British Journal of Pharmacology (2000) 130, 489–494

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Pharmacological characterization of neurogenic responses of the sheep isolated internal anal sphincter

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1 The aim of the study was to establish the nature of the neurogenic responses of the sheep isolated anal sphincter.

2 Isolated strips of sheep internal anal sphincter develop intrinsic contractile tone following the application of stretch tension. On transmural stimulation (1–20 Hz, 10 V pulse strength, 0.5 ms pulse width, 1 s every 180 s) transient relaxations were observed.

3 The amplitude of the relaxations were frequency-dependent reaching a maximal response at 10–20 Hz and were inhibited by tetrodotoxin (0.3 μM). Neither atropine (0.3 μM) nor phentolamine (1 μM) affected control responses.

4 The nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and the selective inhibitor of soluble guanylyl cyclase ODQ, (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one) (1 μM) completely inhibited the neurogenic relaxations and uncovered contractions that were abolished by 1 μM phentolamine and 0.1 μM prazosin. The effect of L-NAME, but not that of ODQ, was partially reversed by the addition of L-arginine (1 mM).

5 Sodium nitroprusside (10 nM–10 μM) caused concentration-dependent inhibition of myogenic tone and this effect was significantly reduced by ODQ. Calcium-free Krebs-Henseleit solution also reduced myogenic tone by 85%.

6 Transmural electrical stimulation of the sheep isolated internal anal sphincter causes a transient relaxation of myogenic tone that appears to involve nitric oxide from non-adrenergic, non-cholinergic nerves and, to a lesser degree, noradrenaline from sympathetic nerves. The characteristics of the preparation compares well with that of human tissue and may prove to be a suitable animal based model for further studies.

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Keywords: Sheep internal anal sphincter; L-arginine; neurogenic relaxation; nitric oxide; N^G-nitro-L-arginine methyl ester; sodium nitroprusside; non-adrenergic; non-cholinergic neurotransmission; adrenergic neurotransmission

Abbreviations: EFS, electrical field stimulation; GTN, glyceryl trinitrate; L-NAME, N^G-nitro-L-arginine methyl ester; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; RX-811059, 2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline; SNP, sodium nitroprusside

Introduction

Over the past 5 years the treatment of chronic anal fissures has been revolutionized by the introduction of pharmacological intervention. This condition, characterized by a persistent elevation of internal anal sphincter muscle tone, has traditionally required surgical intervention to reduce anal sphincter pressure, in order to provide relief from pain and bleeding from the anal passage following defaecation (Hancock, 1997). However, the surgical approach is increasingly being replaced by the topical application of glyceryl trinitrate ointment to the anal passage, so called 'chemical sphincterotomy', which appears to be successful in promoting healing in 70% of cases (Loder *et al*., 1994; Lund & Scholefield, 1997a,b).

The pharmacological basis for the use of glyceryl trinitrate paste has been largely based on the findings of *in vitro* experiments designed to examine the properties of the internal anal sphincter. Work conducted mainly, though not exclusively, on the opossum internal anal sphincter has established that this muscle responds to stretch by developing myogenic tone and, following blockade of sympathetic nerves, to transmural, electrical stimulation by relaxing. The latter response appears to be mediated by intrinsic, non-adrenergic, non-cholinergic nerves that release nitric oxide; the neurogenic relaxations are associated with an elevation in tissue cyclic GMP and are inhibited by selective inhibitors of nitric oxide synthase, e.g. N^G-nitro-L-arginine methyl ester (L-NAME) (Tottrup *et al*., 1992; Rattan & Chakder, 1992; Chakder & Rattan, 1993a,b; Rattan *et al*., 1995). Qualitatively similar results have been obtained in human isolated internal anal sphincter (O'Kelly *et al*., 1993; O'Kelly, 1996) and this has been reinforced by immunohistochemical evidence for the presence of nitric oxide synthase in this tissue (O'Kelly *et al*., 1994).

However, other work on the human isolated internal sphincter have indicated the presence of constrictor α-adrenoceptors (Burleigh & D'Mello, 1983; Regardas *et al*., 1993) that can be activated by noradrenaline released from sympathetic nerves. These observations may account for the recent report that oral administration of indoramin, a selective α_1-adrenoceptor antagonist (Algate & Waterfall, 1978), caused a significant lowering of internal anal sphincter pressure in man (Pitt *et al*., 1999) and, as such, may be a useful adjunct in the treatment of anal fissures.

Ironically, the very success of 'chemical sphincterotomy' with GTN paste has reduced the opportunities for investigators to examine the potential of other pharmacological

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approaches for manipulating human anal sphincter tone. With this problem in mind, we have investigated the pharmacological characteristics of the sheep isolated internal anal sphincter to assess its suitability as a model for man. Specifically, in light of the evidence for constrictor and relaxant neuronal influences on the human isolated anal sphincter, we have examined the sheep isolated internal anal sphincter to establish the principal neurotransmitters and assess the role of cyclic GMP and calcium ions in regulating muscle tone.

Methods

Sheep anal tissue was obtained from a local abattoir, incubated in modified Krebs-Henseleit solution and transported to the laboratory within 1 h of slaughter. The anal canal was opened up by an incision made along the ventral longitudinal axis and pinned down with the mucosal surface up. The mucosa was dissected off to expose the underlying circular muscle. Strips of the distal internal anal sphincter were cut out and further dissected to ensure they contained visible, parallel bundles of muscle fibres only. Suture was tied at both ends of the strip ($10 \times 2 \times 2$ mm) and one end secured to a perspex holder between two parallel platinum wire electrodes. The perspex holder and the anal sphincter strip was then placed in a 20 ml isolated organ bath containing Krebs-Henseleit solution (pH 7.4), gassed (95% O_2/5% CO_2) at 37°C. The upper end of the suture was connected to a Grass FT-03C isometric transducer which in turn was connected to a CED 1902 (Cambridge Electronic Devices, Cambridge, U.K.) unit for amplification and linked *via* a 1401 interface to a 486-33 MHz PC running Spike 2 software (CED). Following application of an initial 2 g tension, the tissue was allowed to equilibrate for 30–40 min. Transmural stimulation of the muscle was achieved using a Digitimer Multistim System D330 (1–20 Hz, pulse strength 10 V, pulse width 0.5 ms, 1 s duration every 180 s). Tissues were allowed a further 30 min to establish reproducible responses on stimulation.

Experimental protocol

All experiments were carried out on tissues that had developed some degree of myogenic tone following the application of 2 g tension (approximately 90% of preparations). Once the myogenic tone had stabilized, preparations were stimulated transmurally with pulses at increasing frequency or, when the pharmacological characteristics of the response was investigated, at 10 Hz for 1 s every 180 s.

In the first set of experiments the effect of either 0.3 μM atropine, 1 μM phentolamine, 100 μM N^G-nitro-L-arginine methyl ester (L-NAME) or 0.3 μM tetrodotoxin were examined alone against the response to 10 Hz, 1 s stimulation. In the second set of experiments the effect of either 1 mM L-arginine or 1 mM D-arginine on responses in the presence of 100 μM L-NAME was investigated, followed by the addition of 1 μM phentolamine. Also the effect of sequential exposure to 1 μM 2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline (RX-811059) and 0.1 μM prazosin was investigated on the responses produced in the presence of L-NAME. A similar series of experiments was conducted in the presence of 1 μM ODQ, (1H-[1,2,4]oxadiazolo[4,3-a]-quinoxalin-1-one), a selective inhibitor of soluble guanylyl cyclase, instead of L-NAME. In the above experiments a minimum of 20 min was allowed for the inhibitors to equilibrate with the preparations.

The effect of cumulatively increasing concentrations of the sodium nitroprusside (10 nM–10 μM) on myogenic tone was tested in paired segments in the presence and absence of 2 μM ODQ. Also, the effect of reducing extracellular calcium ions on myogenic tone was determined by replacing the bathing solution with low calcium (0.1 μM) Krebs-Henseleit solution (Daly *et al*., 1990). In this instance, preparations were washed twice with the low calcium (0.1 μM) solution and myogenic tone assessed 10 min later.

Analysis of data

Unless indicated otherwise, the development of myogenic tone and the magnitude of either the neurogenic relaxations or contractions are expressed in gram weight and shown as the mean ± s.e.mean of n observations. In the case of the effect of sodium nitroprusside against myogenic tone, the pIC_{50} (negative logarithm of the concentration causing 50% inhibition) was also calculated. Differences between groups have been compared using ANOVA with Bonferroni's multiple comparison test and considered statistically significant of $P < 0.05$.

Drugs

The composition of the modified Krebs-Henseleit solution was (mM): NaCl 118, KCl 4.8, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25 and glucose 11.1. For experiments involving low Ca^{2+} Krebs-Henseleit solution, a combination of 0.25 mM Ca^{2+} and 0.5 mM EGTA was used to achieve a Ca^{2+} free concentration of 0.1 μM (Daly *et al*., 1990). L-arginine hydrochloride, D-arginine hydrochloride, L-NAME

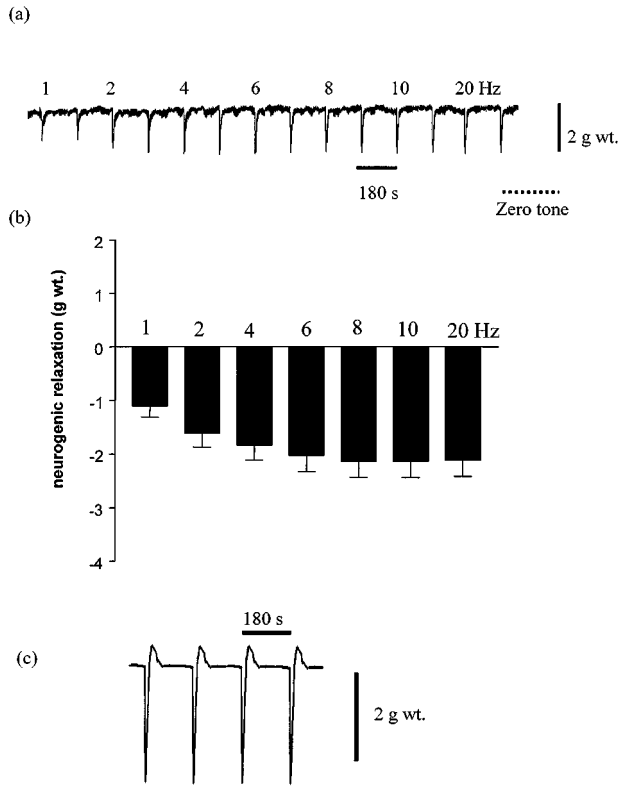

Figure 1 (a) Representative digitized recording of the effect of increasing frequency of stimulation (1–20 Hz for 1 s every 180 s) on the sheep isolated internal anal sphincter. Two responses were obtained at each frequency of stimulation. (b) Histogram of the frequency response relationship for neurogenic relaxations of the sheep isolated internal anal sphincter. Responses are shown as the mean ± s.e.mean of 10 observations. (c) Representative digitized recording showing the occurrence of 'after-contractions' following the initial rapid relaxation on electrical stimulation (10 Hz, 1 s) which was observed in approximately 50% of preparations.

(N^G-nitro-L-arginine methyl ester), atropine sulphate, tetrodotoxin (TTX) were obtained from Sigma-Aldrich (U.K.) and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) was obtained from Tocris (Bristol, U.K.). Sodium nitroprusside (SNP) dihydrate was obtained from David Bull Laboratories (U.K.), while 2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline (RX-811059, Reckitts and Coleman), prazosin HCl (Pfizer) and phentolamine mesylate ('Rogitine', Ciba Geigy) were supplied as gifts. All drugs were dissolved in distilled water and added in a volume less than 0.1% of the organ bath volume (ODQ was made up at concentration of 10 mM in 30% DMSO in water and stored at 4°C).

Results

Following application of 2 g wt initial resting tension, the internal anal sphincter strips slowly developed myogenic tone which stabilized after approximately 30–40 min (3.8 ± 0.2 g, $n=17$) (see Figure 6 for an example of tone development). Electrical field stimulation (EFS) (1–20 Hz, 1 s, every 180 s) caused a transient, frequency-dependent relaxation (Figure 1a,b) of the myogenic tone, with even a single pulse producing 50% of the maximum response. The maximum relaxation response was usually observed following stimulation at 10 Hz (2.3 ± 0.2 g, $n=17$), which was approximately 60% of the developed tone (Figure 1b). At each frequency of stimulation the peak response was obtained with 5 s of stimulation. In approximately 50% of preparations the relaxation to electrical field stimulation was followed by a small 'after contraction' (equivalent to 20% of the inhibitory response), which reached its maximum approximately 20 s after stimulation (Figure 1c). Both components of the response were abolished by 0.3 μM tetrodotoxin ($n=4$). In preliminary experiments we found that increasing the period of stimulation (10–60 s) failed to significantly increase the maximum response to 10 Hz, but was associated with a sustained relaxation equivalent to approximately 40% of the initial response. All further experiments were conducted with a stimulation period of 1 s.

As shown in Figure 2, neither 0.3 μM atropine nor 1 μM phentolamine significantly altered the relaxation produced in response to stimulation at 10 Hz. The addition of L-NAME (100 μM) was associated with a significant increase (33.4 ± 8.8% ($n=17$, $P<0.01$) in myogenic tone, the abolition of neurogenic relaxations and the appearance of a transient contraction (1.10 ± 0.27 g, $n=17$, $P<0.01$) following electrical field stimulation (Figures 2 and 3a). Subsequent addition of L-arginine (1 mM) reversed the effect of L-NAME on responses to electrical field stimulation (10 Hz, 1 s), but the resulting neurogenic relaxations (0.78 ± 0.15 g, $n=4$) were significantly less than those observed in the absence of both agents (Figure 3b). In marked contrast, D-arginine (1 mM) did not significantly alter neurogenic contractions observed in the presence of 100 μM L-NAME (L-NAME: 1.20 ± 0.28 g contractions; L-NAME plus D-arginine: 1.11 ± 0.31 g contractions, $n=4$).

As shown in Figure 3b, the addition of 1 μM phentolamine, following exposure to 100 μM L-NAME and 1 mM L-arginine, failed to significantly alter the neurogenic relaxations. However, in a separate series of experiments we noted that neurogenic relaxations elicited in the presence of 1 μM phentolamine (2.45 ± 0.65 g, $n=4$) were not converted into contractions following exposure to 100 μM L-NAME but significantly reduced by 60.5 ± 5.0% (0.95 ± 0.23 g, $n=4$). In light of these conflicting results, we investigated further the role of α-adrenoceptors in the neurogenic responses. Figure 4 shows that 1 μM RX-811059, a selective α_2-adrenoceptor antagonist (Mallard *et al*., 1992) failed to significantly affect neurogenic contractions observed in the presence of 100 μM L-NAME (1.47 ± 0.18 g, $n=10$), but the subsequent addition of 0.1 μM prazosin, a selective α_1-adrenoceptor antagonist (Massingham *et al*., 1981), abolished the responses and resulted in the re-appearance of small neurogenic relaxations (0.89 ± 0.14 g, $P<0.01$).

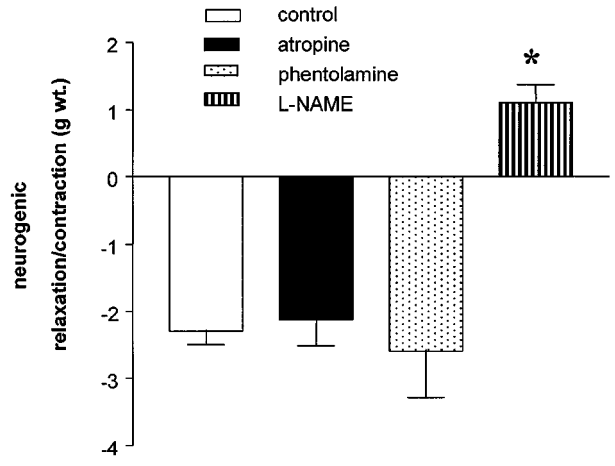

Figure 2 Histogram of the response of the sheep isolated internal anal sphincter to 10 Hz stimulation for 1 s under control conditions ($n=17$) and the presence of 0.3 μM atropine ($n=5$), 1 μM phentolamine ($n=5$) or 100 μM L-NAME ($n=17$). Responses are shown as the mean ± s.e.mean. *Denotes a statistically significant difference from the control value.

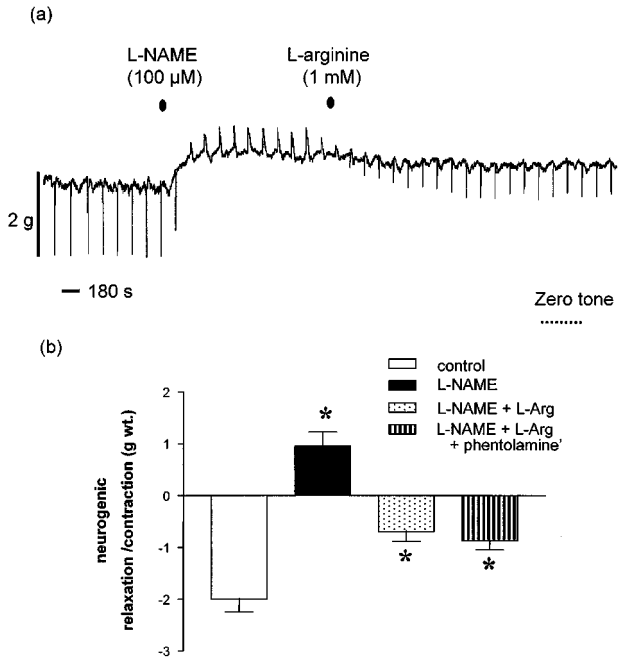

Figure 3 (a) Representative digitized recording of neurogenic responses (10 Hz, 1 s stimulation every 180 s) of the sheep isolated internal anal sphincter under control conditions, in the presence of 100 μM L-NAME and subsequent addition of 1 mM L-arginine. (b) A histogram of the neurogenic responses of the sheep isolated internal anal sphincter under control conditions and sequential addition of 100 μM L-NAME, 1 mM L-arginine and 1 μM phentolamine. The responses are shown as the mean ± s.e.mean of nine observations. *Denotes a statistically significant difference from the control value.

ODQ (1 μM), a selective inhibitor of soluble guanylyl cyclase (Garthwaite *et al*., 1995), abolished neurogenic relaxations (2.3$\pm$0.3 g wt) and uncovered neurogenic contractions (1.17$\pm$0.21 g wt) (Figure 5). As observed with L-NAME, ODQ caused a further increase in the myogenic tone of 12.4$\pm$0.7% (n=7). Whilst L-arginine (1 mM) failed to affect the contractile responses to electrical field stimulation in the presence of ODQ, the subsequent addition of 1 μM phentolamine converted the response to small relaxations (Figure 5).

As shown in Figure 6, sodium nitroprusside (10 nM–10 μM) caused a concentration-dependent inhibition of myogenic tone to zero. Following washout of sodium nitroprusside, myogenic tone was quickly re-established, equivalent to 64.8$\pm$7.7% (n=4) of the pre-sodium nitroprusside value, and electrical stimulation (10 Hz) caused neurogenic relaxations associated with the appearance of a delayed contractile response (0.57$\pm$0.1 g, n=4). As shown in Figure 7, sodium nitroprusside-induced inhibition of myogenic response (pIC$_{50}$ 6.64$\pm$0.05, n=8) was significantly impaired by the presence of 2 μM ODQ (pIC$_{50}$ 5.62$\pm$0.11, n=8). In a separate series of experiments, exposure to low Ca^{2+} Krebs-Henseleit solution reduced the myogenic tone achieved to 87.5$\pm$6.9% of the maximum response produced by sodium nitroprusside, an effect which was reversed by re-exposure to Krebs-Henseleit solution.

Discussion

The results of this study indicate that the isolated internal anal sphincter of the sheep is pharmacologically similar to that found in many species (Lim & Muir, 1985; Tottrup *et al*., 1992; Rattan & Chakder, 1992; Knudsen *et al*., 1995; Cook *et al*., 1999) and, in particular, man (Burleigh & D'Mello 1983; Burleigh, 1992; O'Kelly *et al*., 1993; O'Kelly, 1996). This view is based on several key observations.

First, the primary response of the internal anal sphincter muscle to stretch is the development of myogenic tone that is highly dependent on the presence of extracellular calcium ions and can be abolished by exposure to high concentrations of sodium nitroprusside. Since the effect of sodium nitroprusside was impaired by exposure to ODQ, a selective inhibitor of soluble guanylyl cyclase (Garthwaite *et al*., 1995), cyclic GMP is an important regulator of myogenic tone, as observed in the guinea-pig and opossum anal sphincter (Rattan & Chakder, 1993b). Second, the preparations responded to low frequencies of electrical stimulation by inhibiting myogenic tone through a tetrodotoxin-sensitive mechanism, indicating the involvement of intrinsic nerves. These neurogenic responses were insensitive to both atropine and phentolamine, selective antagonists of muscarinic receptors and α-adrenoceptors, respectively, but abolished by L-NAME, an inhibitor of nitric oxide synthase (Moncada *et al*., 1989). Furthermore, the effect of L-NAME on

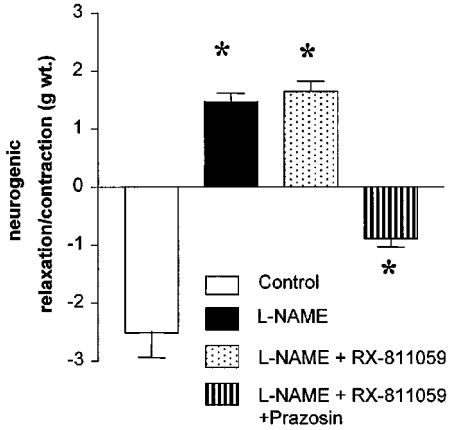

Figure 4 Histogram of the response of the sheep isolated internal anal sphincter to 10 Hz stimulation for 1 s under control conditions and following the sequential addition of 100 μM L-NAME, 1.0 μM RX-811059 and 0.1 μM prazosin (n=10). Responses are shown as the mean$\pm$s.e.mean. *Denotes a statistically significant difference from the control value.

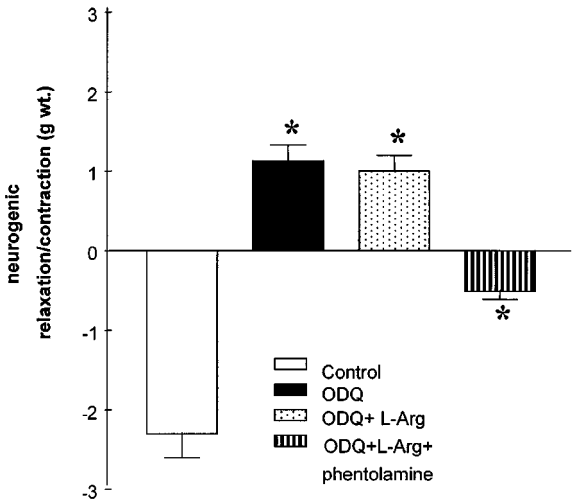

Figure 5 A histogram of the neurogenic responses of the sheep isolated internal anal sphincter under control conditions and subsequent, sequential addition of 1 μM ODQ, 1 mM L-arginine and 1 μM phentolamine. The responses are shown as the mean$\pm$s.e.mean of seven observations. *Denotes a statistically significant difference from the control value.

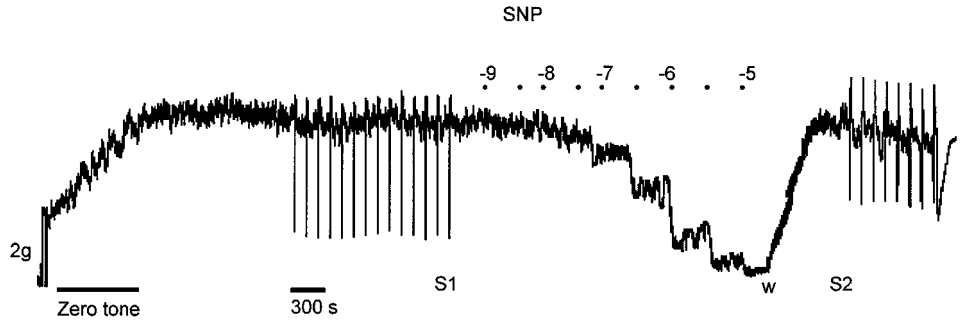

Figure 6 Representative digitized recording of the response of the sheep isolated internal and sphincter to the application of 2 g tension, electrical stimulation (**S1**; 10 Hz, 1 s every 180 s) and subsequent cumulative addition of sodium nitroprusside (10 nM–10 μM). Following washout (w) the preparation was again stimulated electrically (**S2**).

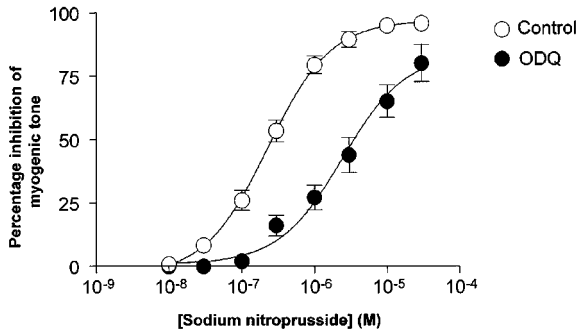

Figure 7 A graph of the effect of cumulative addition of sodium nitroprusside on myogenic tone of the sheep isolated internal anal sphincter under control conditions and in the presence of 2 μM ODQ. The contractile tone has been expressed as a percentage of the contraction prior to the addition of sodium nitroprusside and shown as mean $\pm$ s.e.mean of eight observations.

neurogenic responses was partially reversed by L-arginine, but not by D-arginine, providing further evidence for the involvement of nitric oxide synthase. Third, ODQ produced a qualitatively similar effect to L-NAME on neurogenic relaxations, a finding which further implicates cyclic GMP in the neuronal control of anal sphincter tone (Chakder & Rattan, 1993b). The specificity of the effect of ODQ is underlined by the observation that L-arginine failed to reduce neurogenic contractile response following inhibition of soluble guanylyl cyclase. Finally, both ODQ and L-NAME caused an increase in myogenic tone, suggesting that the intrinsic nerves also release nitric oxide in a spontaneous manner, as has been observed in other isolated gastrointestinal preparations (see: Rand & Li, 1995).

In addition to a role for nitric oxide in regulating anal sphincter smooth muscle tone, we obtained evidence that noradrenaline released from sympathetic nerves may elicit constrictor responses. In this regard, our observations are similar to those reported in man (Burleigh, 1992) and different from that for guinea-pig (Lim & Muir, 1985) and rabbit anal sphincter (Knudsen *et al*., 1995). A small, delayed 'after contraction' was observed in approximately 50% of control preparations and in all preparations following abolition of neurogenic relaxations by either L-NAME or ODQ (see also Burleigh, 1992). It seems likely that the failure to observe similar responses in later studies on human isolated anal sphincter (O'Kelly *et al*., 1993; O'Kelly, 1996) is probably due to the routine use of an adrenergic neurone blocker in the bathing medium. Based on the use of subtype selective α-adrenoceptor antagonists, prazosin and RX-811059, our experiments also revealed that the neurogenic contractions appeared to be mediated by α_1-adrenoceptors. Interestingly, constrictor α_1-adrenoceptors have been identified on human isolated anal sphincter (Burleigh & D'Mello, 1983; Regardas *et al*., 1993) and may contribute to the control of anal sphincter pressure (Pitt *et al*., 1999).

In light of the pharmacological evidence that nitric oxide and noradrenaline exert opposing effects on sheep anal sphincter tone, it is surprising then that phentolamine failed to significantly enhance neurogenic relaxations in either control preparations (Figure 2) or those exposed to a combination of L-NAME and L-arginine (Figure 3). One possible explanation is that nitric oxide modulates the release of noradrenaline from adrenergic nerves in an inhibitory manner and curtails the involvement of sympathetic nerves. However, Rand & Li (1993) have shown that inhibitors of nitric oxide synthase do not increase the release of tritiated noradrenaline from the rat anococcygeus. Alternatively, the markedly different time courses of neurogenic relaxations (approximately 5 s to peak) and the contractions (approximately 20 s to peak) observed in this study, could explain why adrenergic responses exert little effect on the preceding relaxant component. Thus, while inhibition of nitrergic nerves clearly has a profound effect on adrenergic responses in sheep internal anal sphincter, an effect similar to that observed in mouse and rat anococcygeus muscle (Gibson *et al*., 1990; Vila *et al*., 1992), the temporal separation of these responses appears to ensure that the reverse is not true. Interestingly, this explanation can also account for the failure of atropine to modify neurogenic relaxations of the rabbit isolated anal sphincter under control conditions, yet enhance responses in the presence of L-NAME (Knudsen *et al*., 1995). Another factor that may influence the appearance of motor responses to electrical field stimulation is the level of myogenic tone developed by the preparation. Significantly, preparations exposed to sodium nitroprusside and then washed repeatedly failed to re-establish the original myogenic tone. Under these conditions neurogenic responses were more likely to exhibit a biphasic time course, comprising inhibitory and excitatory components.

The involvement of further inhibition transmitters, VIP, PACAP or carbon monoxide, has been suggested for both the rabbit (Knudsen *et al*., 1995) and opossum (Rattan & Chakder, 1993c, Chakder & Rattan, 1998) internal anal sphincter. In both species neurogenic relaxations are not completely inhibited by nitric oxide synthase inhibitors. Similarly, in the present study we noted the reoccurrence of small neurogenic relaxations of the sheep internal anal sphincter following exposure to a combination of phentolamine with either L-NAME or ODQ. A role for carbon monoxide seems unlikely, however, as this putative transmitter elicits relaxations by stimulating guanylyl cyclase and elevating cyclic GMP (Rattan & Chakder, 1993c) and responses in the sheep were detected in the presence of ODQ. Clearly, further studies with putative receptor antagonists for VIP and PACAP receptors appear to be warranted. It should be noted that in this respect the sheep (along with the rabbit and opossum) differs from man since the combination of α-adrenoceptor blockade and inhibition of nitric oxide synthase abolished neurogenic responses (Burleigh, 1992; O'Kelly *et al*., 1993).

In summary we have established a simple model for the study of the internal anal sphincter which possesses many of the pharmacological characteristics of the human internal anal sphincter. This is particularly the case in terms of the principal effects of intrinsic adrenergic and non-adrenergic, non-cholinergic nerves on smooth muscle tone, but also with respect to role of the intracellular messengers cyclic GMP and calcium ions. Since ovine tissue is readily available in large quantities, several complementary approaches, pharmacological, biochemical and anatomical, can be used to develop novel therapeutic strategies for treating anal fissures.

References

ALGATE, D.R. & WATERFALL, J.F. (1978). Action of indoramin at pre- and post-synaptic α-adrenoceptors in pithed rats. *J. Pharm. Pharmacol.,* **30,** 651 – 652.

BURLEIGH, D.E. (1992). N^G-Nitro-L-arginine reduced nonadrenergic, non-cholinergic relaxations of human gut. *Gastroenterology,* **102,** 679 – 683.

BURLEIGH, D.E. & D'MELLO, A. (1983). Neural and pharmacologic factors affecting motility of the internal anal sphincter. *Gastroenterology,* **84,** 409 – 417.

CHAKDER, S. & RATTAN, S. (1998). Involvement of pituitary adenylate-activating peptide in opossum internal anal sphincter relaxation. *Am. J. Physiol.,* **275,** G769 – G777.

COOK, T.A., BRADING, A.F. & MORTENSEN, N.J. (1999). Differences in contractile properties of anorectal smooth muscle and the effects of calcium channel blockers. *Br. J. Surg.,* **86,** 70 – 75.

DALY, C.J., DUNN, W.R., MCGRATH, J.C., MILLER, D.J. & WILSON, V.G. (1990). An examination of the sources of calcium for contractions mediated by postjunctional α_1- and α_2-adrenoceptors in several blood vessels isolated from the rabbit. *Br. J. Pharmacol.,* **99,** 253 – 260.

GARTHWAITE, J., SOUTHAM, E., BOULTON, C.L., NIELSEN, E.B., SCHMIDT, K. & MEYER, B. (1995). Potent and selective inhibition of nitric oxide sensitive guanylyl cyclase by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one. *Mol. Pharmacol.,* **48,** 184 – 188.

GIBSON, A., MIRZAZADEH, S., HOBBS, A.J. & MOORE, P.K. (1990). L-N^G-mono methyl arginine and L-N^G-nitro arginine inhibit non-adrenergic, non-cholinergic relaxation of the mouse anococcygeus muscle. *Br. J. Pharmacol.,* **99,** 602 – 607.

HANCOCK, B.D. (1977). The internal sphincter and anal fissure. *Br. J. Surg.,* **64,** 92 – 95.

KNUDSEN, M.A., GLAVIND, E.B. & TOTTROP, A. (1995). Transmitter interaction in rabbit internal anal sphincter. *Am. J. Physiol.,* **269,** G232 – G239.

LIM, S.P. & MUIR, T.C. (1985). Mechanisms underlying the electrical and mechanical responses of the guinea-pig internal anal sphincter to field stimulation and to drugs. *Br. J. Pharmacol.,* **86,** 427 – 438.

LODER, P.B., KAMM, M.A., NICHOLLS, R.J. & PHILLIPS, R.K.S. (1994). 'Reversible chemical sphincterotomy' by local application of glyceryl trinitrate. *Br. J. Surg.,* **81,** 1386 – 1389.

LUND, J.N. & SCHOLEFIELD, J.H. (1997a). Glyceryl trinitrate is an effective treatment for anal fissure. *Dis. Colon Rectum,* **40,** 468 – 470.

LUND, J.N. & SCHOLEFIELD, J.H. (1997b). A randomised, prospective, double-blind, placebo-controlled trial of glyceryl trinitrate ointment in treatment of anal fissure. *Lancet,* **349,** 11 – 14.

MALLARD, N.J., HUDSON, A.L. & NUTT, D.J. (1992). Characterization and autographical localization of non-adrenoceptor, idazoxan binding site in the rat brain. *Br. J. Pharmacol.,* **106,** 1019 – 1027.

MASSINGHAM, R., DUBOCOVICH, M.L., SHEPPERSON, N.B. & LANGER, S.Z. (1981). *In vivo* selectivity of prazosin but not WB-4101 for postsynaptic α_1-adrenoceptors. *J. Pharmacol. Exp. Ther.,* **217,** 467 – 474.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1989). Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication. *Biochem. Pharmacol.,* **38,** 1709 – 1715.

O'KELLY, T. (1996). Nerves that say NO: a new perspective on the human rectoanal inhibitory reflex. *Ann. Rev. Coll. Surg. Engl.,* **78,** 31 – 38.

O'KELLY, T., BRADING, A. & MORTENSON, N. (1993). Nerve mediated relaxation of the human internal anal sphincter: role of nitric oxide. *Gut,* **34,** 689 – 693.

O'KELLY, T., DAVIES, J.R., BRADING, A. & MORTENSON, N. (1994). Distribution of nitric oxide synthase containing neurons in the rectal myenteric plexus and anal canal. *Dis. Colon Rectum.,* **37,** 350 – 357.

PITT, J., OJO-AROMMOKUDU, O., CRAGGS, M.D. & BOULOS, P.B. (1999). The role of alpha- and beta-adrenoceptors in chronic fissure-in-ano. *Colorectal Disease,* **1,** (Suppl 1): 55.

RAND, M.J. & LI, C.G. (1993). The inhibition of nitric oxide-mediated relaxations in rat aorta and anococcygeus muscle by diphenylene iodonium. *Clin. Exp. Pharmacol. Physiol.,* **20,** 141 – 148.

RAND, M.J. & LI, C.G. (1995). Nitric oxide in the autonomic and enteric nervous system. In *Nitric Oxide in Nervous System.* ed. Vincent, S.R. pp. 228 – 279. London: Accademic Press.

RATTAN, S. & CHAKDER, S. (1992). Role of nitric oxide as a mediator of internal anal sphincter relaxation. *Am. J. Physiol.,* **262,** G107 – G112.

RATTAN, S. & CHAKDER, S. (1993a). Release of nitric oxide by activation of nonadrenergic noncholinergic neurons of internal anal sphincter. *Am. J. Physiol.,* **264,** G7 – G12.

RATTAN, S. & CHAKDER, S. (1993b). Involvement of cAMP and cGMP in relaxation of internal anal sphincter by neural stimulation, VIP and NO. *Am. J. Physiol.,* **264,** G702 – G707.

RATTAN, S. & CHAKDER, S. (1993c). Inhibitory effect of CO on internal anal sphincter: Heme oxygenase inhibitor inhibits NANC relaxation. *Am. J. Physiol.,* **265,** G799 – G804.

RATTAN, S., ROSENTHAL, J. & CHAKDER, S. (1995). Human recombinant haemoglobin (rHb 1.1) inhibits nonadrenergic noncholinergic (NANC) nerve-mediated relaxation of internal anal sphincter. *J. Pharmacol. Exp. Ther.,* **272,** 1211 – 1216.

REGARDAS, F.S.P., BATISTA, L.K., ALBUQUERQUE, J.L.A. & CAPAZ, F.R. (1993). Pharmacological study of the internal anal sphincter in patients with chronic anal fissue. *Br. J. Surg.,* **80,** 799 – 801.

TOTTRUP, A., GLAVIND, E.B. & SVANE, D. (1992). Role of nitric oxide as a mediator of internal anal sphincter relaxation. *Am. J. Physiol.,* **262,** (G107 – G112).

VILA, E., TABERNERO, A., FERNANDES, F. & SALAICES, M. (1992). Effects of neuropeptide Y on adrenergic and non-adrenergic, non-cholinergic responses in the rat anococcygeus muscle. *Br. J. Pharmacol.,* **107,** 66 – 72.

(Received July 3, 1999
Revised December 6, 1999
Accepted February 25, 2000)