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Tachykininergic synaptic transmission in the coeliac ganglion of the guinea-pig

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1 The responses of coeliac ganglion neurones of the guinea-pig to electrical stimulation of the mesenteric nerves and applications of tachykinin receptor agonists were investigated by use of intracellular recording techniques.

2 Ganglion neurones were classified into three groups based on firing patterns in response to a depolarizing current pulse: phasic (38% of the population), tonic (39%) and atypical (23%). In the majority of phasic neurones (91%) a long after-hyperpolarization (LAH) lasting 5–8 s followed action potentials induced by a train of depolarizing current pulses. In contrast, LAH was rarely observed in tonic neurones (5%).

3 In most of tonic neurones (90%) slow excitatory post-synaptic potentials (e.p.s.ps) lasting 3–10 min were evoked by repetitive electrical stimulation of the mesenteric nerves. Prolonged depolarizations were also evoked in most tonic neurones by applications of substance P (SP), neurokinin A (NKA) or senktide, a tachykinin NK_3 receptor agonist.

4 In most of phasic neurones (73%), mesenteric nerve stimulation did not induce an obvious depolarization but induced a prolonged inhibition of LAH lasting 3–10 min. Bath-applied tachykinin receptor agonists similarly induced an inhibition of LAH without causing depolarization in most of the phasic neurones.

5 GR71251 (5 μM), a tachykinin NK_1 receptor antagonist, partially depressed the nerve-evoked slow e.p.s.ps in tonic neurones and the nerve-evoked LAH inhibition in phasic neurones.

6 Capsaicin (0.1–5 μM) induced a prolonged depolarization in tonic neurones and an inhibition of LAH in phasic neurones.

7 A mixture of peptidase inhibitors potentiated the depolarization and the LAH inhibition evoked by nerve stimulation, SP and NKA, but not those evoked by senktide.

8 It is concluded that tonic neurones respond to repetitive mesenteric nerve stimulation preferentially with slow e.p.s.ps and that phasic neurones respond preferentially with LAH inhibition. The present study further suggests that SP and NKA, released from axon collaterals of primary afferent neurones, produce slow e.p.s.ps in tonic neurones and the LAH inhibition in phasic neurones via NK_1 receptors.

Keywords: After-hyperpolarization; slow e.p.s.ps; substance P; neurokinin A; tachykinin receptor; GR71251

Introduction

In mammalian prevertebral sympathetic ganglia repetitive stimulation of pre- or post-ganglionic nerves evokes non-cholinergic slow excitatory postsynaptic potentials (e.p.s.ps) in a subset of the population of neurones. There is evidence suggesting that tachykinins, particularly substance P (SP) and neurokinin A (NKA), contribute to the slow e.p.s.ps (for review, see Otsuka & Yoshioka, 1993). Thus, exogenously applied SP and NKA mimic the slow e.p.s.ps (Tsunoo *et al.*, 1982; Konishi *et al.*, 1983; Saria *et al.*, 1985) and tachykinin receptor antagonists, [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP and [D-Pro[2], D-Trp[7,9]]SP, inhibit the slow e.p.s.ps as well as the SP-induced depolarization (Konishi *et al.*, 1983; Konishi & Otsuka, 1985). In agreement with these results from electrophysiological experiments, biochemical and immuno-histochemical studies have shown the presence of SP-immunoreactivity and SP-containing nerve fibres in prevertebral sympathetic ganglia of the guinea-pig (Konishi *et al.*, 1979; Gamse *et al.*, 1981; Tsunoo *et al.*, 1982; Dalsgaard *et al.*, 1982; Matthews & Cuello, 1984; Lindh *et al.*, 1988). These SP-containing nerve fibres are probably collaterals of primary afferent neurones.

Sympathetic postganglionic neurones are heterogeneous in electrophysiological and morphological properties. For example, neurones in the prevertebral sympathetic ganglia of the guinea-pig were classified into three types based on their firing patterns to a depolarizing current: tonic, phasic and LAH (long after-hyperpolarization) neurones (McLachlan & Meckler, 1989; Jänig & McLachlan, 1992). McLachlan & Meckler (1989) showed that the slow e.p.s.ps were evoked in some phasic and tonic, but not LAH neurones in the guinea-pig sympathetic ganglia. This suggests that non-cholinergic inputs are confined to these phasic and tonic neurones but absent in LAH neurones. Another possibility is that LAH neurones receive non-cholinergic innervation which causes a different type of response.

In a previous study we examined the responses of coeliac ganglion neurones of the guinea-pig to exogenously applied tachykinins (Zhao *et al.*, 1995). We found that two distinct types of responses, depolarization and inhibition of LAH, were evoked in response to SP and NKA. Furthermore, the occurrence of these responses was closely correlated with the electrophysiologically defined types of neurones: these tachykinins evoked depolarization in most tonic neurones, which were one of major types of ganglion neurones, and LAH inhibition in most phasic neurones, another major type of neurones. These results prompted us to investigate: (1) whether repetitive electrical stimulation of the mesenteric nerves, i.e., the postganglionic nerves of the ganglion, evokes LAH inhibition, in addition to the slow e.p.s.ps in ganglionic neurones; (2) whe-

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ther these distinct responses correlate with neurone types; and (3) whether neurally released tachykinins contribute to these responses. Some of the results have been published in a preliminary form (Saito & Konishi, 1993; Zhao *et al.*, 1993).

Methods

Guinea-pigs of either sex weighing 200–250 g were stunned and bled, and the coeliac ganglion with the attached mesenteric nerves (Macrae *et al.*, 1986; or coeliac nerves according to McLachlan & Meckler, 1989) was isolated under a stereomicroscope. The isolated preparation was placed in a recording chamber of 0.5 ml volume, which was perfused at 2 ml min^{-1} with a modified Tyrode solution bubbled with 95% O_2 and 5% CO_2. The composition of the solution was (mM): NaCl 138.6, KCl 3.35, NaH_2PO_4 0.58, $NaHCO_3$ 21.0, $MgCl_2$ 1.16, $CaCl_2$ 2.5, glucose 10. The temperature in the bath was kept at 30°C.

Membrane potential changes were recorded with a glass microelectrode of 80–160 MΩ filled with 2 M potassium acetate and displayed on a storage oscilloscope, a pen recorder and an X-Y plotter. Depolarizing current pulses were injected into neurones through the recording electrode to determine the cell type (a single square pulse of 0.1~0.5 nA with 1.5 s duration) and to examine the presence or absence of LAH (10 pulses of 1 nA with 20 ms duration at 20 Hz). To stimulate the mesenteric nerves, more than two thirds of the mesenteric nerve bundles were held in a tight-fitting suction electrode and stimulated at 20 Hz with 10–120 pulses of 100 V intensity and 500 μs duration. An interval of 10–15 min was allowed between nerve stimulations. To examine the effect of the nerve stimulation on LAH, intracellular current injection was given every 60 s to evoke the LAH and the mesenteric nerves were stimulated 30 s before a test LAH. The magnitude of the LAH was expressed as the area between the after potential and the resting potential. All the data were from the neurones with an initial resting potential larger than 45 mV (absolute value). Data are expressed as mean ± s.e.mean and significant differences determined by Student's *t* test.

Table 1 Neurone types and occurrence of slow e.p.s.ps, long after-hyperpolarization (LAH) and LAH inhibition

Neurone type	*Tonic*		*Phasic*		*Atypical*	
	LAH (+)	*LAH (−)*	*LAH (+)*	*LAH (−)*	*LAH (+)*	*LAH (−)*
Slow e.p.s.ps	1/1	109/121	8/64	1/2	2/3	11/27
LAH inhibition	0/1	NO	48/64	NO	1/2	NO

Number of neurones that responded with slow e.p.s.ps or LAH inhibition to mesenteric nerve stimulation/number of neurones examined; NO, not observed.

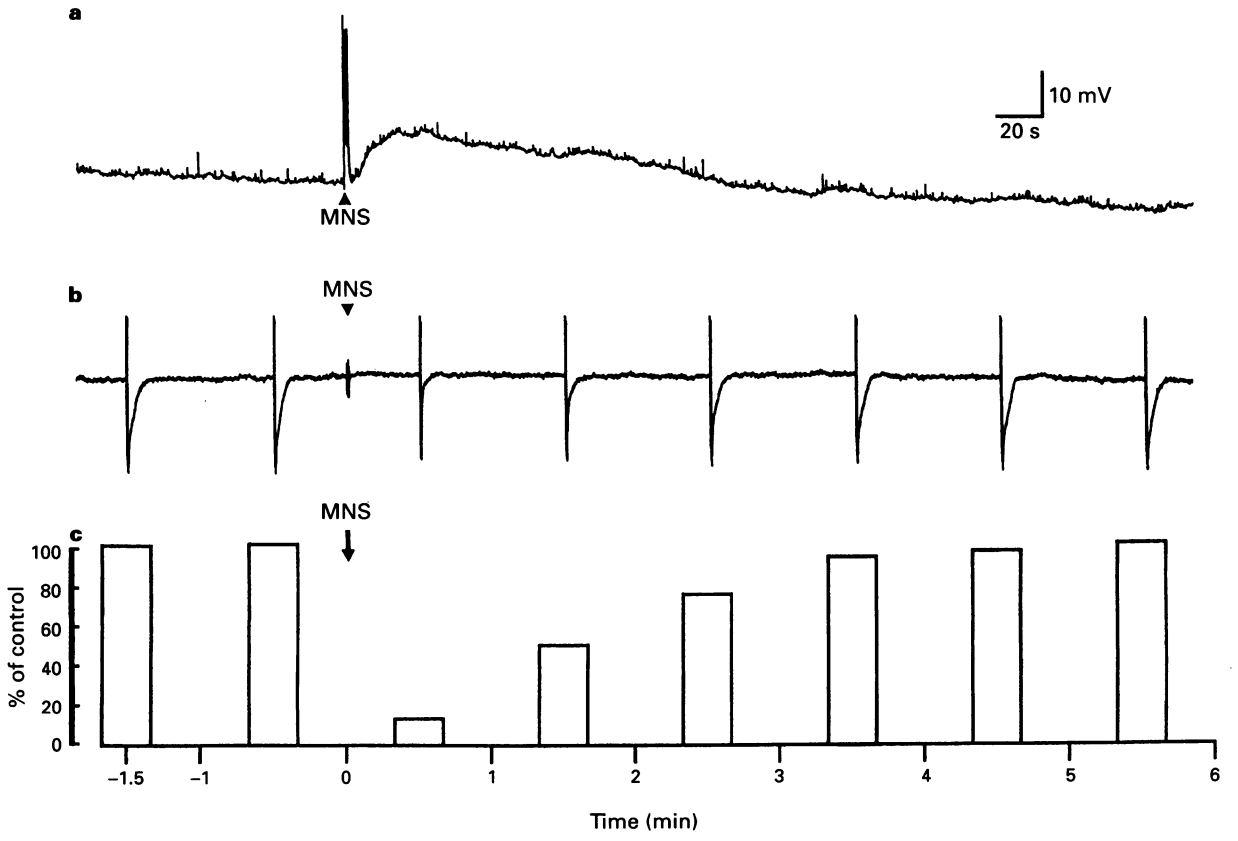

Figure 1 Two types of response to mesenteric nerve stimulation in tonic and phasic neurones. (a) A slow e.p.s.p. in a tonic neurone; (b) inhibition of the long after-hyperpolarization (LAH) in a phasic neurone; and (c) time course of change in the magnitude of LAH in response to nerve stimulation (the same experiment as in (b)). Mesenteric nerve stimulation (MNS; 10 pulses of 100 V intensity and 500 μs duration at 20 Hz) was given at the times marked with filled triangles and downward arrow. Each LAH in (b) and (c) was evoked by a train of depolarizing current pulses (10 pulses of 1 nA and 20 ms at 20 Hz). In (c) the magnitude of LAH was determined as the area between the LAH and resting membrane potential. This area was expressed as a percentage of the average area of the two LAHs immediately before the nerve stimulation. The resting membrane potentials were −81 mV and −56 mV in (a) and (b), respectively.

Table 2 Responsiveness to tachykinins in tonic and phasic neurones

	Neurone type	
Responsivenes to tachykinins	*Tonic neurones with slow e.p.s.ps*	*Phasic neurones with LAH inhibition*
SP (+), NKA (+), Senk (+)	53 (80.3%)	14 (77.8%)
SP (−), NKA (−), Senk (+)	7 (10.6%)	3 (16.7%)
SP (−), NKA (−), Senk (−)	6 (9.1%)	1 (5.5%)

Number of neurones that responded with depolarization (tonic neurones) or long after-hyperpolarization (LAH) inhibition (phasic neurones) to substance P (SP), neurokinin A (NKA) and senktide (Senk). The responses of neurones to mesenteric nerve stimulation were first examined and those to the three agonists were then examined for the tonic and phasic neurones that showed nerve-evoked slow e.p.s.ps (tonic neurones) or LAH inhibition (phasic neurones). The numbers in parentheses are percentages of tonic or phasic neurones examined. First row, neurones that responded to SP, NKA and senktide; second row, neurones that responded only to Senk; and third row, neurones that responded to none of the three agonists.

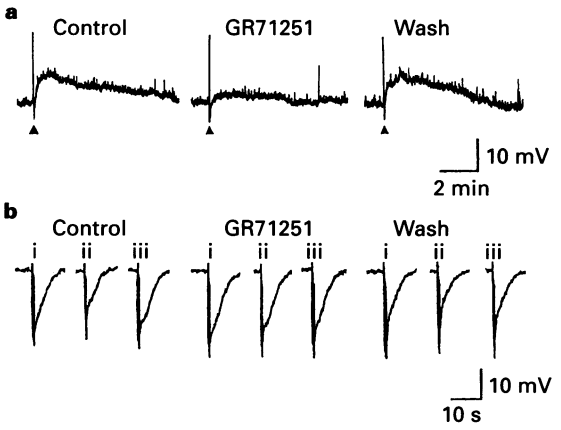

Figure 2 Effects of GR71251 on two types of response evoked by mesenteric nerve stimulation. (a) Slow e.p.s.ps in a tonic neurone. Mesenteric nerve stimulation (20 pulses of 500 μs and 100 V at 20 Hz) was given at filled triangles. Control, response immediately before applying GR71251; GR71251, response 35 min after the start of application of GR71251 (5 μM); and wash, 20 min after washing out the antagonist. (b) Inhibition of the long after-hyperpolarization (LAH) in a phasic neurone. Each LAH was evoked by a train of 10 depolarizing pulses (1 nA and 20 ms at 20 Hz) every 1 min. Mesenteric nerve stimulation was given between (i) and (ii) with 20 pulses of 500 μs and 100 V at 20 Hz. Control, immediately before applying GR71251; GR71251, 15 min after the start of application of GR71251 (5 μM); and wash, 20 min after washing out the antagonist. (i) Immediately before nerve stimulation; (ii) 30 s after the stimulation; and (iii) 4.5 min after the stimulation. The resting membrane potentials were −48 mV and −56 mV in (a) and (b), respectively.

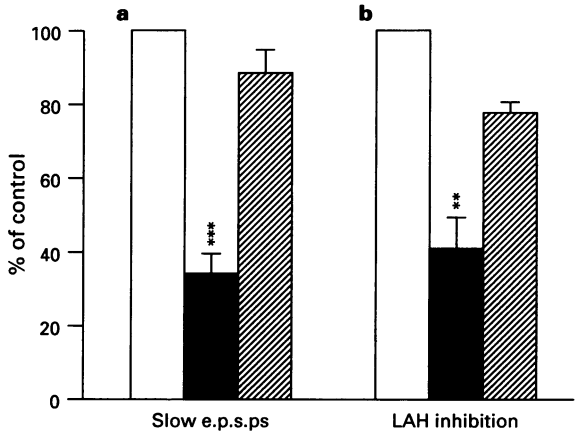

Figure 3 Averaged data showing the effects of GR71251 on nerve-evoked slow e.p.s.ps and the long after-hyperpolarization (LAH) inhibition. (a) Effects of GR 71251 on slow e.p.s.ps in tonic neurones ($n=10$). In each experiment, mesenteric nerve stimulation (40 pulses of 500 μs and 100 V at 20 Hz) was given every 15 min and resulting slow e.p.s.ps were recorded: first in normal solution, then in the presence of GR71251 (5 μM), and finally after washing out the antagonists (for details see the legend of Figure 2). The magnitude of slow e.p.s.ps was determined as area between the slow e.p.s.ps and the resting potential and expressed as percentage of the control response before adding GR71251. Each column shows the mean magnitude of slow e.p.s.ps and vertical lines indicate s.e.mean. Open column, control; solid column, under GR71251 (5 μM); and hatched column, after washing out the antagonist. (b) Effects of GR71251 on LAH inhibition in phasic neurones ($n=4$). Each LAH was evoked by a train of depolarizing current pulses (10 pulses of 1 nA and 20 ms at 20 Hz). Mesenteric nerve stimulation (20–40 pulses of 100 V and 500 μs at 20 Hz) was given every 20 min and resulting inhibition of LAH was recorded: first in normal solution, then in the presence of GR71251 (5 μM), and finally after washing out the antagonist. The magnitude of LAH was determined as area between the LAH and the resting potential. The inhibition of LAH by mesenteric nerve stimulation was expressed as the percentage decrease of the magnitude of LAH 30 s after nerve stimulation. This inhibition of LAH was further normalized by expressing the inhibition as percentage of the control inhibition immediately before GR71251 application. Each column shows the mean extent of LAH inhibition and vertical lines indicate s.e.mean. Open column, control; solid column, under GR71251 (5 μM); and hatched column, after washing out the antagonist. Significantly different from control, ** $P<0.01$, *** $P<0.001$.

Drugs and their sources were: SP, NKA, [MePhe^7]neurokinin B ([MePhe^7]NKB) and [Trp^7, β-Ala^8]NKA$_{4-10}$ (purchased from Peninsular Laboratories, Inc.); L659,877 (cyclo[Gln-Trp-Phe-Gly-Leu-Met]) (from Cambridge Research Biochemicals, Ltd.); senktide (succinyl-[Asp^6, MePhe^8]SP$_{6-11}$) (kindly supplied by Dr Z. Selinger of Hebrew University of Jerusalem); and GR71251 ([D-Pro^9[spiro-γ-lactam]Leu^{10}, Trp^{11}]SP) (kindly supplied by Dr R.M. Hagan of Glaxo Group Research Ltd). Actinonin, captopril, thiorphan (purchased from Peptide Institute Inc., Japan); naloxone, 5-hydroxytryptamine (5-HT) and capsaicin (from Sigma, U.S.A.); acetylcholine and noradrenaline (from Sankyo Co. Ltd., Japan). All drugs were applied to the preparation through perfusion. Senktide, L659,877 and capsaicin were first dissolved in DMSO (dimethyl sulphoxide), but other drugs were dissolved in distilled water. They were further diluted with the perfusion solution to various concentrations before application. Tachykinin receptor agonists and other agonists were applied for 60 s. Tachykinin receptor antagonists were applied for at least 20 min before their antagonistic effects were examined and kept perfused throughout agonist application.

Results

Types of neurones in coeliac ganglion

Neurones in the coeliac ganglion were classified on the basis of their firing patterns in response to depolarizing current pulses. Two major types of neurone were identified (Zhao *et al.*, 1995): (1) neurones that exhibited maintained firing during the depolarizing pulse (tonic neurones) which comprised 37.9% (153

out of 404 randomly sampled neurones) and (2) neurones with an initial burst of discharges (phasic neurones), which comprised 39.3% (159/404). Neurones which exhibited an initial burst of discharges followed by maintained firing or only an initial single spike discharge were classified as atypical neurones (92/404). In most of the phasic neurones (91.2%), a long after-hyperpolarization (LAH) lasting 5–8 s, and 10–20 mV in amplitude was evoked following action potentials induced by a train of depolarizing pulses (see Methods). In contrast, the LAH was observed in only 4.6% of tonic neurones. In the following experiments we analyzed the responses of the two major types of neurone in the ganglion, i.e. tonic neurones without obvious LAH and phasic neurones with typical LAH (lasting more than 5 s with amplitude larger than 10 mV).

Effects of mesenteric nerve stimulation

Repetitive electrical stimulation of the postganglionic mesenteric nerves induced slow e.p.s.ps in most of the tonic neurones (110 out of 122, Table 1; Figure 1a). Action potentials, superimposed on fast e.p.s.ps, usually preceded the slow e.p.s.ps. The amplitude and time course of the slow e.p.s.ps depended on the number of stimulus pulses, reaching a maximum amplitude of 15–20 mV and duration of 3–10 min with 40–80 pulses. An interval longer than 10 min between tests was necessary to produce reproducible slow e.p.s.ps. In about 20% of tonic neurones a burst of action potentials lasting for 1–2 min was superimposed on the peak depolarization of slow e.p.s.ps.

The majority of phasic neurones, in contrast, did not produce slow e.p.s.ps or produced only small amplitude slow e.p.s.ps (<3 mV) in response to the mesenteric nerve stimulation. However, the magnitude of LAH was reduced for a prolonged period following nerve stimulation in 75% of phasic neurones (48 out of 64, Table 1; Figure 1b). The LAH inhibition appeared within 30 s after nerve stimulation and lasted for 3–10 min. Thus, the time course of the LAH inhibition in phasic neurones was similar to that of slow e.p.s.ps in tonic neurones (Figure 1). The extent and duration of LAH inhibition increased with the number of pulses applied to the mesenteric nerves, reaching a maximum at 40–80 pulses. However, the maximum amplitude of stimulation-induced slow e.p.s.ps and the extent of stimulation-induced LAH inhibition as well as their durations were different from neurone to neurone.

Effects of tachykinin receptor agonists

We previously found that bath applications of SP (3 nM–1 μM), NKA (30 nM–1 μM) and senktide (0.1 nM–100 nM), an NK_3-receptor selective agonist (Wormser *et al.*, 1986), induced depolarizations in most of the tonic neurones whereas they induced inhibition of LAH without obvious depolarization in most of the phasic neurones (Zhao *et al.*, 1995). The effects of mesenteric nerve stimulation described above are therefore mimicked by exogenously applied tachykinin receptor agonists. In order to establish a correlation between the effect of tachykinin receptor agonists and the occurrence of nerve-evoked responses, the effects of SP, NKA and senktide were examined in tonic and phasic neurones that had responded with typical slow e.p.s.ps and inhibition of LAH, respectively, to mesenteric nerve stimulation. A high proportion of these neurones (about 80%) responded to all of these tachykinin receptor agonists with the same type of response as those evoked by nerve stimulation (Table 2). However, among the 66 tonic neurones showing e.p.s.ps, 7 neurones were depolarized by only senktide and 6

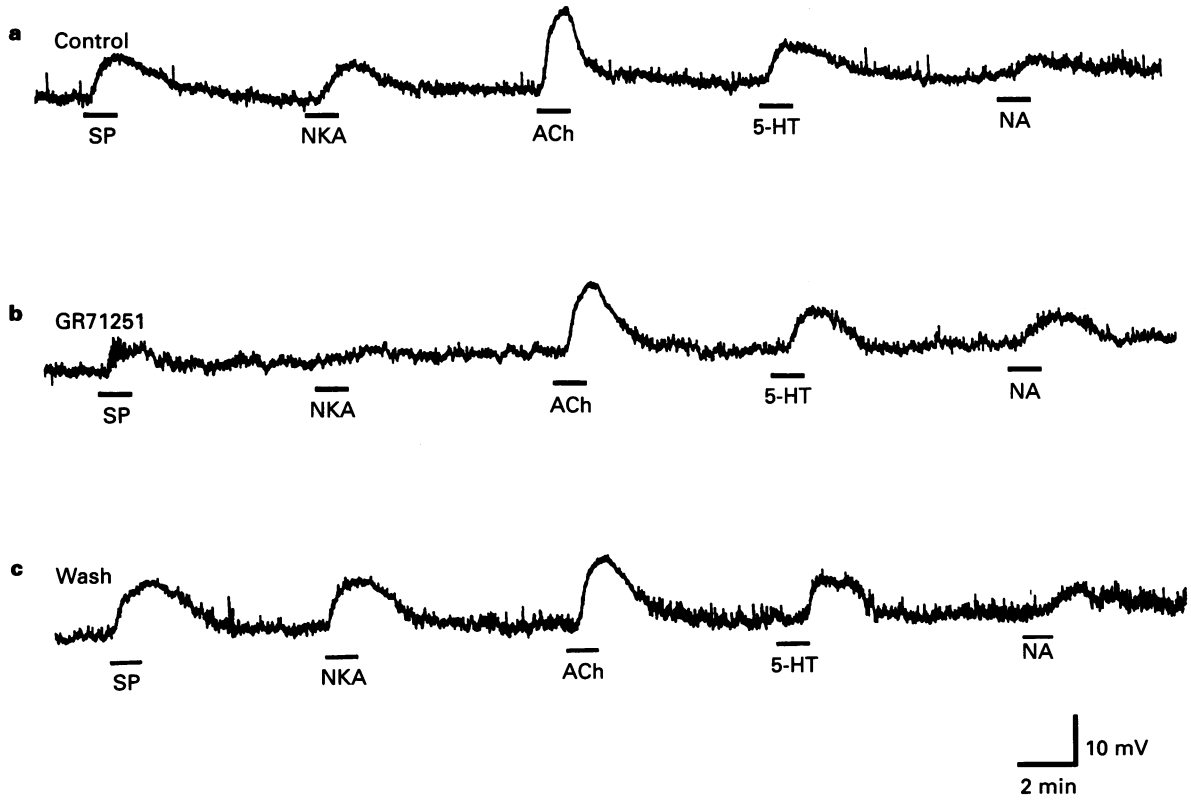

Figure 4 Effects of GR71251 on the depolarizing responses evoked by various agonists in a tonic neurone. (a) Control responses to substance P (SP, 1 μM), neurokinin A (NKA, 1 μM), acetylcholine (ACh, 100 μM), 5-hydroxytryptamine (5-HT, 10 μM) and noradrenaline (NA, 10 μM); (b) responses in the presence of GR71251 (5 μM), the application of which started 15 min before the record; and (c), responses 17 min after washing out the antagonist. Each agonist was applied for 1 min during the period indicated by a horizontal bar. The response to NA was slightly potentiated under GR71251 in this experiment, but was not affected in other two experiments. The resting potential at the start of experiment was −48 mV.

neurones by none of the tachykinin receptor agonists. Similarly, among the 18 phasic neurones showing LAH inhibition, 3 responded only to senktide and one neurone to none of the tachykinin receptor agonists.

Effects of tachykinin receptor antagonists on slow e.p.s.ps and inhibition of LAH

GR71251 (5 μM), a tachykinin NK_1 receptor antagonist (Ward *et al.*, 1990), reduced the magnitude of the mesenteric nerve-evoked slow e.p.s.ps in tonic neurones, expressed as the area between the slow e.p.s.ps and the baseline, to $33.6 \pm 5.5\%$ (2.5% to 70.8%, $n=10$,) of the control (Figures 2a and 3). In the tonic neurones with nerve-evoked slow e.p.s.ps, GR71251 (5 μM) reduced the area of depolarization induced by SP (10–100 nM) and NKA (0.3–1 μM) to $40.8 \pm 4.3\%$ ($n=8$) and $27.8 \pm 5.9\%$ ($n=6$) of the controls, respectively, whereas it did not affect the depolarization induced by senktide (10–100 nM) ($100.4 \pm 9.3\%$, $n=6$)(cf. Zhao *et al.*, 1995).

In phasic neurones, the extent of LAH inhibition induced by the nerve stimulation (for details see the legend to Figure 3) was similarly reduced by GR71251 (5 μM) to $40.6 \pm 8.4\%$ (20.3% to 61.7%, $n=4$) of the control (Figures 2b and 3). GR71251 (5 μM) also depressed the actions of exogenously applied SP and NKA to inhibit LAH in phasic neurones to $42.8 \pm 9.0\%$ ($n=6$) and $58.1 \pm 6.0\%$ ($n=4$), respectively, but not that of senktide ($92.0 \pm 5.7\%$, $n=7$)(cf. Zhao *et al.*, 1995).

In order to examine the specificity of GR71251, effects of the antagonist on responses to various agonists were examined. As shown in Figure 4, the depolarizations induced by SP and NKA were inhibited by GR71251 at 5 μM, whereas those induced by acetylcholine, 5-HT and noradrenaline were not obviously affected ($n=3$) (Figure 4).

L659877 (10 μM, $n=3$), an NK_2 receptor antagonist (McKnight *et al.*, 1991), did not inhibit the depolarizations induced by SP (0.1–1 μM, $n=5$), NKA (0.1–1 μM, $n=7$) and senktide (0.3–30 nM, $n=4$), nor the nerve-evoked slow e.p.s.ps ($n=4$) in tonic neurones.

Effects of capsaicin on tonic and phasic neurones

Capsaicin is known to activate certain primary afferent C neurones and evoke a release of sensory peptides, including SP and NKA (for reviews see Holzer, 1991; Maggi, 1991). Capsaicin (0.1–1 μM) depolarized tonic neurones by 12.3 ± 1.6 mV (Figure 5a, $n=7$), as was shown previously in the inferior mesenteric ganglion neurones (Tsunoo *et al.*, 1982). In phasic neurones, capsaicin (0.3~5 μM) reduced the magnitude of LAH to $24.0 \pm 5.8\%$ of the control ($n=5$) without inducing obvious depolarization (Figure 5b).

Effects of peptidase inhibitors on slow e.p.s.ps and the inhibition of LAH

It has been suggested that endogenous tachykinins released from nerve fibres are degraded by tissue peptidases (Lee *et al.*, 1981, Matsas *et al.*, 1985; Oblin *et al.*, 1988; Yanagisawa *et al.*, 1992; Suzuki *et al.*, 1994). We have therefore ex-

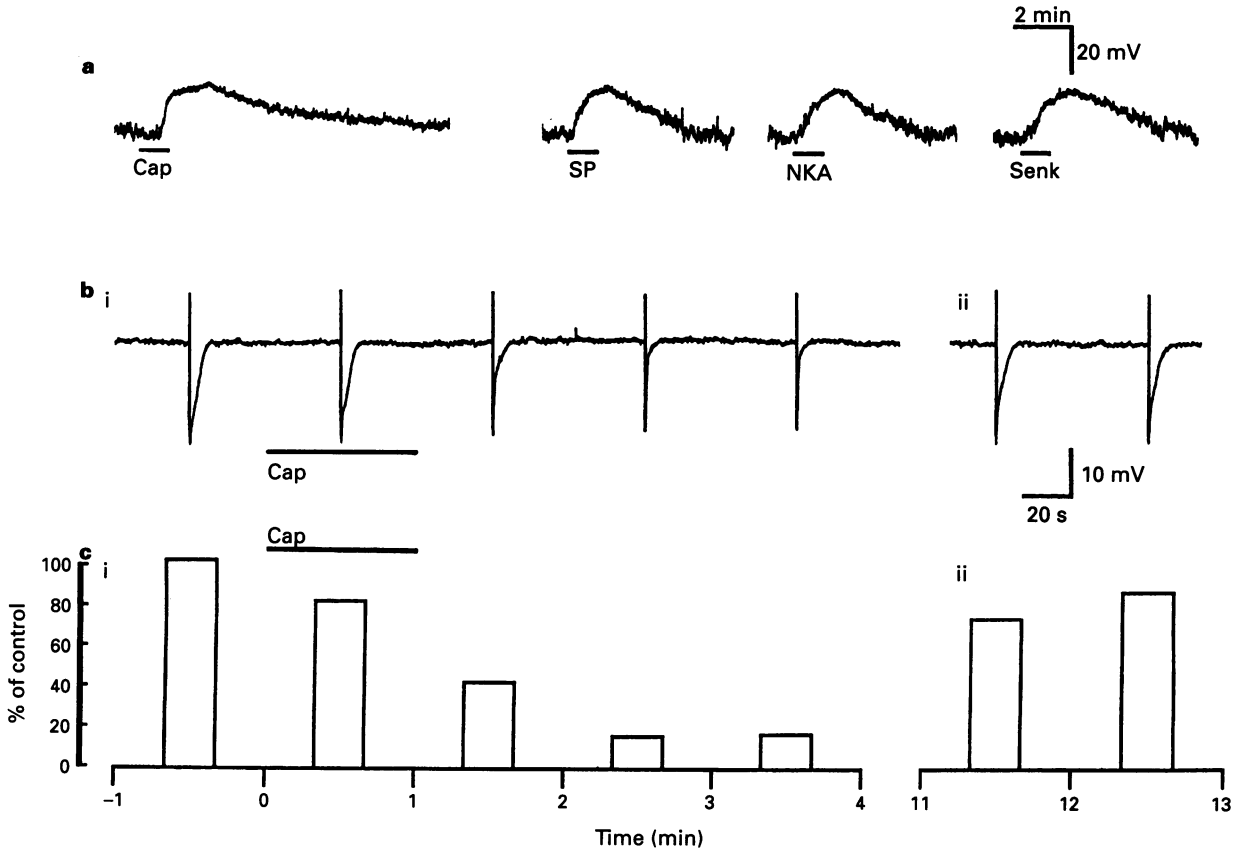

Figure 5 Depolarization and inhibition of the long after-hyperpolarization (LAH) induced by capsaicin. (a) Depolarizations induced by capsaicin (Cap, 0.1 μM), substance P (SP, 1 μM), neurokinin A (NKA, 1 μM) and senktide (Senk; 100 nM) in a tonic neurone. Capsaicin and tachykinin receptor agonists were applied for 1 min during the periods marked by horizontal bars. (b) Inhibition of LAH induced by capsaicin (Cap, 5 μM) in a phasic neurone. Each LAH was evoked by a train of depolarizing current pulses (10 pulses of 1 nA and 20 ms at 20 Hz). (c) Time course of change in the magnitude of LAH (see the legends to Figures 1 and 2). The same experiment as shown in (b). (bii) and (cii) show the recovery after washing out capsaicin. Capsaicin was applied for 1 min during the period marked by horizontal bars in (b) and (c). The resting potentials at the start of experiment were −48 mV in (a) and −56 mV in (b), respectively.

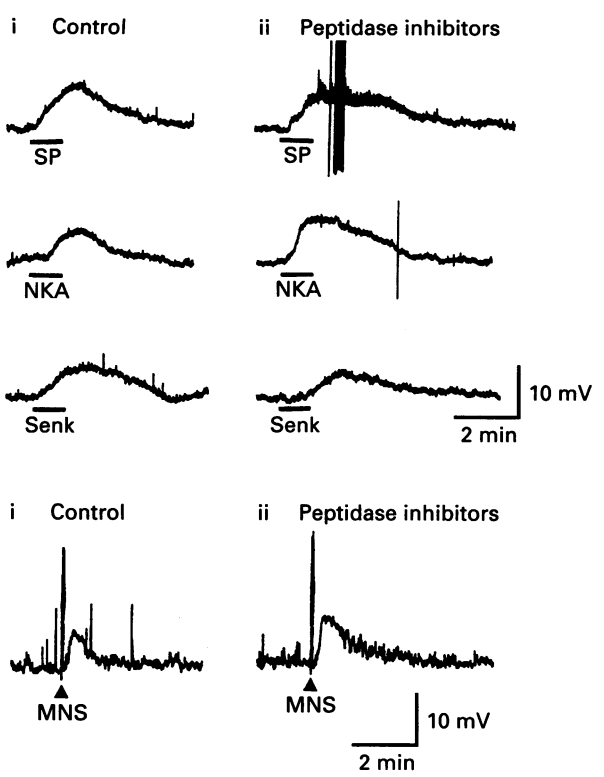

Figure 6 Effect of peptidase inhibitors on the depolarizing responses evoked by tachykinin receptor agonists and slow e.p.s.ps in tonic neurones. (a) Responses to substance P (SP, 0.3 μM), neurokinin A (NKA, 0.3 μM) and senktide (Senk, 30 nM). These agonists were applied for 1 min as indicated by the horizontal bars. (b) Slow e.p.s.ps evoked by mesenteric nerve stimulation (MNS; 40 pulses of 500 μs and 100 V at 20 Hz). (i) Control responses in the presence of naloxone 0.3 μM; (ii) in the presence of actinonin (10 μM), captopril (10 μM), thiorphan (0.3 μM) and naloxone (0.3 μM). The records in (a) were derived from a single neurone, and the records in (b) were from another neurone. Note that the SP-evoked depolarization in (a) was potentiated by the mixture of peptidase inhibitors so that action potentials were superimposed on the depolarization. The peaks of action potentials were cut off. The resting potentials at the start of experiment were −60 mV in (a) and −53 mV in (b), respectively.

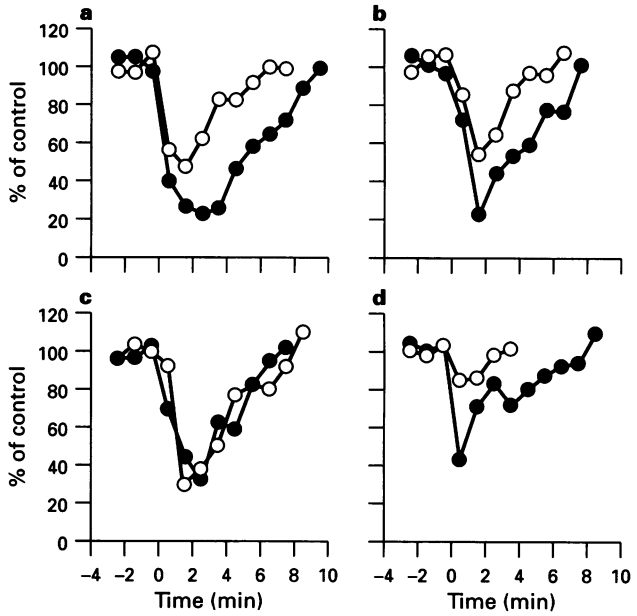

Figure 7 Effect of peptidase inhibitors on the inhibition of the long after-hyperpolarization (LAH) evoked by tachykinin receptor agonists and nerve stimulation in a phasic neurone. Tachykinin receptor agonists were applied for 1 min, starting at time 0 on abscissa scale. (a) Substance P (SP, 0.1 μM); (b) neurokinin A (NKA, 0.3 μM); and (c) senktide (Senk, 10 nM). In (d) the mesenteric nerves were stimulated with 40 pulses of 500 μs and 100 V at 20 Hz at time 0. Ordinate scales, the magnitude of LAH which was determined as area between the LAH and the resting potential level on the record and expressed as percentage of average of the magnitudes of three successive control LAHs immediately preceding the agonist application or the mesenteric nerve stimulation. (○), Time courses of control responses in the presence of naloxone 0.3 μM; (●), in the presence of peptidase inhibitors, actinonin (10 μM), captopril (10 μM), thiorphan (0.3 μM) and naloxone (0.3 μM).

amined the effects of peptidase inhibitors on the mesenteric nerve-evoked responses and those evoked by tachykinin receptor agonists. The experiments were carried out in the presence of naloxone (0.3 μM) in order to exclude the possible involvement of the inhibitory action of endogenous enkephalins, because the peptidase inhibitors may also potentiate the effect of the enkephalinergic component in the nerve-evoked response so that their effect on the tachykininergic component may be masked (Suzuki *et al.*, 1994). A mixture of peptidase inhibitors, consisting of actinonin (10 μM), captopril (10 μM) and thiorphan (0.3 μM), potentiated not only the mesenteric nerve-evoked slow e.p.s.ps in tonic neurones to 305.7±64.5 of the control ($n=5$, Figure 6b) but also the extent of inhibition of LAH in phasic neurones to 179.8±23.5% of the control ($n=4$, Figure 7d). In tonic neurones, the depolarizations induced by exogenously applied SP and NKA were also potentiated by the mixture of peptidase inhibitors to 570.0±127.6% ($n=5$) and 466.3±111.9% ($n=6$), while the depolarization induced by senktide remained unchanged (93.5±27.5%, $n=5$) (Figure 6a). In phasic neurones, the extents of LAH inhibition induced by exogenous application of SP and NKA were also potentiated to 339.0±82.4% ($n=4$) and 410.2±83.2% ($n=3$), respectively, whereas that by senktide was unaffected (95.1±6.0%, $n=4$, Figure 7a,b,c).

Discussion

The present study showed that two different responses of slow time course, i.e. the slow e.p.s.ps and the LAH inhibition are evoked by mesenteric nerve stimulation in guinea-pig coeliac ganglion neurones and that the occurrence of these responses is closely correlated with the electrophysiologically defined types of neurones. Furthermore, this study provided evidence suggesting that tachykinins in sensory fibres contribute to the nerve-evoked slow e.p.s.ps in tonic neurones and LAH inhibition in phasic neurones.

Meckler & McLachlan (1988) suggested that tonic neurones preferentially receive synaptic input from axons of peripheral origin, whereas phasic neurones mainly receive synapses from the greater splanchnic nerve. In the present study the slow e.p.s.ps evoked by mesenteric nerve stimulation were observed in most (90%) of tonic neurones but in only 14% of phasic neurones examined. However, the present study revealed that mesenteric nerve stimulation evoked a prolonged inhibition of LAH in 73% of phasic neurones, but in none of the tonic neurones. This indicates that non-cholinergic innervation is not confined to tonic neurones, but is also present on phasic neurones. The LAH, which follows action potentials, may prevent the neurones from long lasting further excitation (Adams *et al.*, 1986; Yoshimura *et al.*, 1986). Therefore, although these two types of response, the slow e.p.s.ps and the inhibition of LAH, are apparently different, both of the responses may serve to enhance neuronal excitation.

Both the slow e.p.s.ps and the inhibition of LAH evoked by mesenteric nerve stimulation were partially blocked by GR71251 and potentiated by a mixture of peptidase inhibitors. Application of exogenous tachykinins and capsaicin mimicked

the nerve-evoked responses in both tonic and phasic neurones. These results suggest that SP and NKA, released from sensory C fibres act as neurotransmitters and are partially responsible for the nerve-evoked slow e.p.s.ps and the inhibition of LAH. The potency of NKA to induce these responses was about one order of magnitude weaker than SP (Zhao *et al.*, 1995). Furthermore, the amount of NKA in the guinea-pig coeliac ganglion was 76 fmol mg^{-1}protein, which was less than half of SP (177 fmol mg^{-1}protein; unpublished observation). Also, in rat coeliac-superior mesenteric ganglia the content of SP was 3 times higher than that of NKA (Konishi *et al.*, 1989). These results suggest that the contribution of SP may be more important than that of NKA in the generation of the mesenteric nerve-evoked slow e.p.s.ps and the inhibition of LAH.

The actions of SP to induce depolarization and inhibition of LAH were effectively antagonized by the NK_1 receptor antagonist GR71251 (Ward *et al.*, 1990), suggesting that both actions of SP are mediated by NK_1 receptors. Although NKA is known to have a preferential affinity for NK_2 receptors, it also has a weak affinity for NK_1 receptors (for a review see Otsuka & Yoshioka, 1993). GR71251 antagonized the actions of NKA to induce depolarization in tonic neurones and LAH inhibition in phasic neurones with similar potencies to those against the actions of SP, whereas the NK_2-selective antagonist, L659,877 (McKnight *et al.*, 1991), was ineffective in antagonizing the actions of NKA (Zhao *et al.*, 1995 and present study). These results suggest that both the depolarization and the inhibition of LAH induced by NKA are mediated by NK_1 but not NK_2 receptors. All neurones responsive to NKA also responded to SP with the effects of NKA being weaker than those of SP. These results suggest that SP and NKA act on a single subtype of NK_1 receptor. An alternative possibility is that the NK_1 receptors might be divided into further subtypes that can not be distinguished by GR71251. We did not observe any obvious difference between the pharmacological profiles of the tachykinin receptors responsible for the depolarization and those responsible for the inhibition of LAH. This suggests that in both phasic and tonic neurones the tachykinin receptors present are of the same types but they are coupled to different effector mechanisms in the two types of neurones.

This study showed that the selective NK_3 receptor agonist, senktide (Wormser *et al.*, 1986), had a potent effect on large proportions of coeliac neurones, which suggests the presence of NK_3 receptors on these neurones. The existence of neurones responding to senktide but not to SP and NKA suggests that there may be neurones with only NK_3 receptors. Among the endogenous tachykinins neurokinin B has a high affinity to NK_3 receptors. Although [Trp^7, β-Ala^8]NKA_{4-10}, which has been shown to be an NK_3 antagonist (Drapeau *et al.*, 1990), did not inhibit the effects of mesenteric nerve stimulation. This compound, at up to 3 μM, did not antagonize the actions of senktide, either (data not shown). In an additional experiment, [$MePhe^7$]NKB, another selective NK_3 receptor agonist, also evoked depolarizations in the tonic neurones (cf. Zhao *et al.*, 1995) and LAH inhibition in the phasic neurones ($n=3$, data not shown). These results also support the hypothesis that NK_3 receptors exist in the ganglion. However, the contribution of NK_3 receptors in the ganglionic synaptic transmission remains to be confirmed by further experiments with highly selective, potent NK_3 receptor antagonists.

Some neurones responded with slow e.p.s.ps or inhibition of LAH to nerve stimulation, but did not respond to all 3 tachykinin receptor agonists (Table 2). Furthermore, GR71251 only partly reduced the magnitudes of the nerve-evoked slow e.p.s.ps and the inhibition of LAH, and the extent of the reduction varied from neurone to neurone. These results suggest that neurotransmitters other than SP and NKA may also contribute to the slow synaptic responses. In addition to tachykinins, 5-HT, cholecystokinin, vasoactive intestinal polypeptide and calcitonin gene-related peptide have been suggested to be involved in ganglionic transmission (Dun *et al.*, 1984; Love & Szurszewski, 1987; Dun & Mo, 1989).

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