417

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THE INFLUENCE OF THE EPITHELIUM ON CONTRACTILE RESPONSES OF THE INTERNAL ANAL SPHINCTER

Hypothesis / aims of study

Studies have shown that the presence of an epithelial layer has an inhibitory influence on underlying smooth muscle in blood vessels and the bladder. The mechanism of this inhibition may involve nitric oxide (NO) or prostaglandin release but unidentified other factors may also be involved. The aim of the present study is to determine whether the presence of an epithelium in the IAS has a similar inhibitory effect and to investigate the mechanisms involved.

Study design, materials and methods

Paired circular muscle strips were taken from porcine IAS, one strip with the epithelium intact and the other strip denuded. Tissues were mounted in 30ml tissue baths containing Krebs bicarbonate solution and gassed with 95% O_2 and 5% CO_2. Resting tension on the tissue was set at 1g and the tissues allowed to stabilize for 30 minutes. Tissues were then incubated with L-NNA (100nM), indomethacin (5μM), ODQ (10μM) or [D-*p*-Cl-Phe^6,Leu^{17}]-VIP (100nM) for a further 30 minutes. A cumulative concentration-response curve to noradrenaline was then obtained for each pair of tissues, in either the absence of any drug as a control, or in the presence of one of the drugs. Only one concentration-response curve was obtained on each tissue.

Results

Table 1. Potency (pEC_{50})and maximum responses for noradrenaline in the absence and presence of inhibitors

	Epithelium Present		**Epithelium Absent**	
Drug	**pEC_{50}**	**Maximum (g)**	**pEC_{50}**	**Maximum (g)**
Control	5.88 ± 2.57	1.10 ± 0.66*	6.09 ± 1.45	5.50 ± 1.53
Indomethacin	5.71 ± 1.46	1.63 ± 0.66*	5.64 ± 2.40	4.69 ± 1.33
L-NNA	7.35 ± 13.88	1.33 ± 0.29*	5.90 ± 0.73	4.93 ± 1.56
ODQ	4.01 ± 11.84	1.32 ± 0.27*	5.29 ± 0.68	4.71 ± 0.53
[D-*p*-Cl-Phe^6,Leu^{17}]-VIP	5.70 ± 1.24	3.58 ± 0.72*†	5.24 ± 0.82	9.43 ± 1.85

*p<0.05 compared with epithelium-denuded strips
†p<0.05 compared with control tissue (absence of any inhibitor/antagonist)

The presence of an epithelial layer significantly reduced the maximum contractions of the IAS in controls. The addition of L-NNA, indomethacin and ODQ had no significant effect on maximum contraction in either the absence or presence of epithelium. [D-*p*-Cl-Phe^6,Leu^{17}]-VIP had no significant effect in the absence of epithelium compared to controls, but a significant increase in maximum contraction was found when the epithelium was present.

Interpretation of results

The epithelial layer of the IAS appears to have an inhibitory influence on the underlying smooth muscle. L-NNA, a NO-synthase inhibitor, did not reduce the inhibitory effect, indicating that any inhibitory factor is unlikely to be NO. This conclusion is supported by ODQ, an inhibitor of guanylate cyclase, which also rules out carbon monoxide (CO) as a source of the inhibition since CO also acts via cGMP. Prostaglandins are unlikely to be involved, as indomethacin, an inhibitor of prostaglandin synthesis, also failed to reduce the inhibitory effect of the epithelium. The VIP-antagonist [D-*p*-Cl-Phe^6,Leu^{17}]-VIP partially reduced the inhibitory effect of the epithelium, suggesting that the release of VIP may contribute to this inhibitory effect.

Concluding message

The presence of the epithelial layer of the internal anal sphincter has an inhibitory effect on the underlying smooth muscle. This effect is unlikely to involve NO, CO or prostaglandins, but appears to be mediated, at least in part by the release of VIP from the epithelium, which inhibits contraction of the underlying smooth muscle. However, the main mechanism of inhibition remains undetermined.

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