Journal of Physiology (1989), **411**, *pp.* 325–345
With 9 text-figures
Printed in Great Britain

NEUROTENSIN FACILITATES RELEASE OF SUBSTANCE P IN THE GUINEA-PIG INFERIOR MESENTERIC GANGLION

BY W. H. STAPELFELDT AND J. H. SZURSZEWSKI

From the Department of Physiology and Biophysics and Gastroenterology Unit, Mayo Foundation, Rochester, MN 55905, USA

(*Received 8 January 1988*)

SUMMARY

1. Intracellular, electrophysiological techniques were combined with radioimmunological, chromatographic and pharmacological techniques to determine if nerve terminals containing substance P mediated transient depolarizing responses of principal ganglion cells induced by neurotensin. Experiments were performed *in vitro* on guinea-pig inferior mesenteric ganglia.

2. In 61% of principal ganglion cells tested in normal ganglia, neurotensin$^{8-13}$ caused a transient membrane depolarization. In ganglia which were removed from animals which had been pre-treated with capsaicin, transient responses to neurotensin were virtually abolished.

3. In normal ganglia, neurotensin$^{8-13}$ increased the amplitude and duration of non-cholinergic slow EPSPs evoked by electrical stimulation of the lumbar colonic nerve. Such increases were absent in ganglia obtained from animals pre-treated with capsaicin.

4. In guinea-pigs pre-treated with capsaicin, the content of substance P-like material was significantly reduced in inferior mesenteric and coeliac ganglia, dorsal root ganglia and lumbar spinal cord, compared to control animals. The content of substance P-like material in segments of distal colon was slightly reduced. The content of vasoactive intestinal polypeptide-, cholecystokinin- and bombesin-like material in the same tissues from animals pre-treated with capsaicin was not significantly different from control animals.

5. Chromatographic analysis using HPLC (high-performance liquid chromatography) techniques revealed that the material depleted from inferior mesenteric and coeliac ganglia, dorsal root ganglia and lumbar spinal cord by capsaicin pre-treatment co-eluted with synthetic substance P.

6. Electrical stimulation of the lumbar colonic nerve released substance P-like material from isolated inferior mesenteric ganglia as determined by radioimmunoassay of samples of superfusate. Exogenous administration of neurotensin$^{8-13}$ caused a significant increase in the amount of substance P-like material released during nerve stimulation.

7. Transient depolarizing responses evoked by neurotensin were markedly attenuated when ganglion cells were postsynaptically desensitized to exogenously administered substance P.

8. Taken together, these findings suggest that transient depolarizations mediated by an indirect action of neurotensin and facilitation of electrically evoked non-cholinergic slow EPSPs by neurotensin involved presynaptic release of substance P from collateral nerve terminals of primary afferent nerve fibres in the inferior mesenteric ganglion.

9. It was suggested that under normal *in vivo* conditions, neurotensin or a C-terminal-related peptide contained in central preganglionic nerve endings might function as an excitatory neuromodulator to enhance the release of substance P from primary afferent nerve terminals thereby facilitating non-cholinergic peripheral afferent synaptic input to prevertebral ganglion cells.

INTRODUCTION

Neurotensin-like material has been identified immunohistochemically in central preganglionic nerve fibres innervating prevertebral sympathetic ganglia (Lundberg, Hökfelt, Änggard, Uvnäs-Wallensten, Brimijoin, Brodin & Fahrenkrug, 1980; Lundberg, Rökaeus, Hökfelt, Rosell, Brown & Goldstein, 1982; Reinecke, Forssmann, Thiekötter & Triepel, 1983; Heym, Reinecke, Weihe & Forssmann, 1984). Electrophysiological studies have shown that neurotensin increases the excitability of sympathetic neurones by a direct action on the postsynaptic membrane and predominantly by an indirect action on presynaptic nerve terminals to release a non-cholinergic excitatory neurotransmitter (Stapelfeldt & Szurszewski, 1989). Substance P-, vasoactive intestinal polypeptide-, cholecystokinin- and bombesin-like materials are possible candidates mediating the indirect response to neurotensin. Each of these neuropeptides has been demonstrated by immunohistochemical techniques to be present in nerve terminals around sympathetic neurones, each causes a slow excitatory membrane response when exogenously applied and each is thought to mediate slow depolarizing membrane responses following electrical stimulation of afferent nerve trunks and radial distention of the colon (Hökfelt, Elfvin, Schultzberg, Goldstein & Nilsson, 1977; Dalsgaard, Hökfelt, Elfvin, Skirboll & Emson, 1982; Matthews & Cuello, 1982; Tsunoo, Konishi & Otsuka, 1982; Dun & Jiang, 1982; Dun & Kiraly, 1983; Dalsgaard, Hökelt, Schultzberg, Lundberg, Terenius, Dockray & Goldstein, 1983*a*; Mo & Dun, 1984, 1986; Peters & Kreulen, 1984, 1986; Kreulen & Peters, 1986; Schumann & Kreulen, 1986; Love & Szurszewski, 1987).

The aim of the present study was to identify the transmitter(s) involved in the indirect depolarizing action of neurotensin. Studies were performed *in vitro* using intracellular recording techniques in conjunction with pharmacological, radioimmunological and chromatographic techniques.

Evidence will be presented to support the hypothesis that neurotensin acted on primary afferent nerve terminals in guinea-pig inferior mesenteric ganglia to facilitate release of substance P. Some of these findings have been communicated previously (Stapelfeldt, Go & Szurszewski, 1987, 1988).

METHODS

Tissue preparation for radioimmunological analysis and for electrophysiological experiments

Adult male guinea-pigs (300–500 g body weight) were killed by a blow to the head and bled. The abdominal cavity was opened and the vertebral column from Th12 to S1 and attached viscera including the prevertebral ganglia (inferior mesenteric ganglion and coeliac plexus) and a segment of the distal colon were rapidly dissected from the animal and placed in a dissection dish containing ice-cold oxygenated (97% O_2–3% CO_2) Krebs–Ringer Buffer (KRB) of the following composition (mM): Na^+, 137·4; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 134; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2; glucose, 11·5.

After rapidly removing the overlying skin and posterior skeletal muscle of the back, the lumbar vertebral column was laminectomized and the transverse processes of the vertebrae removed to gain access to the spinal cord and dorsal root ganglia. A 2 cm segment of the lumbar spinal cord and dorsal root ganglia from segments L1 to L4 were removed and immediately frozen on dry ice for subsequent radioimmunological analysis for the content of substance P, vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK) and bombesin (BOM). A 3 cm segment of distal colon was removed from its mesenteric attachment and also immediately frozen. Adherent mesenteric adipose and connective tissue sheets were removed from the prevertebral ganglia. The coeliac plexus (containing left and right coeliac and superior mesenteric ganglia) was cut free and immediately frozen. The inferior mesenteric ganglion with its nerve trunks was then transferred to an electrophysiological chamber and superfused with oxygenated KRB solution at 35–37 °C. Bipolar platinum electrodes were attached to the lumbar colonic nerve and connected to a stimulus isolation unit (Grass SIU5A) and stimulator (Grass S88). Intracellular recordings were made from single ganglion cells using methods described previously (Stapelfeldt & Szurszewski, 1989). Slow excitatory postsynaptic potentials (slow EPSPs) were evoked by repetitive stimulation of the lumbar colonic nerve. Rectangular current pulses of 500 μs duration, at just-supramaximal intensities and at a frequency of 15 Hz for 5–8 s duration were used.

Following the electrophysiological experiments, the inferior mesenteric ganglion was removed from the bath and frozen on dry ice for subsequent analysis for substance P, VIP, CCK and BOM. Thus, electrophysiological and radioimmunological data were obtained from the same ganglion. When a ganglion was used for both electrophysiological and radioimmunological experiments, neurotensin$^{8-13}$ (Sigma Chemicals, St Louis, MO, USA) was used instead of neurotensin$^{1-13}$. It has been shown that neurotensin$^{8-13}$ causes similar electrophysiological changes to neurotensin$^{1-13}$ (Stapelfeldt & Szurszewski, 1989). Neurotensin$^{8-13}$ was used so as to avoid any possible interference with the radioimmunoassay for substance P. Although unlikely because of the duration of the experiments, it was nevertheless possible that some of the exogenously added peptide may not have been completely washed out of the preparation or completely degraded by the time a ganglion was removed from the bath and frozen. Neurotensin$^{8-13}$ was safe in this regard since it did not interfere with the substance P radioimmunoassay employed as did authentic neurotensin$^{1-13}$ in the micromolar concentration range used (W. H. Stapelfeldt, V. L. W. Go & J. H. Szurszewski, unpublished observation). When neurotensin$^{8-13}$ was used, it was dissolved in KRB solution to a final concentration of 5 μM and was administered by superfusion. In experiments designed only for intracellular recording, neurotensin$^{1-13}$ and substance P (Sigma Chemicals) were used and were applied by superfusion over the ganglia.

In vivo capsaicin treatment

Six animals were pre-treated with capsaicin *in vivo* before they were killed. The protocol used resembled those described earlier (Gamse, Wax, Zigmond & Leeman, 1981; Dalsgaard, Vincent, Schultzberg, Hökfelt, Elfvin, Terenius & Dockray, 1983*c*; Peters & Kreulen, 1984). Guinea-pigs were anaesthetized with Ketalar (ketamine, 50 mg/kg I.M.). Capsaicin (Sigma Chemicals, St Louis, MO, USA) was dissolved (50 mg/ml) in a vehicle consisting of 10% ethanol: 10% Tween 80 (Sigma Chemicals): 80% physiological saline (v/v/v) and was administered subcutaneously at the back of the neck. Injections were given at a volume of 1 ml/kg on each of two consecutive days to achieve a total dose of 100 mg/kg in each animal. Prior to the first administration, each guinea-pig received an injection of theophylline (5 mg/kg I.P.) and was made to inhale an aerosol of 0·25% isoprenaline hydrochloride (Isuprel®) to alleviate respiratory distress during capsaicin-induced

bronchoconstriction. For the same reason, each animal was placed in a chamber gassed with 95% O_2–5% CO_2 and a mist of isoprenaline hydrochloride for 45 min immediately following the first injection of capsaicin. The animals were killed 5–8 days after the second and final injection of capsaicin.

Radioimmunoassay procedures

Specimens consisting of frozen spinal cord, dorsal root ganglia, coeliac plexus, inferior mesenteric ganglia and distal colon from normal and capsaicin-treated guinea-pigs were individually weighed, dissolved (1:20) in 0·1 M-HCl, boiled for 20 min and homogenized with a Polytron® homogenizer (Brinkmann Instrument Co., Westbury, NY, USA). After centrifugation for at least 20 min, the supernatant of each specimen was decanted and stored at −20 °C for subsequent neuropeptide analysis. Previous studies have shown this extraction procedure to yield reliable recoveries of substance P, VIP and CCK-8 (Yaksh, Abay & Go, 1982) for specific radioimmunoassays.

Radioimmunoassay for substance P. The assay method employed was similar to one previously described (Micevych, Yaksh & Go, 1982). Synthetic (Try^8)-substance P (Peninsula Labs, Belmont, Ca, USA) was ^{125}I-radiolabelled by a modified Greenwood-Hunter method. Purification steps included adsorption to cellulose powder, washing with 0·2 M-glycine (pH 8·8), elution with acidified plasma (10% human plasma, 0·2 M-acetic acid), and gel chromatography over a Bio-Gel P10 column (Bio Rad Labs, Richmond, CA, USA). The assay buffer (pH 6·5) consisted of 0·04 M-KH_2PO_4, 1% bovine serum albumin (BSA), 0·01 M-EDTA, 2% polyethylene glycol (PEG) (8000), 0·25% Trasylol® (aprotinin) and 0·2% thimerosal. Sample volumes of 0·1 ml diluted substance P standard or neutralized tissue extract were incubated with 0·1 ml rabbit substance P antiserum (No. 4892, 1:15000) and 0·7 ml assay buffer for 16 h of pre-incubation. ^{125}I-Substance P diluted in assay buffer containing 4% normal rabbit serum at a specific activity of 8000 c.p.m. per 0·1 ml was added and incubated for an additional 24 h. The bound complex was separated by adding a titred amount of goat anti-rabbit serum (Calbiochem, San Diego, CA, USA), incubated for another 30 min and centrifuged at 2500 r.p.m. for 15 min (double-antibody method). After discarding the supernatant, activity of the bound antibody was counted in a γ-counter for 5 min. The detection threshold (assay sensitivity) was 1·0 pg/tube and the inter- and intra-assay variations were 11 and 5%, respectively. The antiserum showed 100% cross-reactivity with the oxidized molecular form of substance P and less than 1% cross-reactivity with other tachykinins such as kassinin, neurokinin A and substance K.

Radioimmunoassay for VIP. Rabbit VIP antiserum (No. 4823) was raised against natural VIP coupled to BSA, as previously described (Yaksh *et al.* 1982). The VIP antiserum was pre-incubated in a 1:100000 dilution with 200 μl standard or tissue extract samples in 0·01 M-phosphate buffer (pH 7·6) containing 0·05 M-EDTA, 0·2 M-benzamidine and 0·1% (w/v) BSA at a total volume of 0·8 ml for 15–18 h. ^{125}I-VIP, prepared by the chloramine-T method, was added at 1500 c.p.m. per 0·1 ml and incubated for another 24 h. In a similar separation procedure as described above, 0·1 ml of goat anti-rabbit serum, 0·2 ml of 2% normal rabbit serum and 4% PEG (8000) were added, incubated for another 6 h, and the bound activity counted after centrifugation. This procedure yielded an assay sensitivity of 2 pg/tube at inter- and intra-assay variations of 10 and 4% respectively.

Radioimmunoassay for CCK. The radioimmunoassay procedure employed was similar to that previously published (Owyang, Miller, DiMagno, Brennan & Go, 1979) and basically resembled those described above. Synthetic CCK rabbit antiserum (No. 3440) was incubated with the sample and ^{125}I-CCK for 72 h in assay buffer consisting of 0·2 M-sodium barbitone (pH 8·4), 0·02% sodium azide, 0·2% human serum albumin, 0·01 M-EDTA and 2% PEG (8000) before separation. The assay sensitivity was 2 fmol/tube at an 8% intra-assay and 9% interassay variation.

Radioimmunoassay for BOM. Rabbit bombesin antiserum (No. N388) was raised against a synthetic bombesin–BSA conjugate as previously described (Angel, Go & Szurszewski, 1984). The rabbit bombesin antiserum was incubated at a final dilution of 1:8000 in 0·02 M-sodium barbitone buffer (pH 8·4) containing 0·02% sodium azide, 0·2% human serum albumin, 0·01 M-EDTA and 2% PEG (8000) with the sample and ^{125}I-bombesin of a specific activity of 300–700 Ci/nmol for 72 h, followed by double-antibody separation. Intra- and interassay variations were 10 and 15%, respectively, at a detection threshold of 2 pg/tube.

High-pressure liquid chromatography (HPLC)

Pooled extraction samples were lyophilized, reconstituted in the starting solution consisting of 10% acetonitrile in 0·1 M-sodium acetate and 0·1% morpholine and injected on a reverse-phase column (Rainin microsorb C18 Short-One, Rainin Instrument Co., Woburn, MA, USA). At a flow rate of 0·5 ml/min, a linear gradient from 10 to 60% acetonitrile was run over a period of 50 min. Fractions were collected at 1 min intervals, lyophilized, reconstituted in assay buffer, and analysed by radioimmunoassay as described above.

Neuropeptide release experiments

Isolated inferior mesenteric ganglia together with their peripheral nerve trunks were obtained from guinea-pigs using a similar dissection procedure as described above. For each experiment, three ganglia were pinned down in a same small (250 μl) electrophysiological chamber and superfused with oxygenated KRB solution at 35–37 °C. The lumbar colonic nerve trunks of each of these ganglia were attached to bipolar platinum electrodes. All three nerve trunks were stimulated simultaneously. To verify the functional integrity of each preparation, intracellular recordings were obtained from individual ganglion cells before, during and after collection of superfusate. In order to facilitate diffusion of released neuropeptides out of the ganglia into the superfusion medium, the preparations were superfused initially for 10 min with KRB solution containing 0·01% collagenase (Sigma Chemicals) before collection of superfusate. BSA (0·2% w/v) and 500 U/ml Trasylol (aprotinin) were added to the superfusion medium throughout the remainder of the experiment to facilitate recovery and diminish enzymatic break-down of released neuropeptides. The concentration of BSA and Trasylol used in these experiments did not interfere with either electrical responses recorded intracellularly during the experiment (cf. Fig. 6) or the radioimmunoassays employed as determined by a recovery of > 90% of neuropeptides added to blank superfusion medium. Samples of superfusate were collected by carefully aspirating 200 μl after a 2 min period during which the flow was stopped to allow for released neuropeptides to diffuse out of the ganglia. Using this sampling procedure, at least four samples were obtained in each experiment with intervals of at least 5 min. A first sample was collected before nerve stimulation. This served as a control. A second sample was collected 1 min after simultaneous electrical stimulation (15 Hz, 40 V, 1 min) of all three lumbar colonic nerves. A third sample was collected 1 min after the end of a 1 min electrical stimulation with the same stimulation parameters, but in the presence of neurotensin$^{8-13}$ (5 μM). A fourth sample was collected after a 2 min period without nerve stimulation and served as another control. The collected samples were immediately frozen on dry ice for subsequent radioimmunoassay analysis as described above.

It is worth restating that neurotensin$^{8-13}$, up to a concentration of 10 μM, did not cross-react with the substance P antiserum used in this study and did not interfere with the binding characteristics of our assay when added to the substance P standards.

Statistical analysis

A χ^2 test was used to test for significance between membrane responses observed in normal and capsaicin-treated ganglia. Otherwise, paired and unpaired sets of data were compared using Student's t test and expressed as mean ± standard error of the mean (mean ± S.E.M.). P values of less than or equal to 0·05 were considered significant.

RESULTS

Electrophysiological effects of neurotensin$^{8-13}$ in inferior mesenteric ganglia obtained from normal and capsaicin-treated animals

Effect on membrane potential

Intracellular recordings were obtained from twenty-one neurones in fifteen normal ganglia. The mean resting membrane potential of these neurones was $-53{\cdot}0 \pm 1{\cdot}6$ mV, a value similar to one reported previously for ganglion cells in guinea-pig inferior mesenteric ganglia (Stapelfeldt & Szurszewski, 1989). When neurotensin$^{8-13}$ (5 μM)

was superfused over these ganglia, fourteen neurones (67%) responded with a slow depolarization which averaged $4{\cdot}3 \pm 0{\cdot}8$ mV in amplitude. In thirteen of these fourteen neurones, the depolarization was transient even though the concentration of neurotensin$^{8-13}$ was maintained constant (Fig. 1*A*). The mean maximum amplitude of the transient depolarization was $4{\cdot}5 \pm 0{\cdot}8$ mV and it was reached in $1{\cdot}3 \pm 0{\cdot}1$ min. The mean total duration of a transient depolarization was $3{\cdot}6 \pm 0{\cdot}4$ min. This type of transient response has been observed previously and has been shown to be caused by release of a non-cholinergic transmitter from presynaptic nerve terminals (Stapelfeldt & Szurszewski, 1989). One of the fourteen neurones tested responded with a 3 mV depolarization which was slower in onset (2·9 min) but was maintained constant throughout the superfusion of neurotensin$^{8-13}$. This response resembled the steady-state type of response previously described (Stapelfeldt & Szurszewski, 1989). It has been suggested that this type of response was caused by direct action of neurotensin$^{8-13}$on the postsynaptic membrane (Stapelfeldt & Szurszewski, 1989).

Intracellular recordings were obtained from seventeen neurones in six ganglia removed from capsaicin-treated animals. The mean resting membrane potential was $-58{\cdot}0 \pm 2{\cdot}1$ mV. This value was significantly ($P < 0{\cdot}05$, unpaired t test) more negative when compared to the resting membrane potential of neurones in normal ganglia. In the presence of neurotensin$^{8-13}$, two of thirteen neurones tested responded with a slow membrane depolarization which was 2 mV in amplitude in both cells. The depolarization of one neurone followed a time course characteristic for a steady-state type of response, i.e. it was slow in onset (3·5 min) and lasted throughout the presence of neurotensin$^{8-13}$ (Stapelfeldt & Szurszewski, 1989). The depolarization of the other neurone showed a transient time course. The transient depolarization reached maximal amplitude (2 mV) within 1·2 min and then repolarized back to the original resting membrane potential. The response lasted for 3·5 min, even though neurotensin$^{8-13}$ was present thereafter. The depolarizing response of this neurone, therefore, resembled the transient type of response seen in normal ganglia (Stapelfeldt & Szurszewski, 1989). While the occurrence of a steady-state type of response was not significantly ($P > 0{\cdot}05$) different in the two groups of ganglia (one out of twenty-one cells in normal ganglia compared to one out of thirteen cells in ganglia of capsaicin-treated animals) the occurrence of a transient-type of response was significantly ($P < 0{\cdot}001$) decreased in the capsaicin-treated group, when compared by the χ^2 test (thirteen out of twenty-one cells in normal ganglia compared to one out of thirteen cells in ganglia of capsaicin-treated animals). This finding suggested that, as is the case after superfusion of capsaicin *in vitro* (Stapelfeldt & Szurszewski, 1989), the transmitter responsible for the mediation of indirect, transient membrane responses was largely depleted in ganglia of animals that were treated with capsaicin *in vivo*.

To study further the presynaptic action of neurotensin, the effect of neurotensin$^{8-13}$ on electrically evoked slow EPSPs was examined in normal and capsaicin-treated guinea-pigs.

Effect on electrically evoked slow EPSPs

In normal ganglia, stimulation of the lumbar colonic nerve at 15 Hz for 5–8 s at supramaximal intensities just as neurotensin$^{8-13}$ (5 μM) reached the preparation

evoked slow EPSPs which were greater in amplitude and longer in duration compared to slow EPSPs evoked in normal KRB solution (Fig. 1*B*). In seven cells in the presence of neurotensin$^{8-13}$, an electrically evoked slow EPSP had a mean amplitude of $5{\cdot}1 \pm 1{\cdot}1$ mV and a mean duration of $1{\cdot}7 \pm 0{\cdot}3$ min. In these same cells in normal KRB solution without neurotensin, the mean amplitude was $4{\cdot}2 \pm 1{\cdot}3$ mV and the mean duration was $1{\cdot}3 \pm 0{\cdot}1$ min. Thus, both amplitude and duration of an electrically evoked slow EPSP were significantly ($P < 0{\cdot}05$) greater in neurotensin-containing KRB solution.

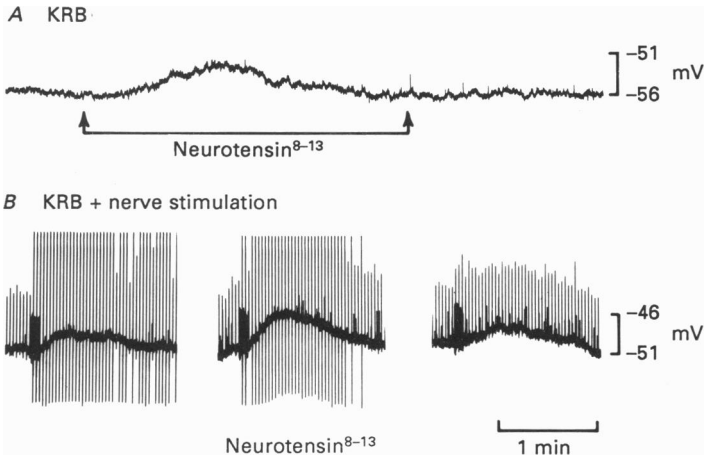

Fig. 1. Effects of neurotensin$^{8-13}$ on two different neurones in an inferior mesenteric ganglion of a normal guinea-pig. *A*, superfusion of neurotensin$^{8-13}$ (5×10^{-6} M) in Krebs–Ringer Buffer (KRB) for the time indicated between the arrows evoked a 4 mV transient depolarization of the membrane. Note that the membrane repolarized to the original resting membrane potential (−56 mV) in the continued presence of neurotensin$^{8-13}$. *B*, in the left-hand panel, a slow EPSP was evoked in a neurone in normal KRB by repetitive stimulation (15 Hz, 5 s) of the lumbar colonic nerve. The trace in the middle panel illustrates a slow EPSP evoked in the same neurone with the same parameters of stimulation just as KRB solution containing neurotensin$^{8-13}$ (5 μM) was washed into the bath. Note that the evoked slow EPSP was increased in amplitude and duration. This facilitatory effect of neurotensin$^{8-13}$ on the slow EPSP was reversible after wash-out (right-hand trace). In the tracings in panel *B*, subthreshold electrotonic potentials were evoked intermittently (0·5 Hz) throughout. Note that most reached threshold to fire an action potential during and shortly after the evoked slow EPSP. Action potentials overshot zero potential but are not shown in their full amplitude because of the limited frequency response of the chart recorder.

Slow EPSPs were studied in ganglia of capsaicin-treated animals using similar stimulation parameters. When evoked in normal KRB solution, electrically evoked slow EPSPs were significantly reduced ($2{\cdot}0 \pm 0{\cdot}3$ mV, $P < 0{\cdot}05$) in amplitude compared to slow EPSPs evoked in normal ganglia ($4{\cdot}2 \pm 1{\cdot}3$ mV). Although their duration was reduced ($1{\cdot}0 \pm 0{\cdot}1$ min), it was not significantly different from that observed in normal ganglia ($1{\cdot}3 \pm 0{\cdot}1$ min, $P > 0{\cdot}05$). When ganglia from capsaicin-treated animals were superfused with neurotensin$^{8-13}$ (5 μM), there was no increase in amplitude of electrically evoked slow EPSPs as seen in normal ganglia. Examples of electrically evoked slow EPSPs recorded in ganglia removed from capsaicin-treated

animals in the presence and absence of neurotensin is illustrated in Fig. 2. A summary of the changes in amplitude and duration of electrically evoked slow EPSPs under the various conditions described is presented in Fig. 3.

The findings presented above suggested the hypothesis that capsaicin treatment depleted an excitatory neurotransmitter whose release was modulated by neurotensin. To identify this capsaicin-sensitive mechanism, the content of some putative

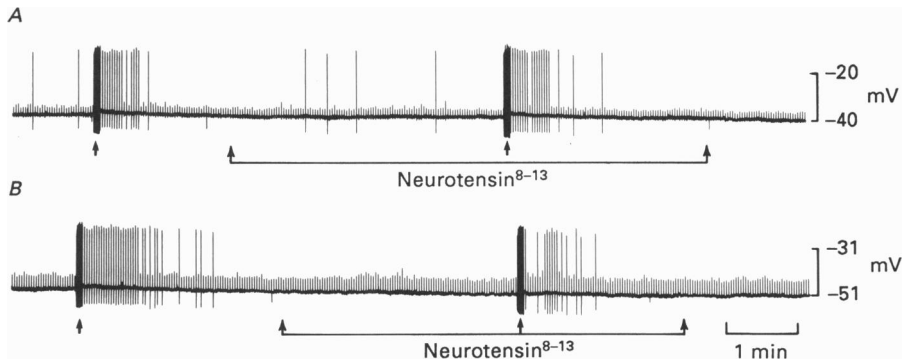

Fig. 2. Lack of effect of neurotensin$^{8-13}$ (5×10^{-6} M) on two neurones in two different inferior mesenteric ganglia from two capsaicin-treated guinea-pigs. Note that both neurones (*A* and *B*) failed to respond to superfusion with neurotensin$^{8-13}$ (5×10^{-6} M). Furthermore, compared to slow EPSPs evoked in ganglia removed from untreated animals, slow EPSPs evoked by stimulation of the lumbar colonic nerve (15 Hz, 5 s, at arrow-heads) in treated animals were either unaffected (*A*) or slightly reduced (*B*) in the presence of neurotensin$^{8-13}$ (5×10^{-6} M). The amplitude of the action potentials was attenuated due to the limited frequency response of the chart recorder.

neurotransmitters was determined in the same ganglia which had been used for electrophysiological experiments. The content of the same neuropeptides was also determined in those anatomical structures known to project nerve fibres to the prevertebral ganglia. These structures included the coeliac plexus, dorsal root ganglia, lumbar spinal cord and distal colon of the same animals.

Neuropeptide content of inferior mesenteric ganglia and associated structures of normal and capsaicin-treated animals

Substance P-, VIP-, CCK- and BOM-like material have been localized by immunohistochemical techniques in nerve terminals in prevertebral ganglia and are thought to mediate slow synaptic responses (slow EPSPs). Therefore, the concentration of each of these neuropeptides was analysed in prevertebral ganglia (inferior mesenteric ganglion and coeliac plexus), distal colon, dorsal root ganglia and lumbar spinal cord. As can be seen in Fig. 4, these peptides were present at particularly high concentrations in prevertebral ganglia of normal animals. Although the concentrations of BOM-, CCK- and VIP-like immunoreactive material of all tissues studied were not significantly different ($P > 0{\cdot}05$) in capsaicin-treated animals compared to control animals, the concentration of substance P-like immunoreactive material in capsaicin-treated animals was slightly reduced in the

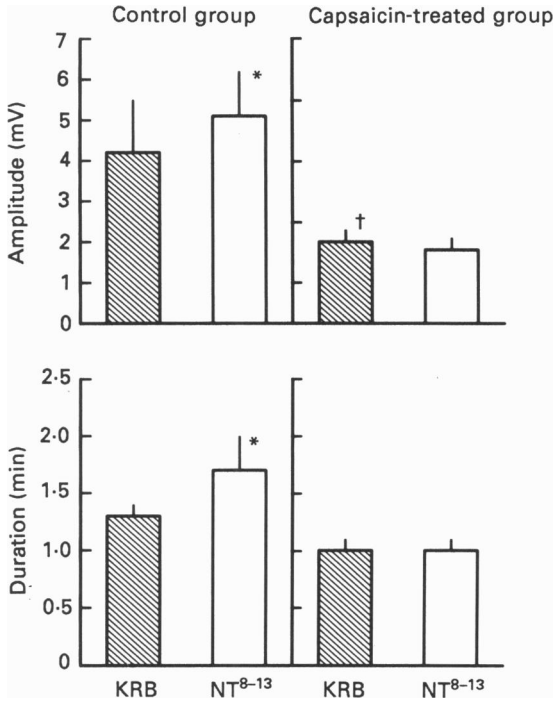

Fig. 3. Effect of neurotensin$^{8\text{–}13}$ (5×10^{-6} M, NT$^{8\text{–}13}$) on slow EPSPs in inferior mesenteric ganglia removed from untreated animals (control group, $n = 6$) and from capsaicin-treated (100 mg/kg, $n = 7$) animals. In all experiments, slow EPSPs were evoked by stimulation of the lumbar colonic nerve (15 Hz, 5–8 s). In ganglia from untreated animals (left-hand side), slow EPSPs were significantly ($P < 0{\cdot}05$) increased in amplitude (top) and duration (bottom) in the presence of neurotensin$^{8\text{–}13}$ (5×10^{-6} M) compared to slow EPSPs evoked with the same stimulation parameters in the absence of neurotensin. In ganglia removed from animals treated with capsaicin (right-hand side), slow EPSPs evoked in normal Krebs–Ringer Buffer (KRB) solution were significantly ($P < 0{\cdot}05$) reduced in amplitude (top, †) compared to slow EPSPs observed in ganglia from untreated animals. The decreased duration of slow EPSPs (bottom) was not significant ($P > 0{\cdot}05$) compared to slow EPSPs observed in the control group of animals. Slow EPSPs in ganglia from capsaicin-treated animals were not affected by NT$^{8\text{–}13}$ (5×10^{-6} M). Data expressed as mean ± standard error of the mean. * †, $P < 0{\cdot}05$.

colon and significantly ($P < 0{\cdot}05$) decreased in inferior mesenteric and coeliac ganglia, dorsal root ganglia and lumbar spinal cord, when compared to control animals (Fig. 4). In order to characterize better the substance P-like material which was present in normal tissues and presumably depleted in capsaicin-treated animals, extracts of normal tissues were reanalysed using high-pressure liquid chromatography (HPLC). As can be seen in Fig. 5, substance P-like material in all tissues studied co-eluted with a synthetic substance P standard, mostly in its authentic molecular form and, to some extent, its oxidized molecular form. These radioimmunological findings, when considered along side the electrophysiological observations, suggested the possibility that the transient depolarization induced by neurotensin in normal ganglia was due to release of substance P from presynaptic

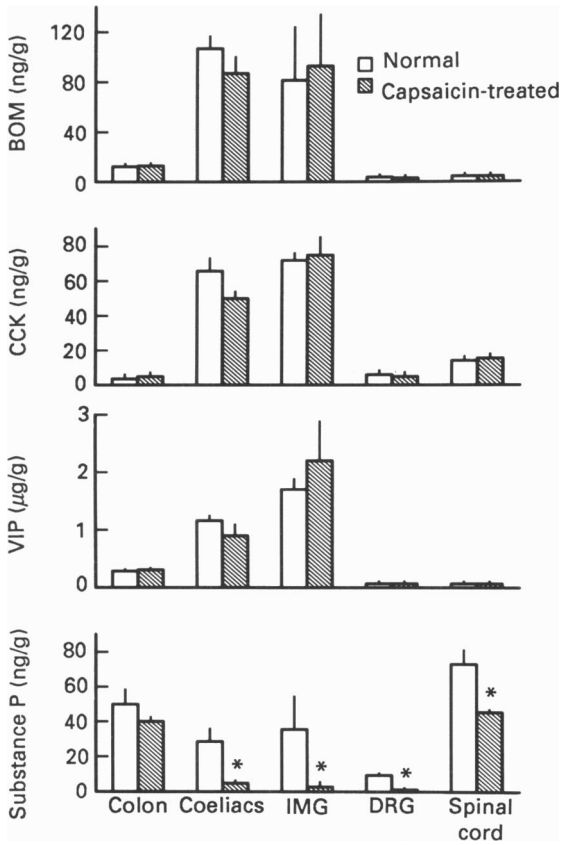

Fig. 4. Tissue content of various neuropeptides in untreated and capsaicin-treated guinea-pigs ($n = 6$). The tissue content of the four neuropeptides was analysed in the same inferior mesenteric ganglia the electrophysiological data were obtained from, and in the coeliac plexus, dorsal root ganglia (L1–L4), lumbar spinal cord and distal colon of the same animals. The content of BOM-, CCK- and VIP-like immunoreactivity was not significantly different (top, second and third rows) in tissues removed from untreated (open columns) and capsaicin-treated animals (hatched columns). The content of substance P-like immunoreactivity (bottom row), however, was significantly ($P < 0{\cdot}05$) reduced in the coeliac plexus (coeliacs), inferior mesenteric ganglion (IMG), dorsal root ganglia (DRG) and lumbar spinal cord. The reduction in content of substance P-like immunoreactivity in the distal colon in capsaicin-treated guinea-pigs was not significantly ($P > 0{\cdot}05$) different from untreated animals. Data expressed as mean ± standard error of the mean. *, $P < 0{\cdot}05$.

terminals. Therefore, the effect of neurotensin$^{8-13}$ on electrically evoked release of substance P from lumbar colonic nerves was studied.

Effect of neurotensin$^{8-13}$ on electrically evoked release of substance P

For these experiments, ganglia were treated with 0·01 % collagenase, as described in the Methods. This treatment had no effect on either resting membrane potential or on the occurrence of evoked fast and slow synaptic responses. In thirty-nine cells from twenty-five ganglia treated with collagenase, the mean resting membrane

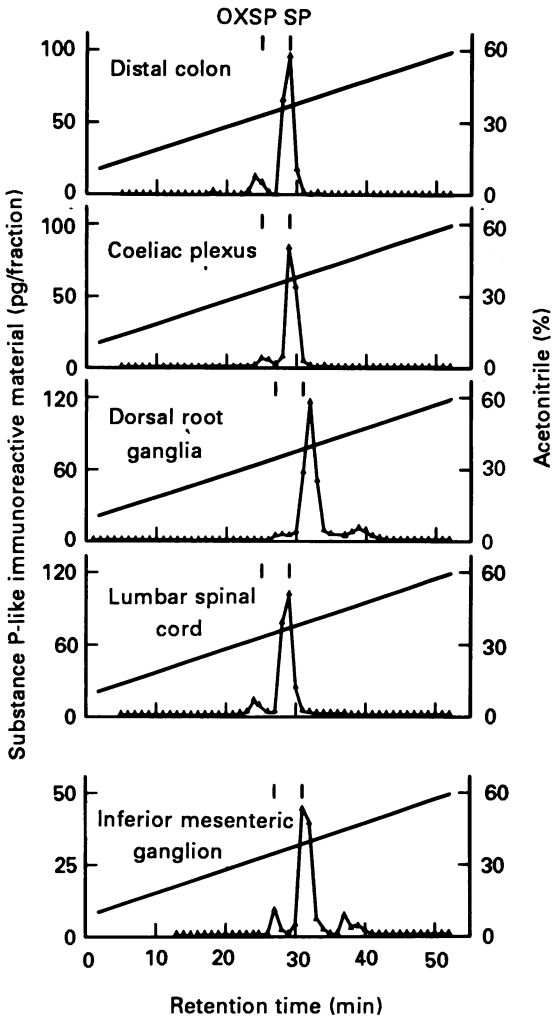

Fig. 5. Profile of substance P-like immunoreactivity in guinea-pig tissues as determined by HPLC. Radioimmunoassay analysis of the HPLC eluate (10–60% acetonitrile gradient) of tissue extracts revealed that the substance P-like material present in the distal colon, coeliac plexus, dorsal root ganglia and lumbar spinal cord recognized by the antiserum used in this study co-eluted with synthetic substance P in mostly its authentic form (SP) and partly in its oxidized (OXSP) molecular form. Bottom panel illustrates results obtained from an analysis of HPLC eluate of extracts from inferior mesenteric ganglia. Not enough extract material of the inferior mesenteric ganglia was left for HPLC analysis after determining the content of the various neuropeptides shown in Fig. 4. In order to determine the profile of the substance P-like material localized to inferior mesenteric ganglia, an additional ten ganglia from untreated guinea-pigs were dissected, pooled and extracted solely for the purpose of HPLC analysis. The resulting chromatogram was similar to those obtained in other tissues showing a major peak which co-eluted with authentic substance P and a minor peak which co-eluted with oxidized substance P.

potential was $-54{\cdot}1 \pm 1{\cdot}0$ mV. This value was not statistically significant from values obtained from ganglion cells in untreated ganglia ($-53{\cdot}0 \pm 1{\cdot}6$ mV, $P > 0{\cdot}05$). Although the effect of collagenase superfusion on synaptic responses was not examined in detail, there was no apparent change in the occurrence and amplitude of evoked fast EPSPs during superfusion of collagenase in five cells from five different preparations studied. In twenty-three cells from twelve ganglia treated with collagenase, repetitive stimulation of attached nerve trunks evoked fast and slow EPSPs indicating the integrity of cholinergic and non-cholinergic synaptic pathways. Furthermore, seven neurones tested in six different collagenase-treated preparations responded to superfusion of either neurotensin$^{1-13}$ (5 μM) or neurotensin$^{8-13}$ (5 μM) with a transient depolarization which ranged from 3 to 7 mV in amplitude, similar to the response observed in normal untreated ganglia (Stapelfeldt & Szurszewski, 1989).

Release of substance P from inferior mesenteric ganglia was determined by measuring the concentration of substance P in superfusate samples collected from the electrophysiological chamber before and after stimulation of the lumbar colonic nerves. The recording chamber contained three isolated ganglia with attached lumbar colonic nerve trunks from three normal animals. Each preparation was pinned to the floor of the chamber. Stimulation of all three lumbar colonic nerves was performed simultaneously. Intracellular recordings were obtained during the course of each experiment from individual neurones in one of the three ganglia. Figure 6 illustrates the results obtained from a typical experiment. Prior to lumbar colonic nerve stimulation, the concentration of substance P-like material in the bath remained below the level of detection of the assay ($\leqslant 15$ pg/ml; Fig. 6*A*). Simultaneous stimulation of all three lumbar colonic nerves for 1 min at 15 Hz caused a slow EPSP in the impaled cell and increased the concentration of substance P-like material in the superfusate to 22 pg/ml (Fig. 6*B*). In the same preparations, stimulation of the lumbar colonic nerves during superfusion of neurotensin$^{8-13}$ (5 μM) increased the amplitude of the electrically evoked slow EPSP and also increased the concentration of substance P-like material in the superfusate to 70 pg/ml (Fig. 6*C*). The concentration of substance P-like material returned to below the level of detection of the assay when the lumbar colonic nerves were again left unstimulated (Fig. 6*D*). The effect of neurotensin$^{8-13}$ on the concentration of substance P-like material in the superfusate in three separate experiments is summarized in Fig. 7.

Superfusion of neurotensin$^{8-13}$ (5 μM) alone without nerve stimulation in two experiments did not cause a measurable increase in the concentration of substance P-like material.

The concentration of CCK-like material, which was also determined in these samples, did not rise above the assay detection threshold of 40 pg/ml throughout the experiment suggesting that the facilitatory action of neurotensin$^{8-13}$ did not include the release of CCK-like material from afferent nerve terminals.

The radioimmunological data provided above suggest that neurotensin$^{8-13}$ facilitated release of substance P from nerve terminals in guinea-pig inferior mesenteric ganglia. The data are consistent with the hypothesis of substance P mediating neurotensin-induced excitatory responses. To test this hypothesis, the effect of postsynaptic desensitization to substance P on neurotensin-induced transient membrane responses was studied.

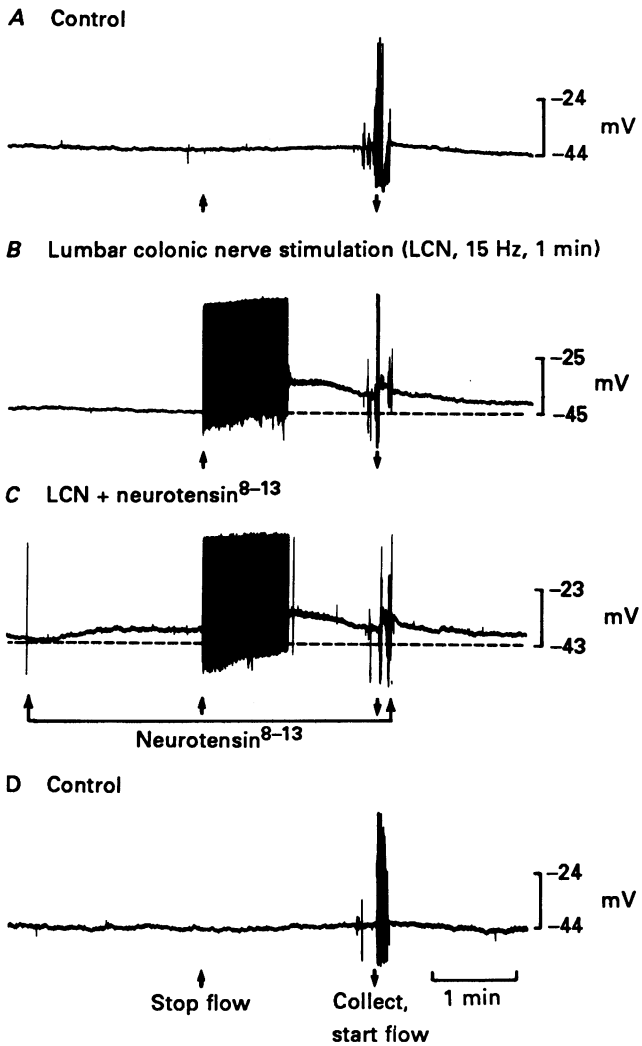

Fig. 6. Simultaneous recording of intracellular electrical activity and measurement of substance P-like material released from isolated inferior mesenteric ganglia. Intracellular electrical activity was recorded continuously from a cell in one of three ganglia co-incubated in an electrophysiological chamber. Superfusate samples were collected from the chamber using the following protocol. In panels *A–D*, flow of KRB solution through the chamber was stopped at the time indicated by the first arrow (↑). After a period of 2 min, superfusate samples (200 μl) were collected at the time indicated by the second arrow (↓), accounting for the artifacts in the recordings. Immediately after collecting the sample the flow was re-established. *A* and *D*, in the absence of nerve stimulation, the concentration of substance P-like material in the superfusate was below the assay detection threshold (< 15 pg/ml). *B*, electrical stimulation of the lumbar colonic nerves (LCN, 15 Hz) for 1 min evoked a train of spikes, a slow EPSP and increased the concentration of substance P-like immunoreactivity in the superfusate to 22 pg/ml (*B*). *C*, electrical stimulation of the lumbar colonic nerves in the presence of neurotensin$^{8-13}$ (5 μM) increased the amount of substance P-like material in the superfusate to 70 pg/ml. All recordings were obtained from the same neurone. Action potentials attenuated due to the limited frequency response of the recorder.

Effect of desensitization to substance P on transient membrane response to neurotensin

To determine if substance P mediated transient, excitatory responses to neurotensin, neurones responding to neurotensin with a transient membrane depolarization were subsequently tested for their sensitivity to exogenously administered substance P and for their response to neurotensin following desensitization to exogenously administered substance P.

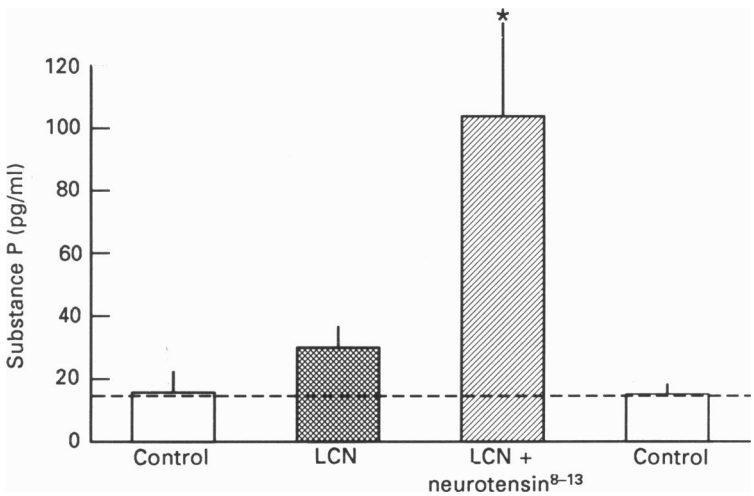

Fig. 7. Effect of neurotensin$^{8-13}$ on release of substance P during electrical stimulation of the lumbar colonic nerves (LCN). In the absence of nerve stimulation and neurotensin$^{8-13}$ (Control), the concentration of substance P-like material in the superfusate was not different from the detection threshold of the radioimmunoassay (15 pg/ml, dashed line). During stimulation of the LCN at 15 Hz for 1 min, the concentration of substance P-like material in the superfusate rose above detection threshold (cross-hatched bar). During stimulation of the LCN at 15 Hz for 1 min in the same preparations in the presence of neurotensin$^{8-13}$ (5 μM), the concentration of substance P-like material in the superfusate increased significantly ($P < 0{\cdot}05$) compared to the increase observed during nerve stimulation in normal KRB solution (hatched bar). Data expressed as mean $\pm$ standard error of the mean of three different release experiments ($n = 3$). In each experiment, three ganglia were present in the same chamber. *, $P < 0{\cdot}05$.

In five neurones from five ganglia, neurotensin (5 μM) caused a transient depolarization which averaged $5{\cdot}4 \pm 1{\cdot}7$ mV (range, 3–10 mV) in amplitude. In these same cells, an equimolar concentration of substance P evoked a sustained depolarization which averaged $3{\cdot}8 \pm 0{\cdot}4$ mV (range, 3–5 mV) in amplitude. An example of depolarizing responses of a neurone to neurotensin and substance P is shown in Fig. 8*A* and *B*, respectively. Four of the five neurones studied were impaled long enough to follow complete desensitization to substance P. In these four neurones, complete desensitization to substance P occurred after $11{\cdot}3 \pm 1{\cdot}4$ min (mean $\pm$ S.E.M.; range, 8–13 min) of continued superfusion of substance P. This period for complete desensitization is consistent with previous observations (Tsunoo *et al.* 1982; Dun & Jiang, 1982; Peters & Kreulen, 1984, 1986). Following desensitization to substance P, the response to neurotensin was completely abolished

in two neurones and markedly reduced from 10 to 2 mV and from 3 to 0·5 mV in the remaining two neurones. The tracing in Fig. 8*C* was made from one of the cells in which the response to substance P was abolished. When the responses in all four neurones are considered together, desensitization to substance P significantly ($P < 0{\cdot}025$) attenuated transient depolarizing responses to neurotensin. Prior to desensitization, the transient depolarization evoked by neurotensin averaged

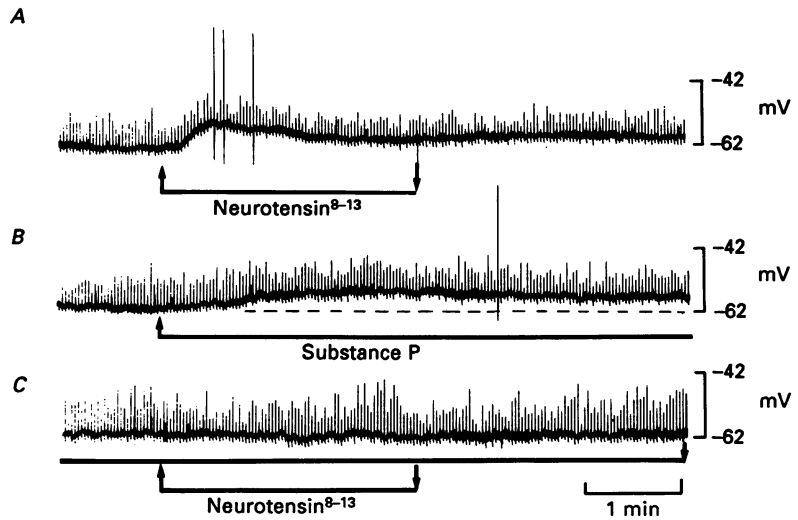

Fig. 8. Effect of desensitization to substance P on the transient membrane depolarization induced by neurotensin. In normal KRB solution (*A*), superfusion of neurotensin$^{8-13}$ (5 μM) elicited a transient membrane depolarization which was 8 mV in amplitude. Superfusion of substance P (5 μM) caused a sustained depolarization which initially was 5 mV in amplitude (*B*). However, in the continued presence of substance P, the membrane potential gradually repolarized over a period of 12 min to the level (−62 mV) recorded before administration of substance P. Addition of neurotensin$^{8-13}$ (5 μM) during desensitization to substance P (*C*) failed to induce a membrane response. Note that the fast EPSPs evoked by electrical stimulation of the lumbar colonic nerve (0·5 Hz) were not affected by desensitization to substance P. All recordings obtained from the same neurone. Action potentials attenuated due to the limited frequency response of the recorder.

6·5 ± 1·8 mV. Following desensitization to substance P, neurotensin-induced transient depolarizations averaged 0·6 ± 0·5 mV. In one neurone, impalement was maintained long enough to follow partial restoration of neurotensin-induced depolarization following wash-out of substance P. At 40 min after removing substance P, the amplitude of the transient depolarization evoked by neurotensin (5 μM) was 5 mV compared to 6 mV observed in normal KRB solution prior to desensitization to substance P.

Although not studied in detail, electrically evoked fast EPSPs (nicotinic responses) were similar in amplitude before and during desensitization to substance P (Fig. 8*A* and *C*). Synaptically evoked action potentials which were evident during neurotensin-induced depolarization prior to desensitization to substance P (Fig. 8*A*) were absent when the depolarizing response to neurotensin was abolished during

desensitization to substance P (Fig. 8*C*). This suggests that the facilitatory action of neurotensin on fast EPSPs was indirectly due to the facilitatory effects of substance P-like transmitter released by neurotensin rather than to a direct action of neurotensin on nicotinic transmission.

The inhibitory effect of postsynaptic desensitization to substance P on transient responses to neurotensin raised the question as to whether transient depolarizations evoked by neurotensin acting through presynaptic elements was possibly due to postsynaptic desensitization to endogenously released substance P or to tachyphylaxis of neurotensin receptors located at presynaptic sites. This possibility was explored by studying the sensitivity of the postsynaptic membrane to substance P during transient responses to neurotensin.

Postsynaptic sensitivity to substance P during transient responses to neurotensin

To determine if the transient nature of indirectly mediated responses to neurotensin was due to postsynaptic desensitization in response to continuously released substance P or to tachyphylaxis at presynaptic terminals releasing substance P during continued superfusion of neurotensin, the sensitivity of neurones to exogenously applied substance P was studied before and immediately after neurotensin-induced transient membrane depolarizations. In these experiments, ganglia were exposed to substance P by injecting substance P into the bath fluid to cause an instantaneous exposure to a final estimated concentration of about 5×10^{-6} M. In three neurones from three ganglia, substance P applied before a neurotensin response caused a depolarization of $6{\cdot}6 \pm 1{\cdot}8$ mV in amplitude. Superfusion with either neurotensin or neurotensin$^{8-13}$ evoked a transient depolarization of $5{\cdot}0 \pm 1{\cdot}2$ mV in amplitude. When the membrane repolarized to the level recorded before application of neurotensin and during the maintained presence of neurotensin, substance P was again added to the bathing solution. The mean maximal depolarization observed was $6{\cdot}0 \pm 1{\cdot}4$ mV. This response was not significantly ($P > 0{\cdot}05$) different from that observed during the first application of substance P. When substance P was applied for a third time after wash-out of neurotensin, the cells responded with a similar depolarization ($6{\cdot}0 \pm 2{\cdot}1$ mV). This response again was not significantly ($P > 0{\cdot}05$) different when compared to the first response to substance P. An example of the responsiveness of a neurone to substance P under the various conditions just described is illustrated in Fig. 9. The transitory nature of indirectly mediated responses to continuous superfusion of neurotensin was, therefore, most probably due to tachyphylaxis of neurotensin receptors located at presynaptic terminals containing substance P rather than to desensitization of the postsynaptic membrane to continuously released substance P.

DISCUSSION

The present study supports the hypothesis that exogenously added neurotensin caused transient depolarizing responses by acting on presynaptic terminals containing substance P. Three lines of evidence support this hypothesis. First, neurotensin$^{8-13}$ simultaneously increased the amplitude of electrically evoked slow EPSPs and the amount of substance P-like material released into the superfusate

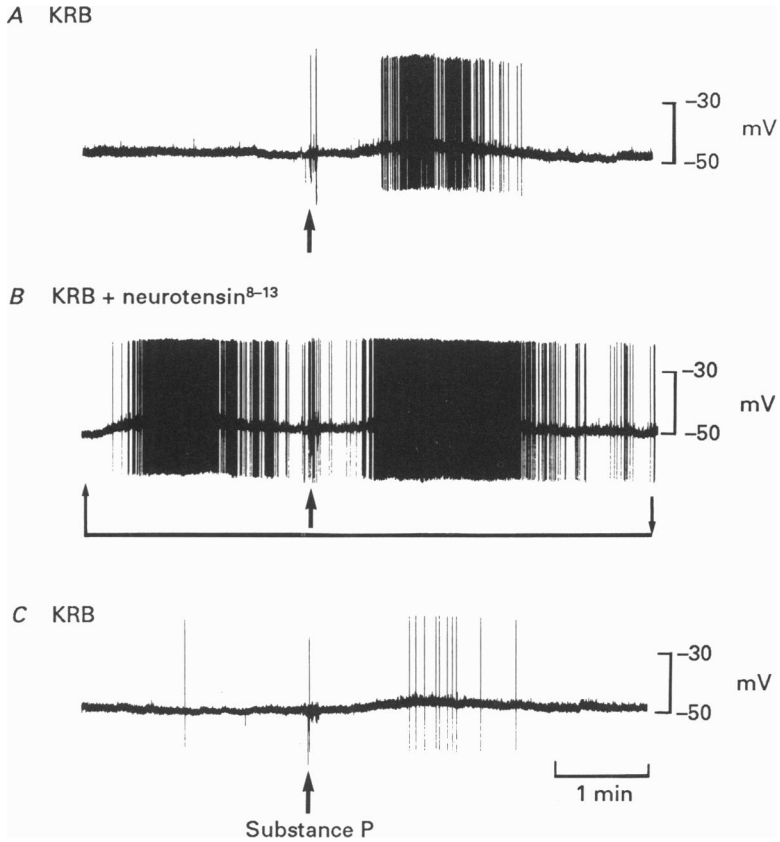

Fig. 9. Effect of continuous perfusion of neurotensin$^{8-13}$ on response to exogenously added substance P. *A*, in normal Krebs–Ringer Buffer (KRB) solution, injection of substance P (final concentration 5 μM) into the bath depolarized the membrane by 5 mV and induced spontaneously occurring action potentials. *B*, superfusion of neurotensin$^{8-13}$ (5 μM) elicited a transient depolarization of the membrane potential which was 7 mV in amplitude. When the membrane had repolarized close to the level recorded before administration of neurotensin$^{8-13}$ (-50 mV) and in the maintained presence of neurotensin$^{8-13}$, substance P depolarized the membrane by 4 mV. *C*, 5 min after wash-out of neurotensin$^{8-13}$, injection of substance P (5 μM) into the bath caused a depolarization which was 4 mV in amplitude. All recordings obtained from same neurone. Action potentials attenuated due to the limited response of the recorder.

during electrical nerve stimulation. The increased release of substance P can be considered direct evidence that the increase in the amplitude of slow EPSPs was mediated by substance P. The failure of neurotensin to cause a measurable increase in substance P in the superfusate in the absence of nerve stimulation suggests that neurotensin promoted release of substance P only from transmitting synapses.

Second, *in vivo* capsaicin treatment abolished transient responses to neurotensin and depleted inferior mesenteric ganglia of substance P without substantially altering the content of other neuropeptides such as CCK-, VIP- and BOM-like material. These latter observations are consistent with earlier semiquantitative immunohistochemical studies which showed that substance P-like immunoreactivity

was reduced by *in vivo* capsaicin treatment whereas CCK-, VIP- and BOM-like immunoreactivity were unaltered (Dalsgaard *et al.* 1983*c*). The failure of exogenously added neurotensin to potentiate the amplitude of electrically evoked slow EPSPs in ganglia depleted of substance P strongly suggests that neurotensin specifically facilitated substance P-mediated slow EPSPs without releasing other neuropeptides which are also thought to mediate slow EPSPs.

And third, neurotensin-induced excitation was markedly attenuated after desensitization of the postsynaptic membrane to substance P.

In addition to substance P, CCK- and VIP-like material are also present in nerve fibres in guinea-pig inferior mesenteric ganglia and both have been implicated as mediators of slow EPSPs (Dalsgaard 1983*a*; Mo & Dun, 1984, 1986; Schumann & Kreulen, 1986; Love & Szurszewski, 1987). It does not appear likely that either neuropeptide mediated indirect actions of neurotensin because no increase in their release into the superfusate was observed. While the failure to detect CCK-like immunoreactivity in these experiments might have been due to an inadequate sensitivity of the assay, the fact that neurotensin failed to increase the amplitude of electrically evoked slow EPSPs in ganglia removed from animals treated with capsaicin suggests that release of CCK-like material was not modulated by neurotensin. The occurrence of slow EPSPs evoked by electrical stimulation of the lumbar colonic nerve in ganglia from capsaicin-treated animals was in all probability mediated by CCK and VIP or closely related peptides. The observation that the content of CCK- and VIP-like material was unchanged in ganglia from capsaicin-treated animals compared to untreated animals supports this notion. The reduction in amplitude of electrically evoked slow EPSPs and the near disappearance of substance P-like material in ganglia from capsaicin-treated animals supports a causal relationship between substance P and slow EPSPs.

In addition to substance P, other tachykinins such as neurokinin A and neuropeptide K, which may be derived from a common precursor molecule (Hua, Theodorsson-Norheim, Brodin, Lundberg & Hökfelt, 1985), and calcitonin-gene related peptide (Gibbins, Furness, Costa, MacIntyre, Hillyard & Girgis, 1985; Gibbins, Furness & Costa, 1987), have also been found in capsaicin-sensitive afferent nerves. Thus, it is possible that they too were released by neurotensin and participated in generating transient depolarizing responses. Both neurokinin A (Saria, Ma & Dun, 1985) and calcitonin-gene-related peptide (W. H. Stapelfeldt & J. H. Szurszewski, unpublished observation) evoke excitatory postsynaptic actions in ganglion cells in guinea-pig inferior mesenteric ganglia. While the possible role of these neural peptides remains to be determined, a role for substance P and/or other substance P-like peptides as a postsynaptic mediator of indirect excitatory responses to neurotensin was indicated by the marked attenuation of neurotensin-induced membrane depolarization after desensitization of the postsynaptic membrane to authentic substance P.

Combined retrograde tracer, immunohistochemical and electronmicroscopical studies have identified substance P-containing nerve terminals in the colon and in prevertebral ganglia as collateral terminals of primary afferent fibres whose cell bodies are located in lumbar dorsal root ganglia (Dalsgaard *et al.* 1982; Matthews & Cuello, 1982; Matthews, Connaughton & Cuello, 1987). This association of substance

P-like material in inferior mesenteric ganglia and colon with the primary afferent nerve pathway is supported by the present findings of capsaicin-induced decreases in substance P along the primary afferent pathway, i.e. marked depletion in dorsal root ganglia, significant reduction in the spinal cord and marginal reduction in the colon. It has been suggested that the central limb of this afferent system mediates visceral nociception and that the collateral nerve terminals in prevertebral ganglia which comprise the peripheral limb of the same afferent system mediate visceral mechanosensory input from the colon (Kreulen & Peters, 1986; Peters & Kreulen, 1986; Stapelfeldt, Go & Szurszewski, 1988). Thus, substance P-containing primary visceral afferent fibres may function as both nociceptive and non-nociceptive sensory receptors. A similar suggestion has been put forward by Cervero (1982) for the biliary system in ferrets. The importance of the present study to this newly emerging concept that some visceral afferent fibres may function as non-specific visceral receptors is that synaptic arrangements in inferior mesenteric ganglia provide a mechanism for modulating the gain of the mechanosensory pathway at the peripheral level without necessarily altering the intensity of afferent nerve input to the central nervous system. Thus, activity along central neurotensin pathways would be expected to facilitate substance P-dependent colonic mechanosensory input to neurones in the inferior mesenteric ganglion without altering input to the lumbar spinal cord. Central enkephalin pathways (Dalsgaard *et al.* 1983*d*; Dalsgaard, Vincent, Hökfelt, Christensson & Terenius, 1983*b*) which are known to act presynaptically to reduce release of substance P (Konishi, Tsunoo & Otsuka, 1979; Konishi, Tsunoo, Yanaihara & Otsuka, 1980), would be expected to inhibit substance P-dependent colonic mechanosensory input to neurones in the inferior mesenteric ganglion without altering the frequency of input to the lumbar cord. In this way, mechanoreception at the peripheral level and visceral sensation at the level of the central neuraxis can be modulated independently even though the same fibres are involved.

Finally, the present findings provide direct support for the hypothesis put forward by others that substance P or a related peptide is an excitatory neurotransmitter in guinea-pig inferior mesenteric ganglia (Konishi *et al.* 1979, 1980; Gamse *et al.* 1981; Tsunoo *et al.* 1982). In previous *in vitro* studies, incubation of pooled prevertebral ganglia with capsaicin and a high concentration of external K^+ caused calcium-dependent release of substance P-like material into the supernatant bathing the isolated ganglia. The present findings that electrical stimulation of the lumbar colonic nerve released substance P-like material from intact preparations and that the substance P-like material in inferior mesenteric ganglia co-eluted with synthetic substance P using HPLC techniques provide direct evidence for substance P as an excitatory afferent neurotransmitter in guinea-pig inferior mesenteric ganglia.

The present work was supported by grants from the National Institute of Health (DK 17632 and DK 34988-H) and Deutsche Forschungsgemeinschaft (DFG Sta 252/1-1) (W.H.S.). The authors particularly express their gratitude to Dr V. L. W. Go for providing substance P antiserum and facilities in his laboratory for the various radioimmunological and chromatographic measurements. The authors are indebted to Jan Applequist, Philip Schmalz, Jewel Brandt, William Anding, Jane Bailey, Sandra Michener, Diane Roddy and Darlene Lucas for expert technical assistance.

REFERENCES

ANGEL, F., GO, V. L. W. & SZURSZEWSKI, J. H. (1984). Innervation of the muscularis mucosae of canine proximal colon. *Journal of Physiology* **357**, 93–108.

CERVERO, F. (1982). Afferent activity evoked by natural stimulation of the biliary system in the ferret. *Pain* **13**, 137–157.

DALSGAARD, C.-J., HÖKFELT, T., ELFVIN, L.-G., SKIRBOLL, L. & EMSON, P. (1982). Substance P-containing primary sensory neurons projecting to the inferior mesenteric ganglion: evidence from combined retrograde tracing and immunohistochemistry. *Neuroscience* **7** (3), 647–654.

DALSGAARD, C.-J., HÖKFELT, T., SCHULTZBERG, M., LUNDBERG, J. M., TERENIUS, L., DOCKRAY, G. J. & GOLDSTEIN, M. (1983*a*). Origin of peptide-containing fibres in the inferior mesenteric ganglion of the guinea-pig: immunohistochemical studies with antisera to substance P, enkephalin, vasoactive intestinal polypeptide, cholecystokinin and bombesin. *Neuroscience* **9** (1), 191–211.

DALSGAARD, C.-J., VINCENT, S. R., HÖKFELT, T., CHRISTENSSON, I. & TERENIUS, L. (1983*b*). Separate origins for the dynorphin and enkephalin immunoreactive fibres in the inferior mesenteric ganglion of the guinea pig. *Journal of Comparative Neurology* **221**, 482–489.

DALSGAARD, C.-J., VINCENT, S. R., SCHULTZBERG, M., HÖKFELT, T., ELFVIN, L.-G., TERENIUS, L. & DOCKRAY, G. J. (1983*c*). Capsaicin-induced depletion of substance P-like immunoreactivity in guinea-pig sympathetic ganglia. *Journal of the Autonomic Nervous System* **9**, 595–606.

DUN, N. J. & JIANG, Z. G. (1982). Non-cholinergic excitatory transmission in inferior mesenteric ganglia of the guinea-pig: possible mediation by substance P. *Journal of Physiology* **325**, 145–159.

DUN, N. J. & KIRALY, M. (1983). Capsaicin causes release of a substance P-like peptide in guinea-pig inferior mesenteric ganglia. *Journal of Physiology* **340**, 107–120.

GAMSE, R., WAX, A., ZIGMOND, R. E. & LEEMAN, S. E. (1981). Immunoreactive substance P in sympathetic ganglia: distribution and sensitivity towards capsaicin. *Neuroscience* **6** (12), 437–441.

GIBBINS, I. L., FURNESS, J. B. & COSTA, M. (1987). Pathway-specific pattern of the coexistence of substance P, calcitonin gene-related peptide, cholecystokinin and dynorphin in neurons of the dorsal root ganglia of the guinea-pig. *Cell and Tissue Research* **248**, 417–437.

GIBBINS, I. L., FURNESS, J. B., COSTA, M., MACINTYRE, I., HILLYARD, C. J. & GIRGIS, S. (1985). Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea-pigs. *Neuroscience Letters* **57**, 125–130.

HEYM, CH., REINECKE, M., WEIHE, E. & FORSSMANN, W. G. (1984). Dopamine-β-hydroxylase, neurotensin-, substance P-, vasoactive intestinal polypeptide- and enkephalin-immunohistochemistry of paravertebral and prevertebral ganglia in the cat. *Cell and Tissue Research* **235**, 411–418.

HÖKFELT, T., ELFVIN, L.-G., SCHULTZBERG, M., GOLDSTEIN, M. & NILSSON, G. (1977). On the occurrence of substance P-containing fibres sympathetic ganglia: immunohistochemical evidence. *Brain Research* **132**, 29–41.

HUA, X.-Y., THEODORSSON-NORHEIM, E., BRODIN, E., LUNDBERG, J. M. & HÖKFELT, T. (1985). Multiple tachykinins (neurokinin A, neuropeptide K and substance P) in capsaicin-sensitive sensory neurons in the guinea-pig. *Regulatory Peptides* **13**, 1–19.

KONISHI, S., TSUNOO, A. & OTSUKA, M. (1979). Substance P and non-cholinergic excitatory synaptic transmission in guinea-pig sympathetic ganglia. *Proceedings of the Japanese Academy* **55**, 525–530.

KONISHI, S., TSUNOO, A., YANAIHARA, N. & OTSUKA, M. (1980). Peptidergic excitatory and inhibitory synapses in mammalian sympathetic ganglia: roles of substance P and enkephalin. *Biomedical Research* 1, 528–536.

KREULEN, D. L. & PETERS, S. (1986). Non-cholinergic transmission in a sympathetic ganglion of the guinea-pig elicited by colon distention. *Journal of Physiology* **374**, 315–334.

LOVE, J. A. & SZURSZEWSKI, J. H. (1987). The electrophysiological effects of vasoactive intestinal polypeptide in the guinea-pig inferior mesenteric ganglion. *Journal of Physiology* **394**, 67–84.

LUNDBERG, J. M., HÖKFELT, T., ÄNGGARD, A., UVNÄS-WALLENSTEN, K., BRIMIJOIN, S., BRODIN, E. & FAHRENKRUG, J. (1980). Peripheral peptide neurons: distribution, axonal transport, and

some aspects on possible function. In *Neural Peptides and Neuronal Communication*, ed. COSTA, E. & TRABUCCI, M., pp. 25–36. New York: Raven Press.

LUNDBERG, J. M., RÖKAEUS, A., HÖKFELT, T., ROSELL, S., BROWN, M. & GOLDSTEIN, M. (1982). Neurotensin-like immunoreactivity in the preganglionic sympathetic nerves and in the adrenal medulla of the cat. *Acta physiologica scandinavica* **114**, 153–155.

MATTHEWS, M. R., CONNAUGHTON, M. & CUELLO, A. C. (1987). Ultrastructure and distribution of substance P-immunoreactive sensory collaterals in the guinea-pig prevertebral sympathetic ganglia. *Journal of Comparative Neurology* **258**, 28–51.

MATTHEWS, M. R. & CUELLO, A. C. (1982). Substance P-immunoreactive peripheral branches of sensory neurons innervate guinea-pig sympathetic neurons. *Proceedings of the National Academy of Sciences of the USA* **79**, 1668–1672.

MICEVYCH, P. E., YAKSH, T. L. & GO, V. L. W. (1982). Opiate-mediated inhibition of cholecystokinin and substance P but not neurotensin from cat hypothalamic slices. *Brain Research* **250**, 283–289.

MO, N. & DUN, N. J. (1984). Vasoactive intestinal polypeptide facilitates muscarinic transmission in mammalian sympathetic ganglia. *Neuroscience Letters* **52**, 19–23.

MO, N. & DUN, N. J. (1986). Cholecystokinin octapeptide depolarizes guinea-pig inferior mesenteric ganglion cells and facilitates nicotinic transmission. *Neuroscience Letters* **64**, 263–268.

OWYANG, C., MILLER, L. J., DIMAGNO, E. P., BRENNAN JR, L. A. & GO, V. L. W. (1979). Gastrointestinal hormone profile in renal insufficiency. *Mayo Clinic Proceedings* **54**, 769–773.

PETERS, S. & KREULEN, D. L. (1984). A slow EPSP in mammalian inferior mesenteric ganglia persists after *in vivo* capsaicin. *Brain Research* **303**, 186–189.

PETERS, S. & KREULEN, D. L. (1986). Fast and slow synaptic potentials produced in a mammalian sympathetic ganglion by colon distention. *Proceedings of the National Academy of Sciences of the USA* **83**, 1941–1944.

REINECKE, M., FORSSMANN, W. G., THIEKÖTTER, G. & TRIEPEL, J. (1983). Localization of neurotensin-immunoreactivity in the spinal cord and peripheral nervous system of the guinea-pig. *Neuroscience Letters* **37**, 37–42.

SARIA, A., MA, R. C. & DUN, N. J. (1985). Neurokinin A depolarizes neurons of the guinea-pig inferior mesenteric ganglia. *Neuroscience Letters* **60**, 145–150.

SCHUMANN, M. A. & KREULEN, D. A. (1986). Action of cholecystokinin octapeptide and CCK-related peptides on neurons in the inferior mesenteric ganglion of guinea-pig. *Journal of Pharmacology and Experimental Therapeutics* **239** (2), 618–625.

STAPELFELDT, W. H., GO, V. L. W. & SZURSZEWSKI, J. H. (1987). Neurotensin facilitates release of substance P from primary afferent nerve terminals in the guinea-pig inferior mesenteric ganglion. *Society for Neuroscience Abstracts* **13**, 1308.

STAPELFELDT, W. H., GO, V. L. W. & SZURSZEWSKI, J. H. (1988). Colonic distension releases substance P in the guinea-pig inferior mesenteric ganglion. *Gastroenterology* **94**, 441.

STAPELFELDT, W. H. & SZURSZEWSKI, J. H. (1989). The electrophysiological effects of neurotensin on neurones of guinea-pig prevertebral sympathetic ganglia. *Journal of Physiology* **411**, 301–323.

SZURSZEWSKI, J. H. (1981). Physiology of mammalian prevertebral ganglia. *Annual Reviews of Physiology* **43**, 53–68.

TSUNOO, A., KONISHI, S. & OTSUKA, M. (1982). Substance P as an excitatory transmitter of primary afferent neurons in guinea-pig sympathetic ganglia. *Neuroscience* **7** (9), 2025–2037.

YAKSH, T. L., ABAY, E. O. & GO, V. L. W. (1982). Studies on the location and release of cholecystokinin and vasoactive intestinal peptide in rat and cat spinal cord. *Brain Research* **242**, 289–290.