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Inhibitory action of calcitonin gene-related peptide (CGRP) in the mouse colon

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Abstract—The effect and mechanism of action of calcitonin gene-related peptide (CGRP) have been investigated on the mouse distal colon. CGRP caused a concentration-dependent relaxation which was not blocked by classical pharmacological antagonists. The response pattern was characterized by a relatively rapid onset and long sustained duration. The results suggest that CGRP itself may contribute to regulating the muscular tone of the mouse colon.

Fontaine et al (1984) provided evidence for the presence of a neurogenic non-adrenergic non-cholinergic (NANC) inhibitory control in the mouse isolated colon. Although elegant attempts have been made to identify the neurotransmitter involved, the nature of this NANC inhibitory control remains unknown (Fontaine & Reuse 1985; Fontaine et al 1986).

Several neuropeptides have been implicated as possible neuromodulators of gastrointestinal motility (Burnstock 1986). One such peptide is calcitonin gene-related peptide (CGRP), a 37-residue peptide translated from the calcitonin gene (Rosenfeld et al 1983). Several studies have documented the presence of immunoreactive CGRP-like material in regions of the gastrointestinal tract of various mammalian species including man (Clague et al 1985; Gibbins et al 1985). Furthermore CGRP-containing nerve fibres have been localized close to and within smooth muscle layers of the gastrointestinal system, suggesting that the peptide is ideally located to regulate muscular tone (Mulderry et al 1985). We have therefore examined the effect of CGRP in the mouse isolated colon to establish the rôle of CGRP in the NANC inhibitory control.

Materials and methods

Male CD1 mice, 30–35 g, were killed by exsanguination following intraperitoneal injection of pentobarbitone sodium (30 mg kg^{-1}). The terminal colon was dissected out and a segment (10 to 12 mm in length) was mounted in a 5 mL organ bath containing Krebs solution gassed with 95% O_2, 5% CO_2 and maintained at 37°C. Longitudinal muscle responses were recorded with isometric force displacement transducers (Grass FT03C) coupled to a Beckman model R511A dynograph. All tissues were placed under an initial load of 2 g and allowed to equilibrate for 60 min with changes of the bathing fluid (Krebs, pH 7·4) every 15 min before experimentation began. Control drugs (carbamylcholine and noradrenaline) were used to test the reactivity of the preparation. CGRP was left in contact with the tissue for periods of 4–8 min at 15 min intervals. Antagonists were added at least 15 min before agonists.

Chemicals and solutions. The Krebs solution had the following composition (mM): NaCl 118·1; KCl 4·7; $MgSO_4 \cdot 7H_2O$ 1·2; KH_2PO_4 1·2; $NaHCO_3$ 25; $CaCl_2$ 2·5 and glucose 11·1. The following drugs were used: carbamylcholine chloride, arterenol hydrochloride (noradrenaline), diphenhydramine hydrochloride, propranolol hydrochloride, atropine sulphate, indomethacin, tetrodotoxin (all from Sigma). Methysergide bimaleate (Sandoz Ltd, Basel, Switzerland) and phentolamine (Dr E. McMullen, Parke Davis and Co., Mtl., Canada) were generous gifts. Rat α-calcitonin gene-related peptide (CGRP) was synthesized in our laboratories.

Statistical analysis. Results are presented as means ± standard error of the mean (s.e.m.). EC50 values were calculated by regression analysis.

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Results

Exogeneously applied rat CGRP from 10^{-10} to 3×10^{-6} M, produced a distinct concentration-dependent relaxation in the mouse isolated terminal colon. From the non-cumulative concentration response curve EC50 value of $3{\cdot}6 \times 10^{-8}$ M was calculated by linear regression analysis. The threshold concentration of CGRP was below 10 nM and the maximum relaxation was observed at 10^{-6} M (Fig. 1). When the same dose of CGRP was applied at 15 min intervals, with repetitive renewals of the bathing-fluid, the relaxant response was fully reproductible, indicating an absence of tachyphylaxis to CGRP. The relaxation was rapid in onset, sustained and accompanied by a reduction in spontaneous activity (Fig. 2).

The CGRP-induced relaxation was not affected by the presence of methysergide (10^{-6} M), propranolol (10^{-6} M),

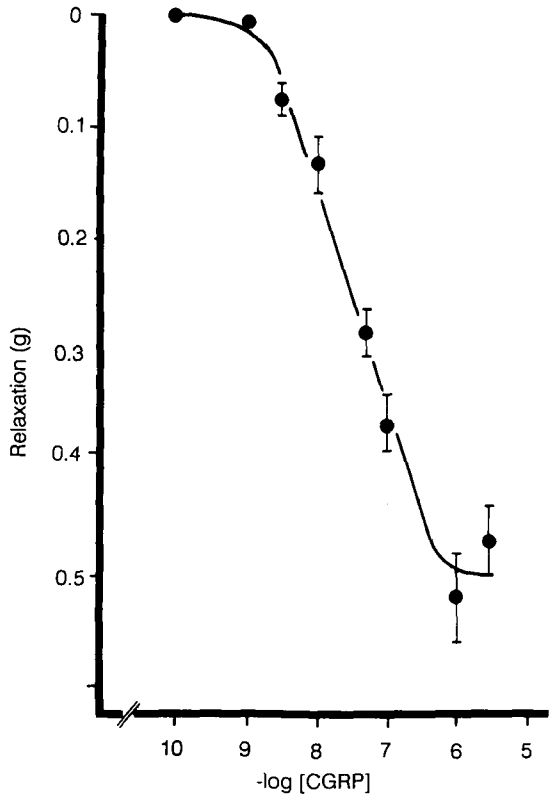

FIG. 1. Mean rat CGRP concentration-response curve plotted as grams of relaxation. Each point represents the mean ± s.e.m. of 7 to 10 determinations.

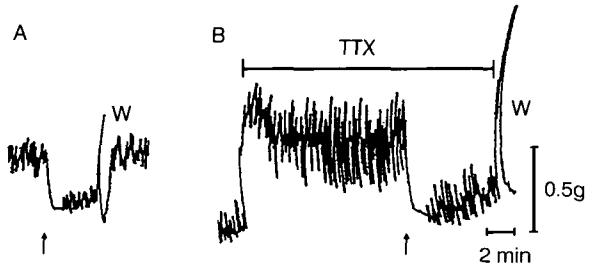

FIG. 2. Relaxant responses to rat CGRP in the mouse colon in the absence (A) and in the presence (B) of tetrodotoxin (TTX-10^{-7} M). Note that tetrodotoxin caused a sustained contraction and increased spontaneous activity in the tissue preparation but did not alter the relaxation induced by CGRP. These two tracings were obtained in the same preparation at 30 min interval. W = washout, Arrows = rat CGRP (10^{-7} M).

phentolamine (10^{-6} M), atropine (10^{-6} M), diphenhydramine (10^{-6} M), indomethacin (10^{-6} M) or tetrodotoxin (10^{-7} M).

Discussion

The present study shows that rat CGRP may cause changes in the resting tension of mouse isolated terminal colon. Its biological activity appears exclusively inhibitory with the intensity of the relaxation being concentration related. The inhibitory response of CGRP was not modified by methysergide, propranolol, phentolamine, atropine, diphenhydramine, indomethacin or tetrodotoxin which suggests that exogeneously applied CGRP produces its relaxant effect through a direct action on smooth muscle cells. This finding supports the hypothesis that CGRP may be involved in the regulation of the smooth muscle tone in the mouse colon. In addition, these results are consistent with the general belief that the predominant action of CGRP in the gastrointestinal tract of various mammals is inhibitory. Hence, CGRP relaxes in a concentration-dependent manner duodenum (Maggi et al 1987), fundus (Katsoulis & Conlon 1989) and colon (Mulderry et al 1988) of the rat; stomach (Katsoulis & Conlon 1989), ileum (Bartho et al 1987; Takaki et al 1989) of the guinea-pig; and lower oesophageal sphincter of the opossum (Rattan et al 1988).

The main features of the CGRP-induced relaxation in the mouse colon are the relatively rapid onset and the long sustained duration. This response pattern is different from those of other inhibitory peptides previously examined in this preparation. For example, neurotensin, which is known to relax duodenum and ileum of the rat and proximal colon of the guinea-pig (Couture et al 1981), exerts potent excitatory effects in mouse distal colon (Fontaine & Lebrun 1985). Vasoactive intestinal polypeptide (VIP), another well established gut inhibitory neuropeptide, exerts two types of effects (contraction and relaxation) in the mouse colon depending on the concentration used. Furthermore the relaxation induced by high doses of VIP develops slowly and does not mimic that induced by electrical field stimulation (EFS) (Fontaine et al 1986). Since CGRP produces a relaxation which presents similarities with that evoked by EFS (Fontaine et al 1984), our data suggest that CGRP might be a candidate as an inhibitory neurotransmitter in this preparation.

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