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Evidence that sensory neurons participate in the non-cholinergic, non-adrenergic contractile response of the guinea-pig ileum

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The possible role of non-cholinergic, non-adrenergic (NCNA) nerves in responses of the guinea-pig terminal ileum to transmural nerve stimulation (TNS) and that of sensory nerves in NCNA responses were investigated. The action of acetylcholine was almost abolished in the presence of histamine, whereas the contractions elicited by TNS were changed to frequency-dependent contraction followed by a secondary relaxation. Guanethidine did not alter the contractions or secondary relaxations. Atropine abolished the action of acetylcholine and transiently suppressed the responses to low (up to 2 Hz) and attenuated (by about 50%) those to high (4 to 20 Hz) frequency stimulation. The remaining complex NCNA response was the sum of the excitatory and inhibitory responses. During desensitization to capsaicin, and in its presence, the NCNA contractions were reduced, whereas the relaxations were not significantly enlarged. The present results suggest that besides the cholinergic innervation, the excitatory and inhibitory NCNA innervation also participates in the responses of the guinea-pig ileum to TNS even without suppression of cholinergic and adrenergic transmission, and that the sensory nerves are, at least to some extent, involved in the NCNA excitatory response.

Substance P-containing neurons in the intestine are of intrinsic and extrinsic origin (Franco et al 1979a; Costa et al 1980, 1981) and substance P, or a related substance, has been proposed as an excitatory non-cholinergic, non-adrenergic (NCNA) transmitter in the guinea-pig intestine (Franco et al 1979b; Leander et al 1981; Bauer & Kuriyama 1982b). Substance P contracts the intestinal smooth muscle directly and indirectly via activation of cholinergic enteric neurons (Katayama et al 1979) and release of acetylcholine (Holzer & Lembeck 1980; Fujisawa & Ito 1982).

NCNA contraction and excitatory junction potentials of the guinea-pig small intestine in response to stimulation of intramural nerves (Bauer et al 1982) were reported to be greatly inhibited by specific desensitization to substance P (Franco et al 1979b; Bauer & Kuriyama 1982b). Recently, cross desensitization between the endogenous excitatory NCNA transmitter and substance P in the guinea-pig ileum was found (Bauer & Matušák 1986). The evoked release of substance P in the guinea-pig ileum could derive not only from autonomic but also from sensory neurons (Barthó et al 1982a). Thus the contraction of the ileum elicited by transmural nerve stimulation (TNS) even in the presence of atropine and guanethidine in bathing fluid could result partly from the activation of sensory neurons.

To investigate the involvement of substance P-containing sensory neurons in the NCNA excitatory response of the guinea-pig ileum to TNS, we used capsaicin, which acts selectively on a population of sensory neurons, as a considerable portion of these contains and releases substance P (Jancsó et al 1967, 1980; Jessell et al 1978; Gamse et al 1979).

METHODS

Male guinea-pigs (250–400 g) were stunned and bled. Segments of the terminal ileum approximately 1·5 cm long taken 0 to 3 cm from the ileocecal junction, were suspended in an organ bath containing 5 ml modified Krebs solution (Na^+ 136·6, K^+ 5·9, Ca^{2+} 2·5, Mg^{2+} 1·2, Cl^- 133·3, HCO_3^- 15·4, $H_2PO_4^-$ 1·2 and glucose 11·5 mmol litre^{-1}) maintained at 37 °C and gassed with a mixture of 95% O_2 and 5% CO_2. After an equilibration period of 30 min at 20 mN tension, the actual experiments were carried out under a tension of about 5 mN. TNS of the guinea-pig terminal ileum with pulses of 0·5 to 1 ms width of supramaximal intensity was given by two platinum electrodes, one placed in the lumen of the preparations and the other in the bath (Paton 1955). The neurogenic nature of the electrically induced responses with and without atropine (0·5 μmol

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litre^{-1}) and guanethidine (10 μmol litre^{-1}) in the bathing fluid, was confirmed by the ability of tetrodotoxin (1·5 μmol litre^{-1}) to abolish the responses.

The drugs used were: atropine sulphate (Spofa), capsaicin (Merck), guanethidine sulphate (Ciba), histamine hydrochloride (Spofa) and tetrodotoxin (Sankyo). Stock solutions of the drugs were prepared in distilled water, except capsaicin, which was dissolved in 96% ethanol to give a 10 mg ml^{-1} stock solution. Before use the final dilution was made with modified Krebs solution. The solvent of capsaicin was ineffective on the ileum in the used concentration range.

Quantitative data are presented as mean ± s.e.m. Differences were tested using Student's *t*-test for paired data. *P* values <0·05 were regarded as significant.

RESULTS

Short train of stimulation: characteristics of contractions and relaxations

As previously described (Paton 1955; Bauer et al 1982) TNS produces longitudinal contraction of the guinea-pig intestine. The amplitude of contractions induced by single stimuli in the terminal ileum reached 40–70% of those elicited by trains of pulses lasting 10 s at the frequencies of 2 to 20 Hz. There were, however, no significant differences ($P > 0·05$; n = 7) among the amplitudes of contractions elicited by trains of stimuli at 4 to 20 Hz frequency and supramaximal intensity (Figs 1A, 2A). On the histamine (2 μmol litre^{-1})-induced tonic contracture, which enables relaxations to be observed, trains of stimuli (4, 8 and 20 Hz for 10 s) produced contractions of a frequency-dependent amplitude accompanied by a secondary relaxation (Fig. 1B).

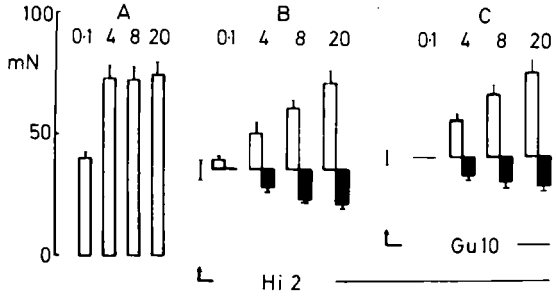

FIG. 1. Effects of histamine (Hi) and guanethidine (Gu) on the responses of the guinea-pig terminal ileum elicited by a single stimulus (0·1 Hz) or trains of pulses for 10 s at a frequency of 4, 8 and 20 Hz. □ Contraction, ■ secondary relaxation. Values are expressed as means ± s.e.m. of 7 experiments. Doses are in μmol litre^{-1}.

Acetylcholine (0·1 to 1 μmol litre^{-1}) either did not change or only minimally increased the smooth muscle tension already enhanced by histamine (n = 5). Guanethidine (10 μmol litre^{-1}), transiently (for 1 to 3 min) enhanced the tension of the untreated (n = 8) and histamine contracted (n = 7) terminal ileum. The muscle contractions elicited by trains of stimuli were not significantly ($P > 0·05$; n = 8 and 7, respectively) affected by 15 min guanethidine treatment (Figs 1C, 2B). Single stimuli in the presence of guanethidine were, however, no longer effective upon the histamine-induced sustained contraction and the amplitude of rebound relaxation was slightly but not significantly reduced ($P > 0·05$; n = 7) (Fig. 1C).

Atropine (0·5 μmol litre^{-1}) only marginally relaxed the guanethidine (10 μmol litre^{-1})-treated terminal ileum but abolished the contractions induced by acetylcholine (0·1 to 1·0 μmol litre^{-1}) and markedly affected the responses elicited by TNS. The contractions induced by single stimuli and trains of low frequency (up to 2 Hz) pulses were gradually suppressed and after 1 to 2 min ceased, whereas the responses induced by high (4 to 20 Hz)-frequency stimulation changed only in their character although high concentrations of atropine (up to 2 μmol litre^{-1}) were applied. In spite of the 5 to 15 min presence of atropine and guanethidine in the bathing fluid, pulses or low frequency stimulation again evoked changes of the smooth muscle tension. These however, were composed of a primary relaxation and contraction followed by rebound contraction at the termination of the TNS. The amplitude of the primary and rebound contraction was increased by increasing the frequency of stimulation from 2 to 20 Hz, whereas that of the primary relaxation was highest at 2 Hz, the lowest stimulation frequency used (Fig. 2C).

Histamine (2 μmol litre^{-1}) contracted the terminal ileum also in the presence of atropine and guanethidine. The initial phasic contraction (80·6 ± 8·1 mN; n = 6) was followed by a sustained tonic contraction (40·7 ± 2·1 mN; n = 6). During the histamine-induced sustained increase of the smooth muscle tension the amplitude of NCNA primary relaxation elicited by TNS was enlarged and that of the primary and rebound contraction reduced (Fig. 2D).

The effect of capsaicin

After control NCNA responses had been determined without and in the presence of histamine (Figs. 3A, C), capsaicin (1 μmol litre^{-1}) was added to the organ bath for 20 min. Capsaicin caused a transient con-

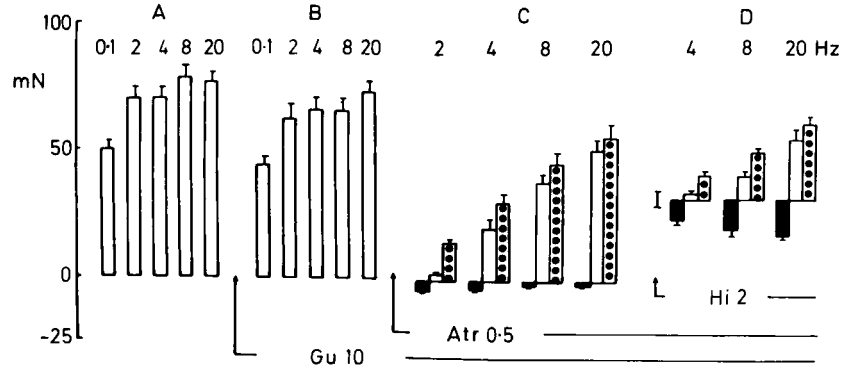

FIG. 2. Effects of guanethidine (Gu), atropine (Atr) and histamine (Hi) on the responses of the guinea-pig terminal ileum elicited by a single stimulus (0·1 Hz) or trains of pulses for 10 s at a frequency of 2, 4, 8 and 20 Hz. □ Primary contraction, ■ primary relaxation, ◉ rebound contraction. Values are expressed as mean ± s.e.m. of 7 to 9 experiments. Doses are in μmol litre^{-1}.

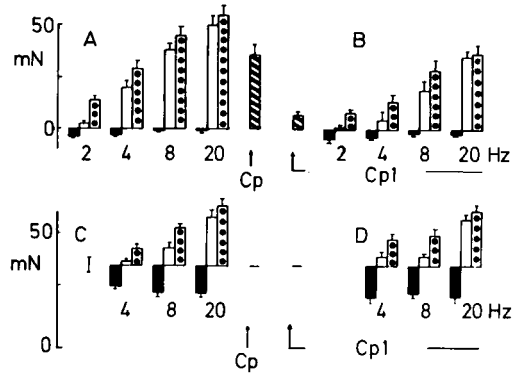

FIG. 3. Effects of capsaicin (Cp) on the NCNA primary relaxation (■), primary contraction (□) and rebound contraction (◉) of the guinea-pig terminal ileum elicited by TNS (2, 4, 8 and 20 Hz for 10 s) under basal conditions (B) and during the histamine (2 μmol litre^{-1})-enhanced basal tension (D). A and C are the corresponding control responses. Atropine (0·5 μmol litre^{-1}) and guanethidine (10 μmol litre^{-1}) were included in the Krebs solution throughout the experiments. Values are expressed as means ± s.e.m. of 5 to 9 experiments.

traction of the atropine- and guanethidine-treated terminal ileum, which had a slow onset, reached its maximum within 61·4 ± 6·6 s and faded away in 157·1 ± 19·9 s (n = 6). When capsaicin (1 μmol litre^{-1}) was again added after repeated washouts, its action was significantly ($P < 0·05$; n = 5) reduced (Fig. 3B), in contrast to the action of histamine (0·5 μmol litre^{-1}) which remained unaltered (n = 5). Consequently, in the presence of histamine (2 μmol litre^{-1}), the already elevated basal tension was not affected by capsaicin, not only at the concentration used in histamine untreated preparations (1 μmol litre^{-1}; n = 6) but also in higher concentrations (up to 10 μmol litre^{-1}; n = 9).

In the presence of capsaicin (1 μmol litre^{-1}) for 20 min, the amplitude of the NCNA primary relaxation was either slightly but not significantly augmented ($P > 0·05$), or not affected in both the untreated (n = 5–7) and the histamine-precontracted (n = 6–7) terminal ileum (Fig. 3B, D). The primary and the rebound contraction were significantly decreased ($P < 0·05$; n = 5–7) when the NCNA nerves were stimulated (2 to 20 Hz for 10 s) at an unchanged basal tension (Fig. 3B), whereas they remained uninfluenced when the preparations were stimulated at the histamine-induced increased basal tension (Fig. 3D). The above changes were similar even during the second application of capsaicin (n = 7 and 6, respectively).

DISCUSSION

It has generally been accepted that the neurogenic contractions of the gastrointestinal smooth muscles due to TNS are mainly cholinergic. Yet we have demonstrated that in different regions of the guinea-pig small intestine NCNA contractions could also be elicited (Bauer et al 1982). NCNA excitatory fibres are more densely distributed in the terminal than in the proximal part of the guinea-pig ileum (Bauer & Kuriyama 1982b). The participation of different substance P-containing nerve endings in the excitatory responses of the guinea-pig ileum to TNS were therefore studied on its terminal segment. In part of the experiments the tone of the tissues was raised with histamine to enable relaxations to be observed. The present results revealed that contractions of the terminal ileum elicited by single stimuli and low frequency (up to 2 Hz) stimulation were abolished by atropine. However, the finding that contractions evoked by trains of pulses at frequencies above 2 Hz

were only partly reduced by atropine, even in concentrations high enough to prevent the action of exogenous acetylcholine (0·1 to 1 μmol litre^{-1}), indicated the contribution of a non-cholinergic mechanism in responses to high frequency stimulation in untreated conditions as well. The possible participation of NCNA mechanisms in tension changes was also supported by the manifestation of secondary relaxation and the appearance of frequency-dependent contractile responses when superimposed on histamine-induced contraction. This frequency dependence was due to the prevalence of NCNA responses over the cholinergic ones, because the histamine-precontracted, but otherwise untreated, preparations were almost insensitive to acetylcholine. Thus, since the NCNA responses reached their maximum at higher frequencies than the cholinergic responses, the frequency dependence was manifested.

The adrenergic mechanism may not account for the non-cholinergic contributions in the responses evoked upon the histamine-increased basal tension, except the contraction elicited by single stimuli and partly in the secondary relaxation, because these were not significantly affected by guanethidine.

The NCNA responses of the guinea-pig small intestine are also rather complex involving the activation of both inhibitory and excitatory nerves, as described earlier (Bauer et al 1982; Bauer & Kuriyama 1982a). Evidence has been provided to show that a substance possessing substance P-like activity might be an excitatory NCNA transmitter (Franco et al 1979b; Leander et al 1981; Bauer & Kuriyama 1982b). This suggestion is supported also by our recent findings of cross desensitization between substance P and the endogenous NCNA excitatory transmitter in the guinea-pig small intestine (Bauer & Matušák 1986). Since a substance P-like peptide, a potential NCNA transmitter, might be released not only from autonomic but also from sensory nerve endings, the NCNA responses were analysed in the presence and absence of capsaicin. Previous studies on the contractile actions of capsaicin on the intestine revealed that its effect is mediated by two distinct mechanisms, i.e. by release of acetylcholine (Barthó & Szolcsányi 1978) and of substance P (Barthó et al 1982a). The effect of capsaicin in the presence of atropine and guanethidine was analogous to that observed in the presence of hyoscine alone (Barthó et al 1982b), indicating that release of endogenous catecholamines does not participate in its action and they are not responsible for the fading of the capsaicin-induced contraction.

Of the substance P-containing neurons, however, capsaicin acts selectively on the sensory neurons (Jancsó et al 1967, 1980; Jessell et al 1978; Gamse et al 1979). As reported earlier (Barthó & Szolcsányi 1978, 1980), a second exposure to capsaicin (10 μmol litre^{-1}) 15–30 min after the first, failed to produce a contraction. A similar desensitization of the terminal ileum to capsaicin (1 μmol litre^{-1}) was observed in the present experiments. Barthó et al (1982b) suggested that the inhibitory effect of high concentrations of capsaicin (10 μmol litre^{-1}) on non-cholinergic contraction and the actions of nicotine, acetylcholine and histamine, might be non-specific. This inhibition disappeared 30–45 min after the removal of capsaicin from the bath. Some discrepancy between the above mentioned results of Barthó et al (1982b) and the suppression of NCNA contractions, even during the second application of capsaicin when the preparations were almost insensitive to its action, may be due to differences in the preparations, concentrations of capsaicin used and the experimental protocol. In the present experiments, a ten times lower concentration of capsaicin than that used by Barthó et al (1982b), reduced its effect on second application (by about 80%) in atropine- and guanethidine-treated terminal ileum. This reduction was specific, because, in contrast to the previously used high concentration (Barthó et al 1982b), capsaicin in low concentration did not affect the action of histamine.

Since capsaicin was without any influence on the smooth muscle tone elevated by histamine, it would appear that the capsaicin-sensitive sensory neurons, or their peripheral endings, were already activated by histamine. Under such circumstances it seems reasonable that capsaicin, which significantly reduced the NCNA contractions in preparations with unchanged basal tension, did not affect the NCNA excitatory responses superimposed on histamine-induced contraction. The reduction of NCNA contractions when preparations were already desensitized to capsaicin might also result from the enhancement of NCNA relaxations. However, this appears not to be the case because, even in histamine precontracted preparations, capsaicin did not enhance the amplitude of NCNA relaxation significantly.

The immunohistochemical investigations have shown that the gut is also innervated by extrinsic substance P-containing sensory fibres, which run in the mesenteric nerves (Costa et al 1980, 1981). Furness et al (1982) have demonstrated that these extrinsic fibres are depleted of substance P by

systemic treatment with capsaicin. Our investigation suggests that the activation of the above sensory nerve fibres participates, at least to some extent, in the NCNA excitatory, but not in the NCNA inhibitory, responses of the guinea-pig terminal ileum to TNS, and that the autonomic NCNA excitatory and inhibitory nerve fibres, in contrast to the sensory ones, are resistant to the action of micromolar concentrations of histamine and capsaicin.

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