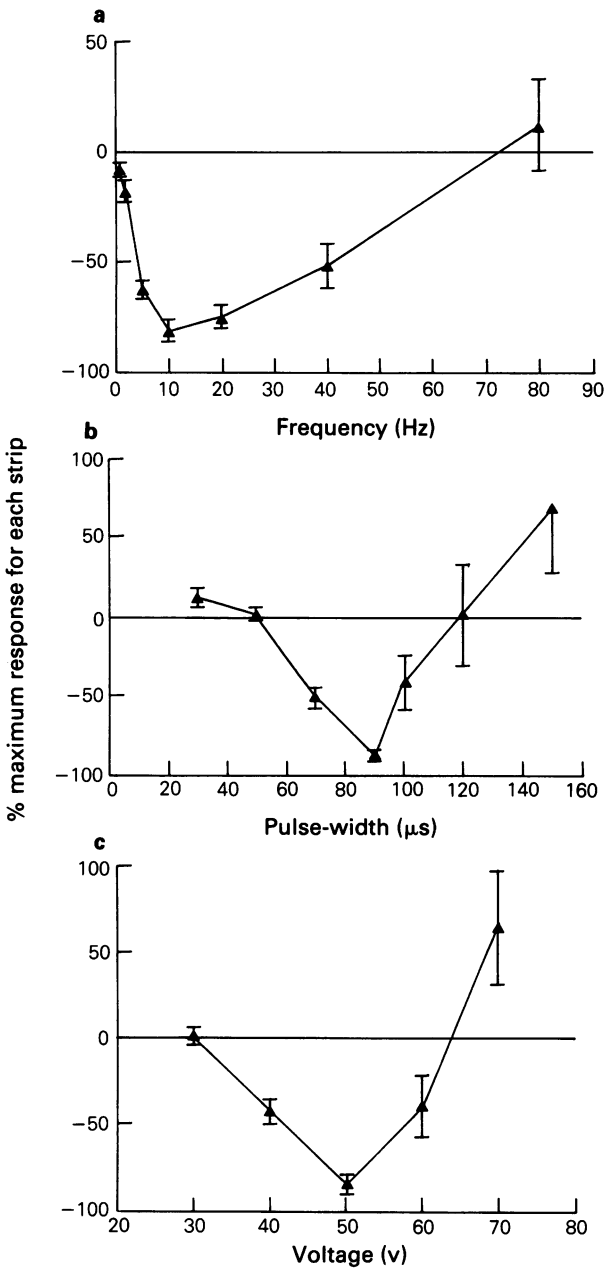

Figure 3 Parameter response curves for relaxation of raised tone (+20 mM KCl) in the presence of atropine (10^{-6} M) (n = 10 patients; 15 s duration stimuli at 5 min intervals). Responses are related to the maximum response for each strip which is taken to be 100%. The precise stimulus parameter at which the relaxation is maximum varied slightly between strips, and so the mean relaxation did not quite reach 100%. Relaxations are expressed as negative values, contractions as positive values. a) Frequency response curve (voltage 50 v, pulse width 90 μs); b) pulse width response curve (frequency 10 Hz, voltage 50 v); c) voltage response curve (frequency 10 Hz, pulse width 90 μs).

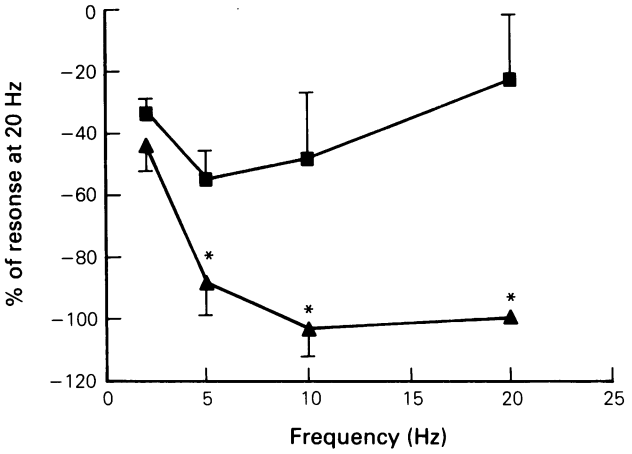

Figure 4 Effect of NOARG on relaxations (n = 8 patients, * = $P < 0.05$ using the Wilcoxon test for each pair of data points; 10 Hz, 90 μs, 50 v, 15 s trains at 5 min intervals). A control frequency response curve was performed (▲), and repeated after exposure to NOARG (10^{-5} M, 30 min, ■). Responses are related to the maximum response for each strip which is taken to be 100%. Relaxations are expressed as negative values. NOARG caused a significant inhibition of relaxation of up to 74.1% (mean).

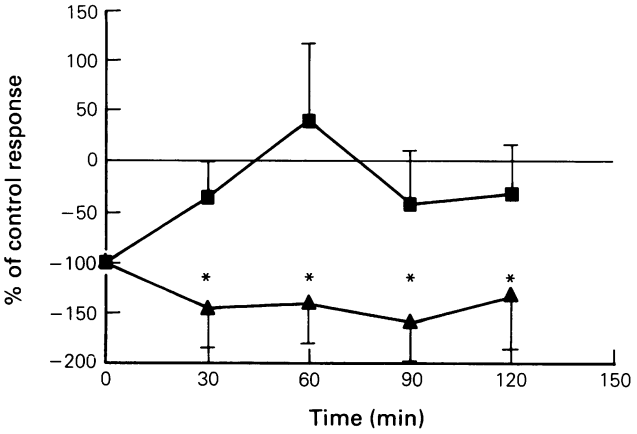

Figure 5 Effect of time and NOARG (10^{-5} M) on relaxations. (n = 6, * = $P < 0.05$ using the Wilcoxon test for each pair of data points; 10 Hz, 90 μs, 50 v, 15 s trains at 5 min intervals). Responses are related to the control response for each strip which is taken to be 100%. Relaxations are expressed as negative values. The relaxations of untreated strips (▲) became larger over the first 30 min, after which they remained constant. NOARG significantly inhibited relaxations (■).

relaxations which was not seen in the untreated strips. Subsequent exposure to NOARG had no effect in the continuing presence of L-arginine, but in the strips not exposed to L-arginine there was a decrease in the relaxations by 50.5%.

Effect of methylene blue

Muscle strips (n = 6) were repeatedly stimulated as described above. The strips were exposed to methylene blue (10^{-5} M) for 30 min after which the relaxations were completely abolished.

Effect of polarity reversal

The electrodes are positioned in the organ bath such that one lies 'upstream' (labelled 'A', Figure 1) and the other 'downstream' (labelled 'B', Figure 1) in the current of perfusate. In order to test the hypothesis that the relaxations may be caused by a product of electrolysis at one or other electrode, the response to 90 μs stimuli was compared at normal and reversed polarity. Polarity change had no effect.

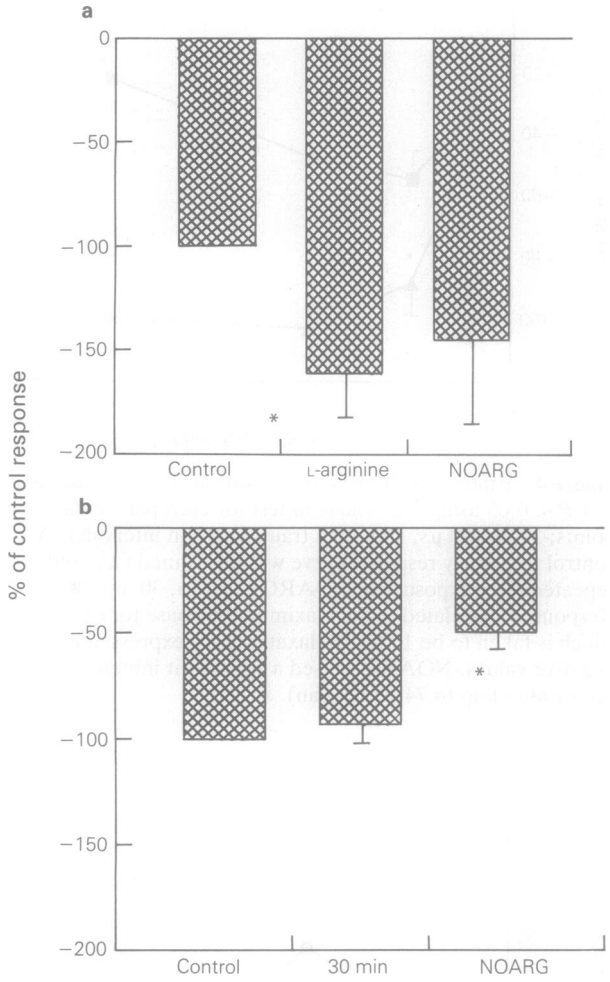

Figure 6 Effect of L-arginine (10^{-4} M, 30 min) and NOARG (10^{-4} M, 30 min) on relaxations ($n = 9$ patients. * = $P < 0.05$ compared with control (Wilcoxon); 10 Hz, 90 μs, 50 v, 15 s trains at 5 min intervals). Responses are related to the control response for each strip which is taken to be 100%. Relaxations are expressed as negative values. a) Half of the strips from each patient were treated with L-arginine for 30 min, and L-arginine + NOARG for the next 30 min. L-arginine produced an enhancement of relaxation by a mean of 61.4%. In the continuing presence of L-arginine NOARG had no effect. b) The other half of the strips from each patient were treated with NOARG from 60 min only. There was no significant change in the relaxation at 30 min, but NOARG inhibited relaxations by a mean of 50.5%.

Discussion

It is well established that contraction of the detrusor is mediated by the cholinergic innervation and, unlike many animal species, it is generally accepted that in man this response is entirely sensitive to atropine (Kinder & Mundy, 1985, 1987; Palfrey *et al.*, 1984; Sibley, 1984). Although many other neurotransmitters have been demonstrated histologically to be present in the bladder, their function is still largely unknown (Gu *et al.*, 1984; Maggi & Meli, 1986; Milner *et al.*, 1987; Polak & Bloom, 1984; Scultety, 1989; Vaalasti *et al.*, 1986).

This report describes a method for reproducing relaxation of human isolated detrusor *in vitro* and the characteristics of these relaxations. The stimulation parameters which were found to give the largest magnitude relaxation are those which would be expected to be associated with a nerve-mediated response. At the stimulation parameters and concentration of TTX used, a contractile response (in the absence of atropine) would be reduced by about 80%. The finding that relaxation was TTX-resistant was therefore unexpected. One possibility which could account for this is that the response is mediated by ionic channels other than the voltage-operated sodium channels blocked by TTX. If this were the case the response might be due to stimulation of excitable membranes, and possibly nerves, which are not dependent on these sodium channels. However direct smooth muscle stimulation is perhaps more likely to be the explanation, given that the stimulation parameters are slightly higher than those demonstrated to be associated with a purely nerve-mediated response. The TTX-resistant nature of these relaxations distinguishes them from other electrically-induced (and presumably nerve-mediated) relaxations which have been observed in smooth muscle from the human trigone (Speakman *et al.*, 1988) and from pig bladder neck, trigone and bladder (Klarskov, 1987). The authors are unaware of any studies which report relaxation of human detrusor to electrical field stimulation.

One concern about the experimental conditions utilised is that the use of KCl to increase tone may be causing widespread depolarisation in all excitable tissues making the whole situation rather 'unphysiological'. We have, however, also demonstrated similar relaxant responses of both spontaneous tone in the presence of atropine, and of carbachol-induced tone in the presence of TTX (see Figure 2).

All the NANC relaxations mediated by NO reported in the literature are sensitive to tetrodotoxin (TTX), but a number of earlier studies have also demonstrated relaxations in response to electrical field stimulation which are presumed to be non-neurogenic, mostly on the basis of TTX-resistance (in canine coronary artery (Feletou & Vanhoutte, 1987; Gantzos *et al.*, 1983; Lamb & Webb, 1984; Rooke *et al.*, 1982), rabbit and monkey pulmonary artery (Frank & Bevan, 1983), rat tail artery (Lamb & Webb, 1984), bovine coronary artery (Kalsner & Quillan, 1989), rat, rabbit, cat, pig, dog, monkey and human pial arteries together with a variety of peripheral vessels from rat, rabbit and cat (Hardebo *et al.*, 1989), and rat oesophageal smooth muscle (Bieger & Triggle, 1985)). These relaxations appeared to be of two sorts; a rapidly developing, rapidly reversible relaxation to short duration stimuli of moderate strength, and a more slowly developing, irreversible relaxation produced by long duration (5 min or more) higher intensity stimulation. Whilst being resistant to TTX, and to conventional methods of blocking neuromuscular transmission, the reversible, short duration relaxations were slightly enhanced by agents which reduce the number of oxygen free radicals present, such as superoxide dismutase. This finding would be consistent with the relaxations being mediated by nitric oxide. In contrast, the slower, irreversible relaxations were inhibited by these agents, and are therefore likely to be caused by free radical-induced tissue damage. A number of explanations have been put forward to account for these responses; Hardebo *et al.*

(1989) proposed that they were due to the formation of chlorine gas by electrolysis due to the electrical field. In our perfused organ bath we were able to test this hypothesis, because the two ring electrodes are arranged such that one is upstream of the tissue and the other downstream. Any product of electrolysis would therefore be carried past the tissue with the polarity in one direction, and away from it with the polarity in the opposite direction. We observed no change in the relaxations with a change of polarity and therefore conclude that they are unlikely to be due to a product of electrolysis. Some of the short-duration reversible responses described in the reports above are very similar to those described in this study, often with a biphasic form; in addition, the stimulation parameters in several of these studies are similar to those in the present one, being slightly higher than those needed to give a purely nerve-mediated (TTX sensitive) contraction.

NOARG has been shown to be a potent inhibitor of other NANC responses thought to be mediated by nitric oxide (Gibson *et al.*, 1990; Tucker *et al.*, 1990). Nitric oxide is produced from L-arginine by the enzyme nitric oxide synthase (Palmer *et al.*, 1988), and NOARG acts by competing with L-arginine in this reaction. Incubation of the detrusor muscle strips with NOARG resulted in an inhibition of the relaxations which suggests that at least part of the response is mediated via the production of NO. This suggestion is supported by the finding that L-arginine increased the relaxations, and antagonised the inhibitory effect of subsequent exposure to NOARG. The lack of reversibility when L-arginine was added after NOARG may be due to differences in the permeability of NOARG and L-arginine, or the relative affinity of each for the enzyme nitric oxide synthase.

NO exerts its relaxant effects by the stimulation of soluble guanylate cyclase with a consequent rise in cyclic GMP levels (Waldman & Murad, 1988), an effect which is blocked by methylene blue. Our finding that methylene blue abolished the relaxations is therefore entirely consistent with the suggestion that these relaxations involve the generation of NO.

Although NO is most familiar as an endothelium-derived relaxing factor, evidence is beginning to emerge that smooth muscle cells are themselves able to synthesise NO (Salvemini *et al.*, 1991). In the absence of atropine, the stimulation parameters used to produce the relaxations were at the borderline beween those producing purely nerve-mediated contraction and those producing direct muscle stimulation. Perhaps the most likely explanation for the TTX resistance of these relaxations is therefore that they are a result of NO generation at the level of the smooth muscle cells. Evidence is also emerging that smooth muscle cells possess both a baseline capacity to synthesise NO, and can increase their release of NO over a relatively short time (hours), possibly by enzyme induction (Salvemini *et al.*, 1991). This may explain the observed increase in the size of the relaxations with time observed in this study.

The effect of indomethacin of reducing tone makes it difficult to comment on the contribution of prostaglandins to the relaxant response, as the inhibition of relaxation may simply be due to the loss of tone rather than a direct effect on the relaxant mechanism. However, the finding that relaxations still develop in the presence of indomethacin suggests that the role of prostaglandins in these relaxations is not a major one.

In summary, we have characterised relaxations of human isolated detrusor strips to electrical field stimulation. These relaxations were resistant to tetrodotoxin but inhibited by nitroarginine and by methylene blue. These results suggest that the relaxation is not dependent on voltage-activated sodium channels, is mediated by activation of guanylate cyclase, and is at least partly mediated by nitric oxide.

If relaxation mechanisms are involved in the control of the human detrusor then it is possible that an alteration of these mechanisms would affect normal bladder function. In particular, if relaxation were impaired this might result in detrusor overactivity of the sort seen in the clinical condition of detrusor instability.

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References

Bieger, D. & Triggle, C. (1985). Pharmacological properties of mechanical responses of the rat oesophageal muscularis mucosae to vagal and field stimulation. *Br. J. Pharmac.*, **84**, 93–106.

Brading, A. F. & Sibley, G. N. A. (1983). A superfusion apparatus to study field stimulation of smooth muscle from mammalian urinary bladder. *J. Physiol.*, **334**, 11p–12p.

Feletou, M. & Vanhoutte, P. M. (1987). Relaxation of canine coronary artery to electrical stimulation: limited role of free radicals. *Am. J. Physiol.*, **253**, H884–H889.

Frank, G. W. & Bevan, J. A. (1983). Electrical stimulation causes endothelium-dependent relaxation in lung vessels. *Am. J. Physiol.*, **244**, H793–H798.

Gantzos, R. D., Ebeigbe, A. B. & Webb, R. C. (1983). Ca^{2+}, histamine antagonists and relaxation to electrical impulses in dog coronary artery. *Eur. J. Pharmac.*, **89**, 287–291.

Gibson, A., Mirzazadeh, S., Hobbs, A. J. & Moore, P. K. (1990). L-NG-monomethyl arginine and L-NG-nitro arginine inhibit non-adrenergic, non-cholinergic relaxation of the mouse anococcygeus muscle. *Br. J. Pharmac.*, **99**, 602–606.

Gu, J., Blank, M. A., Huang, W. M., Islam, K. N., McGregor, G. P., Christofides, N., Allen, J. M., Bloom, S. R. & Polak, J. M. (1984). Peptide-containing nerves in human urinary bladder. *Urology*, **24**, 353–357.

Hardebo, J. E., Kahrstrom, J. & Owman, Ch. (1989). Characterisation of dilatation induced by electrical field stimulation in mammalian cerebral and peripheral vessels. *Quart. J. exp. Physiol.*, **74**, 475–491.

Kalsner, S. & Quillan, M. (1989). Nonneurogenic relaxation to field stimulation in coronary arteries. *J. Pharmac. exp. Ther.*, **250**, 461–469.

Kinder, R. B. & Mundy, A. R. (1985). Atropine blockade of nerve mediated stimulation of the human detrusor. *Br. J. Urol.*, **57**, 418–421.

Kinder, R. B. & Mundy, A. R. (1987). Pathophysiology of idiopathic detrusor instability and detrusor hyper-reflexia. An *in-vitro* study of human detrusor muscle. *Br. J. Urol.*, **60**, 509–515.

Klarskov, P. (1987). Non-cholinergic, non-adrenergic inhibitory nerve responses of bladder smooth muscle *in vitro*. *Br. J. Urol.*, **60**, 337–342.

Kluck, P. (1980). The autonomic innervation of the human urinary bladder, bladder neck and urethra. *Anat. Rec.*, **198**, 439–447.

Lamb, F. S. & Webb, R. C. (1984). Vascular effects of free radicals generated by electrical stimulation. *Am. J. Physiol.*, **247**, H709–H714.

Maggi, C. A. & Meli, A. (1986). The role of neuropeptides in the regulation of the micturition reflex. *J. Auton. Pharmac.*, **6**, 133–162.

Milner, P., Crowe, R., Burnstock, G. & Light, J. K. (1987). Neuropeptide Y- and vasoactive intestinal polypeptide-containing nerves in the intrinsic external urethral sphincter in the areflexic bladder compared to detrusor-sphincter dyssynergia in patients with spinal cord injury. *J. Urol.*, **138**, 888–892.

Palfrey, E. L. H., Fry, C. H. & Shuttleworth, K. E. D. (1984). A new *in-vitro* microperfusion technique for investigation of human detrusor muscle. *Br. J. Urol.*, **56**, 635–640.

Palmer, R. M. J., Ashton, D. S. & Moncada, S. (1988). Vascular endothelial cells synthesise nitric oxide from L-arginine. *Nature*, **333**, 664–666.

Palmer, R. M. J., Ferrige, A. G. & Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.

Pickard, R. S., Powell, P. H. & Zar, M. A. (1991). Evidence that nitric oxide mediates human penile erection. *Br. J. Pharmac.*, **104**, 755–759.

Polak, J. M. & Bloom, S. R. (1984). Localisation and measurement of VIP in the genitourinary system of man and animals. *Peptides*, **5**, 225–230.

Rooke, T., Cohen, R. A., Verbeuren, T. J. & Vanhoutte, P. M. (1982). Non-neurogenic inhibitory effect of electrical impulses in isolated canine coronary arteries. *Eur. J. Pharmac.*, **80**, 251–254.

Salvemini, D., Mollace, V., Angaard, E. & Vane, J. (1991). Regulation of platelet reactivity by vascular smooth muscle cell-derived nitric oxide. *Fund. clin. Pharmac.*, **5**, 397.

Scultety, S. (1989). Neuropeptides in urology. *Int. Urol. Nephrol.*, **21**, 39–45.

Sibley, G. N. A. (1984). A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. *J. Physiol.*, **354**, 431–443.

Shikano, K., Long, C. J., Ohlstein, E. H. & Berkowitz, B. A. (1988). Comparative pharmacology of endothelium-derived relaxing factor and nitric oxide. *J. Pharmac. exp. Ther.*, **247**, 873–881.

Speakman, M. J., Walmsley, D. & Brading, A. F. (1988). An *in vitro* pharmacological study of the human trigone—a site of non-adrenergic, non-cholinergic neurotransmission. *Br. J. Urol.*, **61**, 304–309.

Tucker, J. F., Brave, S. R., Charalambous, L., Hobbs, A. J. & Gibson, A. (1990). L-Ng-nitro arginine inhibits non-adrenergic, non-cholinergic relaxations of guinea-pig isolated tracheal smooth muscle. *Br. J. Pharmac.*, **100**, 663–664.

Vaalasti, A., Tainio, H., Pelto-Huikko, M. & Hervonen, A. (1986). Light and electron microscope demonstration of VIP- and enkephalin-immunoreactive nerves in the human male genitourinary tract. *Anat. Rec.*, **215**, 21–27.

Waldman, S. & Murad, F. (1988). Biochemical mechanisms underlying vascular smooth muscle relaxation; the guanylate cyclase-cyclic GMP system. *J. cardiovasc. Pharmac.*, **12** (suppl. 5), S115–S118.

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