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In vitro response of the human anal canal longitudinal muscle layer to cholinergic and adrenergic stimulation: evidence of sphincter specialization

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A study was performed to determine the in vitro *response of the longitudinal smooth muscle layer (conjoined longitudinal coat) of the human anal canal to cholinergic and adrenergic stimulation, and to compare this with similar features of the internal anal sphincter (IAS) and rectal smooth muscle. Tissue was obtained from abdominoperineal and anterior resection specimens, and strips of muscle mounted for isometric tension recording in superfusion organ baths. Both conjoined longitudinal coat and IAS strips generated and maintained spontaneous myogenic tension (mean(s.e.m.) 0·10(0·01) and 0·48(0·04) g per mg tissue respectively), whereas equivalent rectal smooth muscle did not. Stimulation of muscarinic (cholinergic) receptors caused contraction of strips from the conjoined longitudinal coat and rectal smooth muscle layers in a dose-dependent manner (10^{-7}–10^{-4} mol/l); however, IAS strips relaxed (10^{-6}–10^{-4} mol/l). Activation of α-adrenergic receptors in conjoined longitudinal coat and IAS strips produced concentration-dependent contraction (10^{-7}–10^{-4} mol/l). In comparison, rectal smooth muscle relaxed. All muscle layers relaxed in response to β-adrenoceptor stimulation. These data indicate that in the anal canal both the conjoined longitudinal coat and IAS are specialized sphincteric smooth muscles.*

There are two predominant smooth muscle structures within the anal canal: the internal anal sphincter (IAS) and the conjoined longitudinal coat. The anatomy of these structures has been described previously and is well reviewed[1,2]. The IAS is the terminal part of the circular smooth layer of the gastrointestinal tract and consists of fascicles of smooth muscle fibres ensheathed in connective tissue. The conjoined longitudinal coat, on the other hand, is the most caudal extension of the longitudinal muscle layer of the gut and consists of much less smooth muscle than the internal sphincter. At the pelvic floor, the conjoined longitudinal coat is joined by some (scant) striated muscle fibres from the levator ani (puboccoccygeus) and then traverses the anal canal between the internal and external sphincters. As it descends, it becomes progressively fibroelastic and gives off several septa which ramify through both the IAS and the lowermost part of the external sphincter, to gain attachment to the dermis of the anal and circumanal skin. In addition, other septa pass outward, away from the anal canal, to blend with the connective tissue of the ischiorectal fossa and perianal space.

Much is known about the activity of the IAS. It is recognized that it plays an important role in anorectal continence, being principally responsible for the generation and maintenance of resting anal canal pressure[3,4]. The IAS contracts spontaneously, developing a high degree of myogenic tone, and relaxes to allow the passage of stool, to permit anorectal sampling and in response to rectal distension. The conjoined longitudinal coat can now be imaged by endoanal ultrasonography and is seen during intersphincteric dissection of the anal canal but, compared with the IAS, little is known about its behaviour. Its true function is unknown; indeed, as far as the authors are aware, the activity of the human conjoined longitudinal coat has not been examined previously.

This paper describes studies in which aspects of the behaviour of the two smooth muscle layers of the anal canal were compared *in vitro*. In particular, their spontaneous activities and how these are modified by cholinergic and adrenergic drugs were examined. These agents were chosen to mimic the actions of important neurotransmitters in the gastrointestinal tract that are released from intrinsic enteric neurones as well as from extrinsic nerves of the sympathetic and parasympathetic nervous systems. To characterize the behaviour of anal canal smooth muscle further, similar experiments were also carried out on the equivalent muscle layers of the rectum.

Patients and methods

Sphincter tissue was obtained from patients undergoing abdominoperineal resection of the rectum and anal canal for low rectal carcinoma (three men, four women; median age 65 (range 62–75) years). Rectal smooth muscle was derived from this source and from patients with more proximal rectal tumours undergoing low anterior resection with coloanal anastomosis (four men; median age 63 (range 60–65) years). To maintain consistency in tissue collection, internal sphincter and conjoined longitudinal coat smooth muscle was obtained from adjacent sites in the anal canal, the most cranial part of which was defined by the attachment of the striated muscle of the pelvic floor. Rectal circular and longitudinal smooth muscle was acquired from the 'body' of the rectum, which was assumed to be midway along the craniocaudal axis of the resection specimen.

Once suitable tissue had been selected, the epithelium and submucosa were removed with the aid of a dissecting microscope. Adjacent strips containing parallel smooth muscle bundles were then excised, each measuring approximately 1 × 1 × 10 mm and weighing

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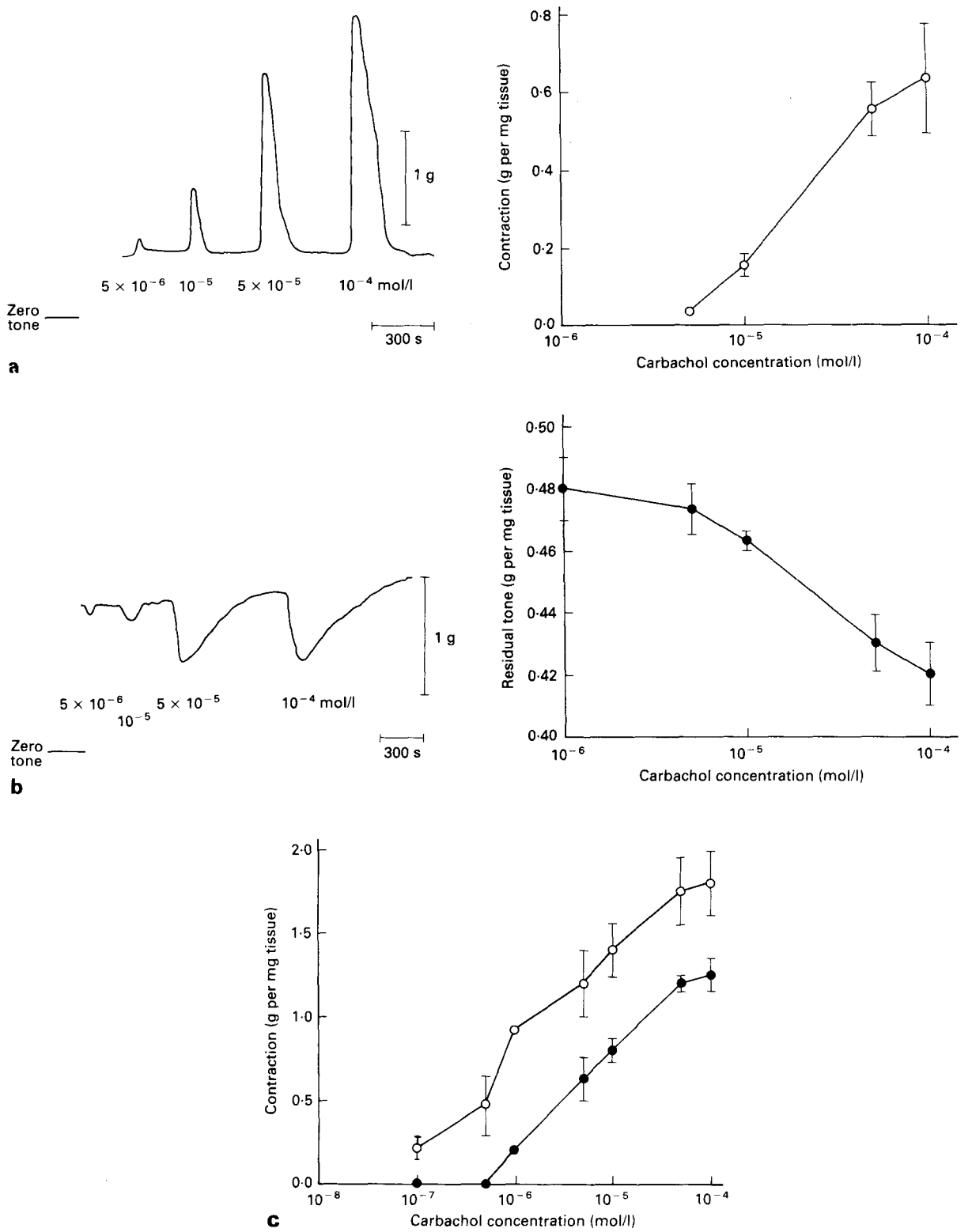

Figure 1 **a** *Response of smooth muscle strips from the conjoined longitudinal coat to increasing concentrations of carbachol, illustrated by a characteristic trace and a dose–response curve. Each point represents the mean(s.e.m.) of all strips tested* (n = *18(3)*). *Zero tone in this and subsequent figures represents the tension present in calcium-free solution.* **b** *Response of smooth muscle strips from the internal anal sphincter to increasing concentrations of carbachol, illustrated by a characteristic trace and a dose–response curve* (n = *30(6); values are mean(s.e.m.)*). *Residual tone is the tension remaining in the strips after application of the drug.* **c** *Responses of longitudinal* (○) *and circular* (●) *smooth muscle strips from the rectum to increasing concentrations of carbachol* (n = *18(4); values are mean(s.e.m.)*)

5–8 mg. Threads were tied at each end of the strips, which were mounted for isometric tension recording in superfusion organ baths (capacity 0·2 ml). The strips were continuously superfused with Krebs solution (37°C) at a rate of 1 ml/min. Drugs and other agents being investigated were added to the Krebs solution before it was placed in the bath. The apparatus allowed six strips to be studied simultaneously. Tension was measured using Pioden transducers (Pioden Controls, Canterbury, UK) and recorded on a six-channel Teckman pen recorder (Teckman Electronics, Leamington Spa, UK). The strips were placed initially under 1·0 g resting tension and allowed to equilibrate for at least 1 h before the start of experiments. For calculation of spontaneous and stimulated tension, the tension present in strips held in calcium-free solution was regarded as the baseline (zero) and was subtracted from the observed tension.

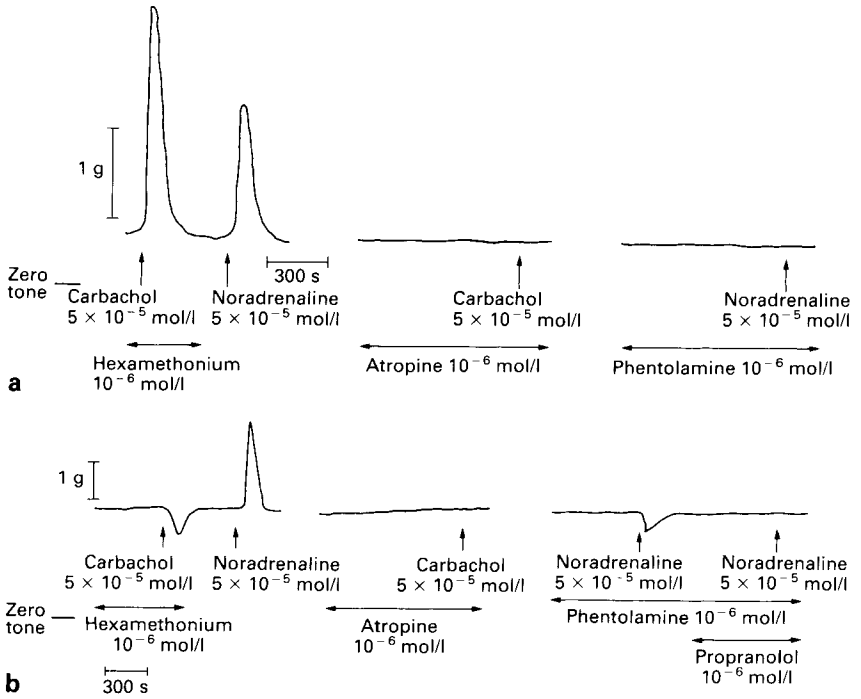

Figure 2 *Analysis of the actions of carbachol and noradrenaline on an isolated strip of human* **a** *conjoined longitudinal coat and* **b** *internal anal sphincter. Similar responses were seen in all strips tested* (n = *12(2)*)

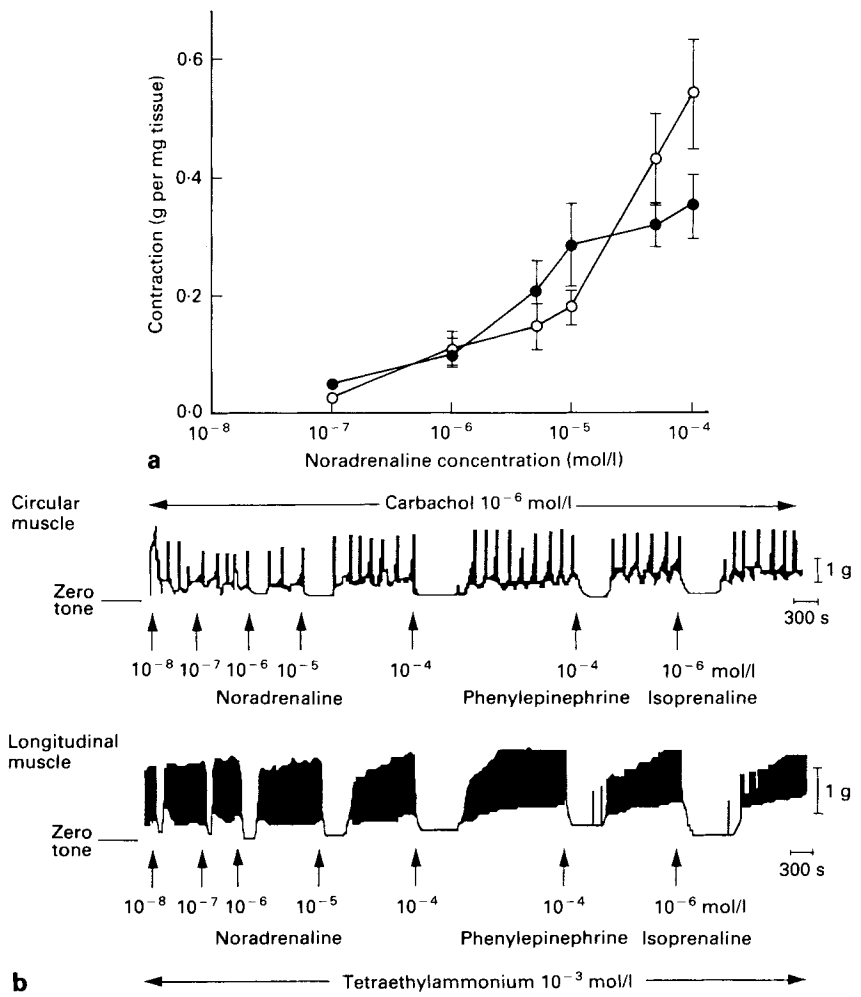

Figure 3 **a** *Response of the conjoined longitudinal coat* (n = *18(5)*; ○) *and internal anal sphincter* (n = *18(3)*; ●) *to increasing concentrations of noradrenaline. Values are mean(s.e.m.).* **b** *Response of the circular and longitudinal muscle layers of the rectum to increasing doses of noradrenaline, phenylephrine (an α-adrenoceptor agonist) and isoprenaline (a β-adrenoceptor agonist). Carbachol and tetraethylammonium were applied to increase basal tone and stimulate spontaneous activity, so revealing the inhibitory actions of the sympathomimetic agents. Similar responses were seen in all 12(2) preparations studied*

Standard Krebs solution was equilibrated with 97 per cent oxygen–3 per cent carbon dioxide. Drugs and agents used were: carbamylcholine chloride (carbachol); hexamethonium bromide; isoprenaline sulphate; phentolamine hydrochloride; phenylephrine hydrochloride; atropine sulphate; tetraethylammonium chloride; noradrenaline; propranolol hydrochloride; and tetrodotoxin.

Experimental results are expressed as mean(s.e.m.) and, where appropriate, statistical differences assessed with Student's unpaired t test. $P < 0{\cdot}05$ was considered significant. Numbers (n) are given in the form $x(y)$, where x is the number of strips and y the number of patients.

Results

Spontaneous tension

During the period of equilibration, muscle strips taken from the IAS contracted spontaneously and developed a mean(s.e.m.) steady-state tension of 0·48(0·04) g per mg tissue ($n = 30(6)$). By comparison, those from the conjoined longitudinal coat relaxed spontaneously and maintained a mean(s.e.m.) steady-state tension of only 0·10(0·01) g/mg ($n = 24(5)$). This difference was highly significant ($P < 0{\cdot}001$). In neither case did the nerve toxin tetrodotoxin (3×10^{-6} mol/l) reduce spontaneous tension, confirming that the effect was myogenic in origin. Both layers of smooth muscle from the rectum ($n = 24(4)$) completely relaxed in the organ baths and had no tension once the steady state was achieved (equal to that present in calcium-free solution).

Responses to cholinergic agonist

Carbachol is a cholinergic agonist; *Figure 1* shows the response of the various muscle strips to a 10-s application of this agent. Samples from the conjoined longitudinal coat ($n = 18(3)$) and both layers of the rectum ($n = 18(4)$) contracted in a dose-dependent manner on addition of carbachol, whereas those from the IAS relaxed ($n = 30(6)$). The responses were not modified by hexamethonium (10^{-6} mol/l) ($n = 12(2)$), a ganglionic (nicotinic) cholinergic receptor antagonist, but they were antagonized by atropine (10^{-6} mol/l) ($n = 12(2)$), which blocks the action of cholinergic agents at muscarinic (cholinergic) receptors (*Figure 2*).

Responses to adrenergic agonist

Noradrenaline is the principal neurotransmitter released by postganglionic sympathetic nerves. Its application to muscle strips from the conjoined longitudinal coat ($n = 18(5)$) and internal sphincter ($n = 18(3)$) for 20 s caused dose-dependent contractions (*Figure 3a*). In the presence of phentolamine, an α-adrenoceptor antagonist, the response of conjoined longitudinal coat strips to 5×10^{-5} mol/l noradrenaline was abolished ($n = 12(2)$); however, in the case of IAS strips, relaxation was revealed ($n = 12(2)$) (*Figure 2*). This modified internal sphincter response was abolished on addition of propranolol, a β-adrenoceptor antagonist ($n = 6(2)$). Both IAS and conjoined longitudinal coat strips relaxed in response to application of isoprenaline, a β-adrenergic agonist (10^{-9}–10^{-6} mol/l).

In their relaxed state, strips from neither layer of rectal smooth muscle responded to noradrenaline ($n = 18(3)$). If, however, tension and activity were raised by the addition of carbachol to circular strips ($n = 12(2)$) and tetraethylammonium to longitudinal strips ($n = 12(2)$), then noradrenaline was seen to have an inhibitory effect on both the frequency and amplitude of the resulting contractions. This inhibition could be reproduced by stimulation of both α- and β-adrenoceptors with phenylephrine or isoprenaline respectively ($n = 12(2)$ for each layer; *Figure 3b*).

These results are summarized in *Table 1*.

Table 1 *Summary of results*

	Tone	Agonist effect	
		Cholinergic	α-Adrenergic
Internal anal sphincter	High	Relaxation	Contraction
Conjoined longitudinal coat	Low	Contraction	Contraction
Rectal smooth muscle	Absent	Contraction	Relaxation

Discussion

The importance of the smooth muscle components of the anal canal is becoming increasingly apparent[5] and a greater understanding of their properties will provide new insight into the mechanisms of continence and defaecation, and how they are altered to produce clinical syndromes such as anorectal incontinence. Such knowledge might also suggest novel ways in which sphincter activity can be altered pharmacologically.

Some aspects of human conjoined longitudinal coat and IAS muscle behaviour are similar. First, isolated strips from both layers generate and maintain spontaneous myogenic tension, although this is much less in the conjoined longitudinal coat. The mechanism responsible for this has been explored by Bouvier and Gonella[6]. Using the sucrose gap technique, they examined tension and electrical activity in circular and longitudinal muscle taken from the anal sphincter area of the cat. In the former they noted slow variations in smooth muscle membrane potential called slow waves. In longitudinal muscle, spike potentials were superimposed on these. Each slow wave (internal sphincter) or spike potential (conjoined longitudinal coat) was associated with an increase in basal tension. Both slow waves and tension disappeared in calcium-free solution and in the presence of manganese ions, indicating that electromechanical coupling was calcium dependent. Research is required to confirm whether human anal canal smooth muscle behaves in a similar fashion and also to elucidate further the processes responsible for spontaneous tone. This area is likely to be fertile ground for the pharmacological manipulation of sphincter activity in the future.

The conjoined longitudinal coat and IAS are also similar in their response to exogenous adrenergic agonists. Both contract when α-adrenoceptors are stimulated, but relax when β-adrenoceptors are stimulated. This behaviour is characteristic of sphincter-specialized gastrointestinal smooth muscle[7]. Non-sphincteric gastrointestinal smooth muscles, as evidenced by the response of both layers of rectum, relax when either of these receptors is stimulated[7]. The differences between responses of the circular smooth muscles of the anal canal and rectum have been described previously in the cat[8] and vervet monkey[9]. The mechanisms that underlie these differences in activity were not determined in the present study.

In its response to exogenous cholinergic agonists, the conjoined longitudinal coat contracts and so behaves like both layers of the rectum and smooth muscle elsewhere in the gastrointestinal tract[7]. The dose-dependent relaxation of the internal sphincter to muscarinic receptor activation is therefore outstanding, and currently not explained. It has been noted previously[10] and could result from a direct cholinergic effect on IAS smooth muscle. It is also conceivable, however, that cholinergic stimulation releases another agent that produces the ultimate mechanical response. Similar relaxations are seen in some vascular smooth muscle preparations, where they are thought to be mediated by the release of nitric oxide from endothelial cells[11,12]. Nitric oxide is a recently discovered endogenous bioactive substance with many functions[13–15], including that of an inhibitory neurotransmitter within the gastrointestinal tract[16]. As intrinsic nerves and small blood vessels are present in the muscle strips used in these studies, it is possible that nitric oxide mediates IAS relaxation in response to cholinoceptor stimulation. Further research is required to explore this phenomenon as it clearly has an important bearing on understanding of how enteric and parasympathetic cholinergic nerves interact with the internal sphincter.

Caution is required when interpreting the findings of *in vitro* experiments. However, on the basis of the present results, we believe that the conjoined longitudinal coat, like the IAS, shows

true sphincter specialization both in its ability to generate spontaneous tension and in its pharmacological behaviour. No firm conclusions can be made about the function of the conjoined longitudinal coat, but the present findings are consistent with previous suggestions that it helps to maintain the structural integrity of the anal canal throughout the defaecation cycle, and contracts during the passage of faeces to shorten the anal canal and evert the anal margin[17,18]. *In vivo* as well as further *in vitro* experiments are required to confirm this suggestion and to explore the actions of the conjoined longitudinal coat more fully.

Acknowledgements

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