

## INHIBITORY EFFECT OF THE MUCOSA ON CONTRACTILE RESPONSES OF THE INTERNAL ANAL SPHINCTER

### Hypothesis / aims of study

Faecal incontinence affects over 18 million adults in the US and has a huge impact on the quality of life of patients. The internal anal sphincter smooth muscle is central in maintaining faecal continence, contributing up to 85% to the anal resting pressure (1). Thus, understandably, it is a potential target for drugs to treat faecal incontinence. In the lower urinary tract, the lining of the bladder, the urothelium/lamina propria, releases a number of factors thought to be involved in bladder control. Specifically of interest, the urothelium/lamina propria releases a factor which inhibits contraction of the smooth muscle (2, 3). The aim of this study was to examine whether a similar mechanism operates to inhibit contractility of the smooth muscle of the internal anal sphincter.

### Study design, materials and methods

Strips of internal anal sphincter were prepared from fresh porcine anal tissue. Half the strips were left intact, whilst the other half was carefully denuded of mucosa. Strips were then mounted in tissue baths at 37°C in Krebs-bicarbonate solution. Contractile responses to the  $\alpha_1$ -adrenoceptor agonist phenylephrine were obtained, in the absence and presence of suramin (P2-receptor inhibitor, 100 $\mu$ M), zinc protoporphyrin IX (heme oxygenase inhibitor, inhibitor of carbon monoxide production, 10 $\mu$ M), ODQ (guanylyl cyclase inhibitor, 10 $\mu$ M) and LNNA (nitric oxide synthase inhibitor, 100 $\mu$ M). All data were expressed as mean  $\pm$  SEM. Data was compared via Student's t test, with  $P < 0.05$  was considered significant.

### Results

Intact strips of internal anal sphincter showed significantly lower maximum contractile responses to phenylephrine than strips denuded of mucosa (Figure 1,  $P < 0.001$ ). The mean percentage inhibition of contraction caused by the mucosa was  $65.1 \pm 10.8\%$ . The potency of phenylephrine was unaffected by removal of the mucosa ( $pEC_{50}$   $5.06 \pm 0.11$  and  $5.10 \pm 0.05$ , intact vs denuded respectively). Inhibition of purinergic P2 receptors and guanylyl cyclase, as well as inhibition of the production of NO and CO, did not significantly affect the inhibitory effect of the mucosa on contractile responses to phenylephrine (Figure 2). Responses remained significantly ( $P < 0.001$ ) depressed in intact tissues in the absence and presence of suramin ( $76.3 \pm 8.0\%$  vs  $86.3 \pm 6.4\%$ ), ODQ ( $50.3 \pm 12.8\%$  vs  $41.4 \pm 8.9\%$ ), and Zn PPIX ( $82.3 \pm 5.7\%$  vs  $69.5 \pm 10.5\%$ ).

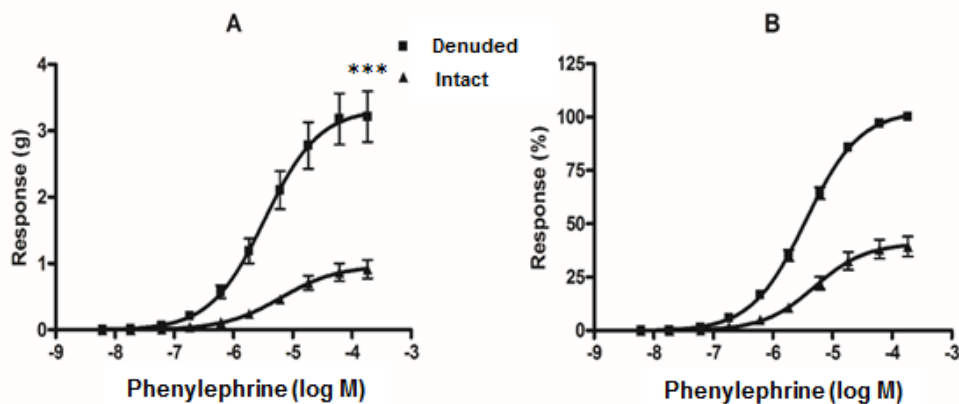


Figure 1. Effect of removal of mucosa on phenylephrine induced contraction of the internal anal sphincter. Data are mean  $\pm$  SEM ( $n=17$ ). Responses are expressed in g (A) and as a percentage of the maximum response of denuded tissues (B). \*\*\* $P < 0.001$  vs intact tissues

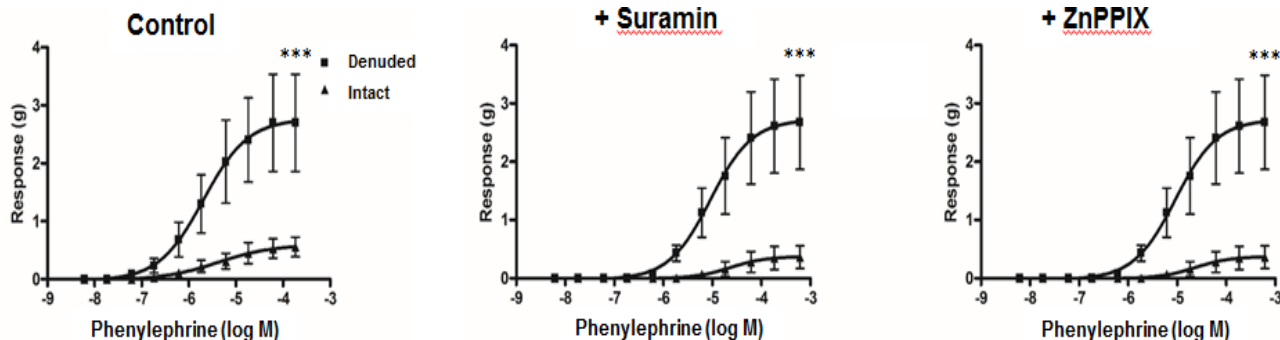


Figure 2. Effect of suramin and zinc protoporphyrin IX on phenylephrine responses of intact and denuded strips of the internal anal sphincter. Data are mean  $\pm$  SEM ( $n=6$ ). \*\*\* $P < 0.001$  vs intact tissues

### Interpretation of results

Contractile responses of the smooth muscle of the internal anal sphincter to the  $\alpha_1$ -adrenoceptor agonist phenylephrine are greater in tissues that have been denuded of their mucosa, compared to those of intact tissues. The degree of inhibition of contraction was approximately 65%, which is greater than the inhibition of bladder contractions by the urothelium, around 55% (2, 3). The inhibitory effect could not be prevented by blockade of purinergic P2 receptors or guanylyl cyclase, or by inhibiting the production of nitric oxide and carbon monoxide.

### Concluding message

This study shows for the first time an inhibitory effect of the mucosa on contractile responses of the internal anal sphincter. This inhibition does not involve ATP acting via purinergic P2 receptors and is not nitric oxide or carbon monoxide. Whilst the precise mechanism of mucosal inhibition remains to be elucidated, it may provide a novel target for development of drugs to increase tone in the internal anal sphincter and thus treat faecal incontinence.

### References

1. Lestar et al. (1989) Int J Colorectal Dis 4(2):118-22
2. Hawthorn et al. (2000) Br J Pharmacol 129(3):416-9
3. Templeman et al. (2002) J Urol 167(2 Pt 1), 742-745

### Disclosures

**Funding:** Bond University **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Pig **Ethics not Req'd:** No ethical approval needed - abattoir tissue