Effects of α - and β -calcitonin gene-related peptides upon ion transport in rat descending colon

¹H.M. Cox, J.A. Ferrar & A.W. Cuthbert

Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ

Both α - and β -calcitonin gene-related peptide (CGRP) exhibit concentration-dependent dual actions upon rat colonic epithelia. At low concentrations (i.e. 0.03–10 nM) both CGRPs produce antisecretory effects that are abolished by pretreating tissues with tetrodotoxin (TTX). At higher concentrations (30–200 nM) secretory responses predominate and these effects are not abolished by TTX. It is suggested that the mediator of the antisecretory actions of CGRPs is somatostatin.

Introduction Two calcitonin gene-related peptides (CGRPs) exist in the enteric nervous system (Mulder et al., 1988). The first was described in rat brain (Rosenfeld et al., 1983) and designated α -CGRP while the second, β -CGRP which differs from α -CGRP by only one of its 37 amino acids was predicted following the discovery of a novel mRNA from rat thyroid carcinoma (Amara et al., 1985). The rat gene coding for α -CGRP also encodes the precursor of the calcium-regulating hormone, calcitonin (Rosenfeld et al., 1983). In contrast there is no evidence for a second mature transcript from the gene encoding β -CGRP (Amara et al., 1985). Both peptides appear to be coexpressed in the CNS, while in the enteric nervous system it is α -CGRP which coexists with substance P (SP) in sensory neurones (Ekblad et al., 1988; Mulder et al., 1988) and β -CGRP is thought to be located in intrinsic submucous and myenteric neurones (Mulder et al., 1988).

Detailed investigations of CGRP containing neurones in the rat colon (Ekblad et al., 1988) have identified several populations of CGRP containing nerves. Some perivascular CGRP fibres contain SP and are extrinsic in origin and probably sensory, whilst other perivascular fibres contain both CGRP and vasoactive intestinal peptide. Another more extensive CGRP-containing population of neurones is present in both intramural ganglia, the cell bodies present in the submucous plexi projecting varicose fibres towards the mucosa. These cells do not appear to contain any other neuropeptides. In contrast a

further subpopulation of CGRP positive cells present only in the submucous plexi also contain somatostatin (SOM) and these neurones issue fibres which are located in the submucosa and around the basal areas of crypts. The presence of CGRP containing nerves so close to the mucosa is suggestive of a role for this peptide in the regulation of ion transport. Rat colon epithelium responds to a variety of endogenous substances which cause either anion secretion and increase the short circuit current Y (SCC) e.g. kinins (Cuthbert & Margolius, 1982) or they exhibit antisecretory effects resulting in a fall in SCC, e.g. neuropeptide Y (NPY) (Cox et al., 1988). This study describes the effects of both α - and β -CGRP upon rat colonic epithelial ion transport.

Methods Preparations of descending colon were removed from male Sprague-Dawley rats (200–250 g) and the overlying smooth muscle layers were removed (together with the accompanying myenteric plexi). Mucosal sheets were placed between two halves of perspex Ussing chambers with a window size of 0.6 cm² and bathed in oxygenated Krebs-Henseleit solution at 37°C as described previously (Cox et al., 1988). Four adjacent preparations of colon mucosa were routinely obtained, all exhibiting similar basal SCC when automatically voltage clamped (WP Instruments, New Haven, CT, U.S.A.). The resultant SCC was continuously recorded. EC₅₀ values are expressed as geometric means with 95% confidence limits.

Both α - and β -CGRP were purchased from Peninsula Laboratories Europe Ltd (St Helens, Merseyside). Tetrodotoxin (TTX) was obtained from Sigma (Poole).

Results Complex concentration-response profiles were obtained following addition of either α - or β -CGRP to the basolateral bathing fluid of untreated colonic preparations. Between concentrations of 0.03 nM and 10 nM α - and β -CGRP, reductions in SCC were recorded (Figure 1a) from

¹ Author for correspondence.

the original stable baseline. Addition of higher concentrations (30–200 nM) resulted in increases in SCC, reversing the effects of the lower doses of both peptides. Following application of β -CGRP at the highest concentration, the elevated SCC returned, not to the original baseline but to the lower SCC achieved after 10 nM β -CGRP. Similar profiles were obtained with α -CGRP (data not shown). Application of 100 nM TTX to colon preparations reduced the baseline SCC by $-12.0 \pm 3.2 \mu\text{A}/0.6 \text{ cm}^2$ ($n = 6$, from $20.5 \pm 4.1 \mu\text{A}/0.6 \text{ cm}^2$ to $8.5 \pm 1.1 \mu\text{A}/0.6 \text{ cm}^2$). Subsequent addition of either α - or β -CGRP (using the same concentration range) resulted in a loss of the antisecretory effects previously observed with low concentrations (Figure 1b) and only the secretory responses remained. These increases in SCC were observed with threshold concentrations of 1 nM for α -CGRP and 3 nM for β -CGRP. EC_{50} values of 13.9 nM (10.7, 18.2; $n = 3$) and 65.0 nM (25.6, 165.3; $n = 3$) were obtained for α - and β -CGRP respectively in TTX pretreated tissues. Taking the antisecretory responses from control tissues in isolation, threshold concentrations were 0.1 nM for both α - and β -CGRP. EC_{50} values of 0.3 nM (0.1, 1.1; $n = 3$) and 2.0 nM (0.4, 10.9; $n = 3$) were obtained for α - and β -CGRP respectively. It does appear that α -CGRP is the more potent secretory and antisecretory agent of these two peptides, although the antisecretory effects of β -CGRP were greater.

Discussion Both α - and β -CGRP produce biphasic effects upon colonic epithelial ion transport. At low concentrations, the antisecretory effects were found to be sensitive to TTX while at higher concentrations, secretory responses were observed which were insensitive to TTX. The antisecretory responses could be mediated by one or more of three known antisecretory agents (all of which are located in neurones innervating the mucosa), either noradrenaline, NPY or SOM. The last of these three is perhaps the most likely candidate as it is by far the most potent antisecretory agent in the rat colon. In addition, preliminary data indicate that preincubation of tissues with 30 nM SOM results in loss of the antisecretory effects of CGRPs, while in the presence of 50 μM yohimbine these effects are still observed. Interestingly in two other gastrointestinal systems, CGRP has been found to stimulate SOM release, namely rat stomach (Yamatani *et al.*, 1986) and canine stomach and intestine (Dunning & Taborsky, 1987). The TTX sensitivity of low-concentration CGRP responses in the rat colon, therefore, indicate the presence of CGRP receptors on submucous neurones, which are retained in our preparations. If SOM is the antisecretory mediator, then it is important to note that the only SOM-containing neurones innervating the

Figure 1 The effect of α -calcitonin gene-related peptide (α -CGRP) (○) and β -CGRP (●) upon electrogenic ion transport in mucosal preparations of rat colon. (a) A representative record of changes in SCC following application of increasing concentrations of β -CGRP (added to basolateral surface only) in the absence of tetrodotoxin (TTX). Note that the direction of changes in SCC is reversed between 10 and 30 nM β -CGRP. (b) Pooled data from 3–8 separate experiments showing the differences in concentration-response curves to α - and β -CGRP either in the presence or absence of TTX. Peak changes in SCC from the original baseline with or without TTX were recorded as $\mu\text{A}/0.6 \text{ cm}^2$. Each point represents the mean of 3–8 observations. Standard errors (which are not shown for reasons of clarity) ranged from 5–25% of the means.

mucosa also contain CGRP (Ekblad *et al.*, 1988). Only one other study has so far investigated the effects of α -CGRP upon intestinal ion transport (McCulloch & Cooke, 1989). Using guinea-pig distal colon these authors observed only TTX-sensitive secretory responses in full thickness preparations. They concluded from their observations that α -CGRP activated myenteric neurones which then by stimulation of submucous neurones released a secretory neurotransmitter. Their findings together with ours, highlight the significant differences now being observed between species in neuropeptide control of mucosal ion transport.

We conclude that α - and β -CGRP elicit both indirect, antisecretory and direct, secretory effects upon colonic epithelia. The antisecretory agent mediating the low concentration effects of CGRPs has yet to be

positively identified; nevertheless SOM is a likely candidate.

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