Multiple inhibitory mechanisms mediate non-adrenergic non-cholinergic relaxation in the circular muscle of the guinea-pig colon

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Received June 29, 1992/Accepted December 11, 1993

Summary. The mechanisms responsible for nerve-mediated, non-adrenergic, non-cholinergic (NANC) relaxation in mucosa-free circular muscle strips from the proximal colon of the guinea-pig were investigated. Electrical field stimulation (EFS, 1–20 Hz, trains of 5 s duration, 100 V, 0.25 ms pulse width) in the presence of atropine (1 μmol/l) and guanethidine (3 μmol/l) evoked a triphasic motor response consisting of: (a) a primary relaxation, (b) a rebound contraction and (c) a secondary relaxation. These three responses were abolished by tetrodotoxin (1 μmol/l). Both apamin (0.01–0.3 μmol/l), a known blocker of low conductance, calcium-activated potassium channels in smooth muscles, and L-nitroarginine (L-NOARG) (1–100 μmol/l), a known blocker of nitric oxide (NO) synthase, increased the tone of the strips. Maximum effects on tone were observed with 0.1 μmol/l apamin (21 ± 3% of KCl-induced contraction) and 30 μmol/l L-NOARG (26 ± 4% of KCl response). The combined administration of 0.1 μmol/l apamin and 30 μmol/l L-NOARG produced an increase in tone (47 ± 5% of KCl response) that was larger than that produced by either compound alone. Neither apamin (0.1 μmol/l) nor L-NOARG (30 μmol/l) affected the isoprenaline-induced relaxation.

Apamin (0.1 μmol/l) depressed, but did not abolish, the primary relaxation to EFS at all frequencies without affecting the secondary relaxation. Apamin also enhanced the rebound contraction at a frequency of 1 Hz. L-NOARG (30 μmol/l) depressed, but did not abolish, the primary relaxation to EFS at all frequencies, had no effect on the rebound contraction and inhibited the secondary relaxation evoked at frequencies of 1–5 Hz, but not 10–20 Hz.

L-arginine (300 μmol/l) reversed the effect of L-NOARG on tone and the inhibitory effect on the EFS-evoked relaxation. In the presence of apamin and L-NOARG, the primary relaxation was suppressed at all frequencies; the secondary relaxation was inhibited at 1–5 Hz and unchanged at 10–20 Hz, as observed with L-NOARG alone. We conclude that three distinct mechanisms mediate the NANC relaxation of the circular muscle of the proximal colon of the guinea-pig in response to EFS. One mechanism can be operationally defined as apamin-sensitive and a second as L-NOARG-sensitive, the latter implying a possible role of NO as an inhibitory transmitter. A third NANC inhibitory mechanism, which is apamin- and L-NOARG-resistant, is also suggested.

Key words: Non-adrenergic – Non-cholinergic – Inhibitory neuromuscular transmission – Apamin – Nitric oxide – Guinea-pig colon – Circular muscle

Introduction

The complex anatomical organization and neurochemical coding of the enteric nervous system enables the occurrence of a variety of intrinsic motor reflexes which subserve efficient propulsion of digesta in the caudal direction (Bayliss and Starling 1899; Furness and Costa 1987). The basic unit of this function is the peristaltic reflex, which is composed of an ascending excitatory and descending inhibitory reflex (Trendelenburg 1917; Costa and Furness 1976). For any significant degree of radial stretch of the gut wall, a propulsive contraction of the circular muscle occurs oral to the site concomitantly with a receptive relaxation caudal to the point of distension (Costa and Furness 1976). Available evidence implicates acetylcholine, acting via muscarinic receptors, and peptides of the tachykinin family as the main, final, excitatory mediators of the ascending excitation (Franco et al. 1979; Costa et al. 1985; Bartho et al. 1982, 1992; Holzer 1989). Intrinsice inhibitory neurotransmission in the gut has much attracted the attention of researchers, leading to the recognition that prominent non-adrenergic, non-cholinergic (NANC) inhibitory neural responses can be easily demonstrated in almost all sections of the mammalian gut. The identity of the transmitters responsible
for such NANC relaxation has for a long time, been a matter of debate. Adenosine triphosphate (ATP), vasoactive intestinal polypeptide (VIP) (see McKirdy 1988 for review) and, more recently, nitric oxide (NO) (Bult et al. 1990; Maggi et al. 1991; for reviews see Rand 1992; Sanders and Ward 1992) have been proposed, at various times, as the main mediators responsible for NANC enteric relaxation in different sections of the mammalian gut. In several studies, NANC relaxant responses of the gut have been shown to be abolished or reduced by apamin, a polypeptide from the venom which blocks low-conductance, calcium-activated potassium channels in smooth muscles (Vladimirova and Shuba 1978; Maas and Den Hertog 1979). In some instances, the action of apamin has been interpreted to support a role for ATP as transmitter of the evoked, nerve-mediated relaxation (e.g. Maggi et al. 1984).

Overall, it appears possible that more than one transmitter/mechanism may determine enteric NANC relaxation (e.g. Costa et al. 1986; Manzini et al. 1986), although one of the above mentioned transmitters may play a dominant role in distinct segments of the intestine of some species. A few studies have identified the contribution of multiple mechanisms, which act, to various extents, to induce NANC relaxation in a given gut segment (e.g. NO and VIP in rat gastric fundus, Li and Rand 1990; VIP and ATP in circular muscle of guinea-pig ileum, Cris et al. 1992).

In this study, the use of apamin and L-nitroarginine (L-NOARG), a potent inhibitor of NO synthase (Hobbs and Gibson 1990), has enabled us to identify three distinct inhibitory NANC mechanisms that lead to relaxation of the circular muscle of the guinea-pig proximal colon.

**Methods**

Male albino guinea-pigs (300–350 g) were stunned and then killed by exsanguination. A segment (1.0–1.5 cm long) of the proximal colon, taken 2–3 cm distant from the end of the caecum, was excised and placed in oxygenated (96% O2 and 4% CO2, pH 7.4 at 37 °C) Krebs solution. The segment was opened along the mesenteric border and pinned flat in a Petri dish. The mucosa was gently removed and circular muscle strips prepared for isotonic tension recording (load 10 mN) in 5 ml baths for isolated organs. In some experiments four parallel strips were excised from each segment and studied concurrently (see below). Unless otherwise stated, all experiments were done in the presence of atropine (1 μmol/l) and guanethidine (3 μmol/l).

Experiments were started after 120 min equilibration time. The contractile response to electric nerve stimulation of control strips averaged 21 ± 3% of the KCl response (n = 8) (Fig. 1). With 3 out of 8 strips, apamin also caused the appearance of a phasic, low-amplitude, rhythmic, contractile activity.

**Results**

**General**

After they were set up in the organ bath, circular muscle strips from the guinea-pig proximal colon developed a high intrinsic tone, which reached a steady state within 90 min, and was well maintained throughout the experiment.

Addition of KCl (80 mmol/l) produced, first, a transient relaxation which was followed by a sustained, maximal contraction of the strip. The amplitude of this contraction was determined for each strip to be used as an internal standard. Isoprenaline (10 nmol/l–1 μmol/l) produced a concentration-dependent relaxation. The response to 30 nmol/l isoprenaline (about 40% of the maximum relaxation produced by 1 μmol/l isoprenaline) was routinely determined with each strip and used as a second internal standard. A submaximal concentration of isoprenaline was selected for this standard because the amplitude of the maximal relaxation to isoprenaline was far in excess of the relaxation observed in response to nerve stimulation, owing to the high intrinsic tone of the strips. When normalized with respect to the submaximal relaxant response to isoprenaline, relaxation in response to electrical nerve stimulation of control strips averaged 30–40% of the relaxation to the internal standard (see below).

**Effect of apamin and L-NOARG on resting tone**

Apamin (0.01–0.3 μmol/l) produced a concentration-dependent increase in the tone of the strips, which reached a peak within 5–10 min of its introduction into the bath and then declined towards the baseline. A maximum effect was produced with 0.1 μmol/l, which averaged 21 ± 3% of the KCl response (n = 8) (Fig. 1). With 3 out of 8 strips, apamin also caused the appearance of a phasic, low-amplitude, rhythmic, contractile activity.

L-NOARG (1–100 μmol/l) produced a concentration-dependent increase in tone of the strips which reached a peak within 15 min and then declined towards baseline. The maximum effect was produced by 30 μmol/l and averaged 26 ± 4% of the KCl response (n = 8, Fig. 1). The effect of L-NOARG on tone was rapidly reversed (70–100%) by the addition of L-arginine (L-Arg, 300 μmol/l, n = 4).

The combined administration of apamin (0.1 μmol/l) and L-NOARG (30 μmol/l) produced a prompt increase in tone of the strips (Fig. 1) which was significantly larger than that produced by apamin, or L-NOARG, alone (47 ± 5% of KCl response, P < 0.05, n = 8).

**Statistical analysis.** All data in the text and figures are given as mean ± SEM. Statistical analysis was by Student's t-test for paired data (effect of isoprenaline before and after addition or apamin of L-NOARG) or by analysis of variance.

**Drugs used were:** Atropine HCl and isoprenaline (Serva); guanethidine sulphate (ICF); L-NOARG, l-arginine and apamin (Sigma, St. Louis, Mo., USA).