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Neurotensin-induced Cl^- current in guinea-pig dorsal root ganglion cells

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Abstract

In guinea-pig dorsal root ganglion cells held under voltage-clamp at −80 mV, neurotensin elicited an inward current (I_{NT}) whose amplitude increased with increasing neurotensin concentration (40–4000 nM). The effect was blocked by a nonpeptide neurotensin antagonist. I_{NT} occurred in the absence of the extracellular Na^+, but not in the absence of the intracellular Cl^-, and it was outward directed by reversing the driving force for Cl^-. I_{NT}, like the γ-amino-butyric acid (GABA)-induced Cl^- current (I_{GABA}), remained little changed after virtual elimination of cytosolic free-ionized Ca^{2+} or after treatment with a Ca^{2+}-activated Cl^- channel blocker, but, in contrast to I_{GABA} it was resistant to the I_{GABA} blocker picrotoxin, slower in time course and more easily desensitized when repeatedly elicited. I_{NT} and I_{GABA} were additive to each other. AG-protein inhibitor markedly reduced I_{NT}, and a G-protein activator produced an inward current during which no current could be elicited by neurotensin. These results show that neurotensin exerts an effect to activate Ca^{2+}-insensitive Cl^- channels distinct from those activated by GABA in guinea-pig dorsal root ganglion cells, and the effect may arise through a G-protein-dependent mechanism.

Keywords: Neurotensin; SR 48692; Neurotensin receptor; Cl^- current; Dorsal root ganglion

1. Introduction

Neurotensin, which was first isolated from bovine hypothalamus and then synthesized (Carraway and Leeman, 1973, 1975), is a tridecapeptide and largely located in neural tissue (Schultzberg et al., 1980). Neurotensin has been suggested to play a role as a neurotransmitter (Snyder, 1980; Goedert et al., 1984; Komori et al., 1986) or a neuromodulator (Kalivas et al., 1983; Audinat et al., 1989; Seutin et al., 1989).

Furthermore, neurotensin has been demonstrated to have an antinociceptive effect (Clineschmidt et al., 1979; Yaksh et al., 1982; Spampinato et al., 1988). Clineschmidt et al. (1979) reported that, in mice, intracisternal administration of neurotensin resulted in an increase in the reaction time in the hot-plate test and a decrease in writhing response to acetic acid. Recently, Zhang et al. (1995) argued that neurotensin basically exerted an antinociceptive effect at the spinal level. In rats implanted with an indwelling intrathecal catheter, injection of neurotensin into the lumbar spinal cord through the catheter resulted in an increase of the nociceptive threshold in the hot-plate test and writhing response test (Yaksh et al., 1982). Demonstration of the modulation of central functions by neurotensin, together with the localization in of neurotensin the central nervous system, suggests that the peptide may play an important role in decreasing nociceptive effects.

Sensory neurons, which serve as primary afferent nociceptors, have their cell bodies in dorsal root ganglions, and their central processes connect synaptically to neurons in the sensory pathway in the central nervous system (Levine et al., 1993). Thus, dorsal root ganglion cells may function as a key element to transmit nociceptive stimuli to the central nervous system. A study in rats revealed a population of dorsal root ganglion cells having mRNA for neurotensin receptors, and the localization of neurotensin-immunoreactive neurons in the superficial dorsal horn of the spinal cord (Zhang et al., 1995). This suggests that such dorsal root ganglion cells may receive inhibitory inputs from the spinal neurons, which contain neurotensin. Xu et

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al. (1997) observed that, in rats, neurotensin produced an outward current in C-type and an inward current in A-type dorsal root ganglion cells, but after transection of the sciatic nerve, it produced an inward current in C-type cells as well as A-type cells.

We have now investigated the effect of neurotensin on the membrane properties of dorsal root ganglion cells from the guinea-pig using the whole-cell patch clamp technique in an attempt to examine the possible involvement of neurotensin in antinociceptive effects.

2. Materials and methods

2.1. Preparation of cells

Male guinea-pigs, weighing 300–450 g, were stunned and bled to death. Dorsal root ganglions were carefully removed from both sides along the cervical region of the spinal cord with scissors and placed in physiological salt solution (PSS; for composition see below). The isolated ganglions were washed in Ca^{2+}-free PSS three times, incubated for 5 min at 37°C in the solution, and then digested by incubation in PSS containing 30 μM Ca^{2+} with collagenase (0.3 mg ml^{-1}) and papain (0.6 mg ml^{-1}) for 20 min at 37°C. After enzymatic digestion, the tissues were placed in a test tube filled with fresh 120 μM Ca^{2+}-containing PSS and washed gently with the solution three times to stop the enzyme action. They were gently agitated by drawing them in and out of a blunt glass pipette some 80 times to dissociate dorsal root ganglion cells. The resulting suspension of the cells was filtered through a fine nylon mesh to remove the debris and centrifuged at 600 rpm for 2 min to collect the cells. The cells were resuspended in 1 ml PSS containing 0.5 mM Ca^{2+}, penicillin (10 unit/ml) and streptomycin (0.01 g/ml). Small aliquots of cell suspension were placed on coverglasses and kept at 37°C in a CO_2 incubator for 3–8 h until use. Single dispersed cells with a diameter of 20–30 m were chosen for the present experiments, because most of them (80–95% in different cell batches) were sensitive to neurotensin. In general, the cells responded to membrane depolarization with a Na^+ current, of which the peak amplitude was little changed by 1 μM tetrodotoxin ($n = 7$), and were classified as either Aδ- or C-type neurons (Villiere and McLachlan, 1996).

2.2. Recording of membrane currents

A coverglass with cells was placed in a 1-ml organ bath on the stage of an inverted microscope (CK2, Olympus, Shibuya-ku, Tokyo), and the organ bath was perfused with PSS at a rate of 5–10 ml/min for 1–2 min to wash away the debris. The cells were then equilibrated with PSS for several min before experiments were started.

Recordings of whole-cell membrane currents were made at room temperature (23–27°C), using standard patch clamp techniques (Hamill et al., 1981). Patch pipettes had resistances of 3–5 MΩ when filled with pipette solution. A constant patch clamp condition throughout each experiment was tested by applying a hyperpolarization pulse of 200-ms duration at 10 mV again at the end of the experiment.

The current signals in response to neurotensin and γ-amino-butyric acid (GABA) were amplified by a patch-clamp amplifier (CEZ-2300, Nihon Kohden, Shinjuku-ku, Tokyo) and stored on a PCM data recorder (RD-111T, TEAC, Musashino City, Tokyo) for later analysis and illustration. Data analysis was performed on a computer (Apple Macintosh Centris 610), using a data acquisition and analysis instrument (MacLab4, AD Instruments, Castle Hill, NSW, Australia). The current signals were filtered at a cut-off frequency of 1 kHz and digitized usually with a sampling rate of 20 kHz. Temporal parameters of current responses to neurotensin and GABA, such as time-to-peak and half-decay time, were estimated in a simple way without fitting.

The reversal potential for currents induced by neurotensin was determined using a ramp pulse protocol that consisted of voltage change from +60 to −100 mV over 350 ms (see Fig. 4C). The ramp pulse was applied to cells held at −80 mV before (control) and during exposure to neurotensin, and a net current induced by the peptide was measured with a subtraction method. No compensation for the capacitive current and series resistance was made, because the peptide-induced current was relatively slow in its activation rate, compared with the capacitive current, and small in size.

2.3. Drugs

The drugs used were bovine neurotensin (from Peptide Institute, Osaka, Japan), 2-[(1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxy-phenyl)Pyrazol-3-yl) carbonylamino]tricyclo(3.3.1.^{3,7})decan-2-carboxylic acid (SR 48692) (kindly supplied by Sanofi Researche, Montpellier, France), GABA, EGTA, HEPES (from Wako, Osaka, Japan), ATP (magnesium salt), guanosine 5′-triphosphate (GTP, sodium salt), 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid (BAPTA), collagenase (Type XI), papain, guanosine 5′-*o*-(2-thiodiphosphate) (GDPβS), guanosine 5′-*o*-(3-thiotriphosphate) (GTPγS), 4,4′-disothiocyanato-stilbene-2,2′-disulfonic acid (DIDS) and picrotoxin (from Sigma, St. Louis, MO, USA).

A stock solution of SR 48692 was dissolved in 100% dimethyl sulphoxide, stock solutions of DIDS and picrotoxin in 0.1% and 100% ethanol, respectively, and stock solutions of other drugs in distilled water. They were made up at 1000 or more times higher concentrations than those used for experiments and stored at 4°C or −20°C.

Extracellular application of drugs was made by replacing the bath solution with drug-containing solution five to

seven times within 30 s using a pair of syringes, one for injection and the other for suction. As one cycle of the injection and suction procedure allowed 70–80% of bath solution to be exchanged, complete replacement with the drug-containing solution was achieved within 10 s or so. Removal of drugs and exchange of bath solution for other purposes were performed substantially in the same way.

Intracellular application of drugs was performed by allowing them to diffuse from the patch pipette into the cell; specifically, EGTA or BAPTA (20 mM) was allowed to diffuse for at least 5 min, and GDPβS (2 mM) for 10 min, before application of neurotensin or GABA (Currie and Scott, 1992; Komori et al., 1992).

2.4. Solutions

2.4.1. Bath solutions

The PSS used in the experiments had the following composition (mM); NaCl 126, KCl 6, $CaCl_2$ 2, $MgCl_2$ 1.2, glucose 14, HEPES 10.5 (titrated to pH 7.2 with NaOH). Na^+-free solution was obtained by replacing 126 mM Na^+ in the PSS with equimolar $tris^+$ (titrated to pH 7.2 with HCl).

2.4.2. Pipette solutions

Four kinds of pipette solution were used: A KCl-based solution, which had the following composition (mM); KCl 134, ATP 1, EGTA 0.05, glucose 14, HEPES 10.5, GTP 0.1, (titrated to pH 7.2 with KOH), a Cl^--free solution, in which 134 mM Cl^- in the KCl-based solution was replaced by equimolar $glutamate^-$, a 5-mM Cl^--containing solution, which had the following composition (mM); L-glutamic acid 129, CsCl 5, ATP 1, EGTA 0.05, glucose 14, HEPES 10.5, GTP 0.1, (titrated to pH 7.2 with CsOH) and a 19-mM Cl^--containing solution, which consisted of L-glutamic acid 115 mM, CsCl 19 mM and the other constituents in the same concentrations as those of the 5 mM Cl^--containing solution.

EGTA (0.05 mM) added in the pipette solutions is not high enough to buffer intracellular Ca^{2+}, and it is generally used for whole-cell patch clamp studies in other cell types such as smooth muscle cell (Komori et al., 1992). The use of CsCl, but not KCl, for the preparation of the Cl^--deficient pipette solutions was effective to block Ca^{2+}-dependent and/or voltage-dependent K^+ currents, which could be activated in experiments at a holding potential of 0 mV and those with a ramp pulse protocol.

2.5. Statistical analysis

The values in the text are expressed as means ± S.E.M., in which n refers to the number of cells used for measurements. Statistical significance was tested using paired or unpaired Student's t-test or Welch's t-test, and differences were considered significant when $P < 0.05$.

3. Results

3.1. Membrane current response to neurotensin

Single dorsal root ganglion cells were patched with pipettes filled with a KCl-based solution and held under voltage clamp at −80 mV, which was close to the resting

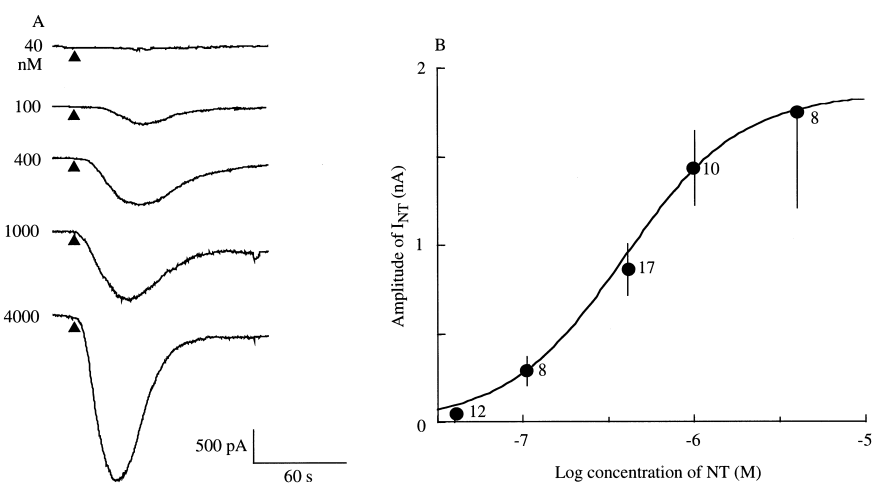

Fig. 1. Membrane current responses to neurotensin (NT) of dorsal root ganglion cells. Single isolated cells from guinea-pig dorsal root ganglions were bathed in PSS and held under voltage clamp at −80 mV using patch pipettes filled with KCl-based solution. (A) Current responses to NT at concentrations as indicated in different cells. NT was applied at the closed triangles and allowed to act during each current trace. NT elicited an inward current (I_{NT}), the amplitude of which increased as the NT concentration increased. Note that I_{NT} declined with time after reaching a peak even in the continued presence of NT. (B) A plot of the mean peak amplitude of I_{NT} against NT concentration. Each point represents the mean ± S.E.M. (vertical bars) of 8–17 measurements. The points were fitted by the Hill equation; $Y = Y_{max}/[1 + (EC_{50}/C)^n]$, where Y_{max}, EC_{50}, C, and n are the maximum amplitude of I_{NT}, concentration required for NT to produce half the maximum I_{NT}, NT concentration and Hill coefficient, respectively. The EC_{50} and n for the curve were 396 nM and 1.3, respectively.

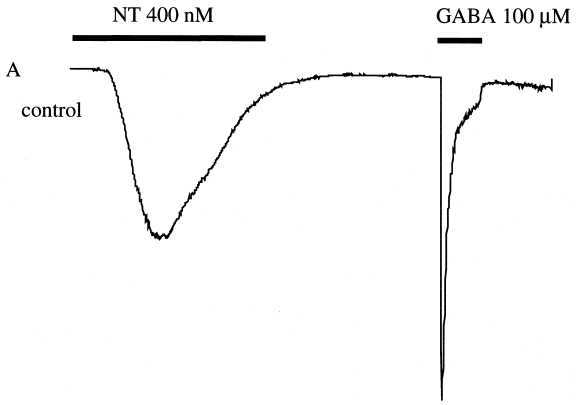

Fig. 2. Effect of SR 48692 on neurotensin-induced and GABA-induced currents. Current responses to neurotensin (NT) and GABA were recorded from cells held at −80 mV. The bath and pipette solutions were the same as in Fig. 1. Application of NT (400 nM) and GABA (100 μM) is indicated by solid lines above current traces. (A) Current responses to NT and GABA in one cell. (B) Current responses to these agonists in another cell exposed to SR 48692 (100 nM) for 5 min before and throughout their application. Note that NT was without effect in the SR 48692-treated cell.

membrane potential (Song and Narahashi, 1995). When neurotensin was applied at concentrations ranging from 40 to 4000 nM, it produced an inward current (I_{NT}) as shown in Fig. 1A. The I_{NT} developed with varying latencies to reach a peak and then declined with time even in the continued presence of the peptide. The peak amplitude of I_{NT} increased as the concentration of neurotensin increased (Fig. 1B). Mean peak amplitudes of I_{NT} activated at concentrations of 40 ($n = 12$), 100 ($n = 8$), 400 ($n = 17$), 1000 ($n = 10$) and 4000 nM ($n = 8$) were 58 ± 9, 325 ± 43, 886 ± 119, 1457 ± 203 and 1744 ± 536 pA, respectively. The concentration–response relationship obtained from the pooled data gave an EC_{50} value of 396 nM.

In the following studies to determine the ion channels responsible for I_{NT}, neurotensin was used at a concentration of 400 nM, close to the EC_{50} value, and GABA (100 μM) was used as a reference drug. GABA is well established to induce an inward current through activation of the Cl^- channel-including $GABA_A$ receptor in dorsal root ganglion cells (Valeyev et al., 1996).

To confirm mediation of I_{NT} via a specific receptor for neurotensin, the effect of a nonpeptide neurotensin receptor antagonist, SR 48692, on I_{NT} was examined. The antagonist was used at a concentration of 100 nM, which was about 30 times higher than the K_d value for antagonism of the contractile and relaxant responses to neurotensin in rat duodenum and colon (Mule et al., 1996), and in guinea-pig ileum and chick rectum (Unno et al., 1999). Pretreatment with SR 48692 for some 5 min prevented all tested cells ($n = 5$) from producing I_{NT} with no appreciable change in the inward current produced by GABA (I_{GABA}) (Fig. 2).

3.2. Ion channels responsible for I_{NT}

Under the ionic condition used, activation of nonselective cation channels or Na^+ channels would elicit an inward current. When a Na^+-free solution instead of PSS was used as bath solution, neurotensin was still effective in eliciting an inward current similar in shape and size to I_{NT} in PSS (Fig. 3A and B). The mean peak amplitude of I_{NT} in the Na^+-free solution was 999 ± 301 pA ($n = 7$), which was not significantly different from that of I_{NT} in PSS (886 ± 119 pA; $n = 17$) (Fig. 3C). Therefore, it seems likely that activation of nonselective cation channels or Na^+ channels does not contribute to I_{NT}.

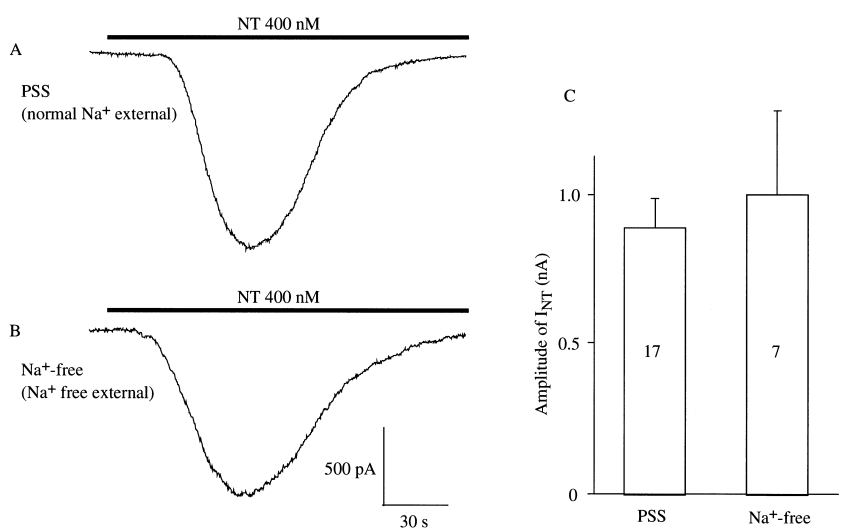

Fig. 3. Effect of depletion of Na^+ from bath solution on neurotensin-induced current (I_{NT}). Cells were patched with a pipette filled with KCl-based solution and held at −80 mV. Neurotensin (NT; 400 nM) was applied as indicated by solid lines above current traces. (A) I_{NT} in one cell bathed in normal PSS. (B) I_{NT} in another cell bathed in a Na^+-free solution prepared by replacing the Na^+ in normal PSS with $Tris^+$. (C) Comparison between I_{NT} amplitudes in normal PSS and Na^+-free solution. Each column indicates the mean ± S.E.M. (vertical bars) of measurements in attached numbers of cells. See text for details.

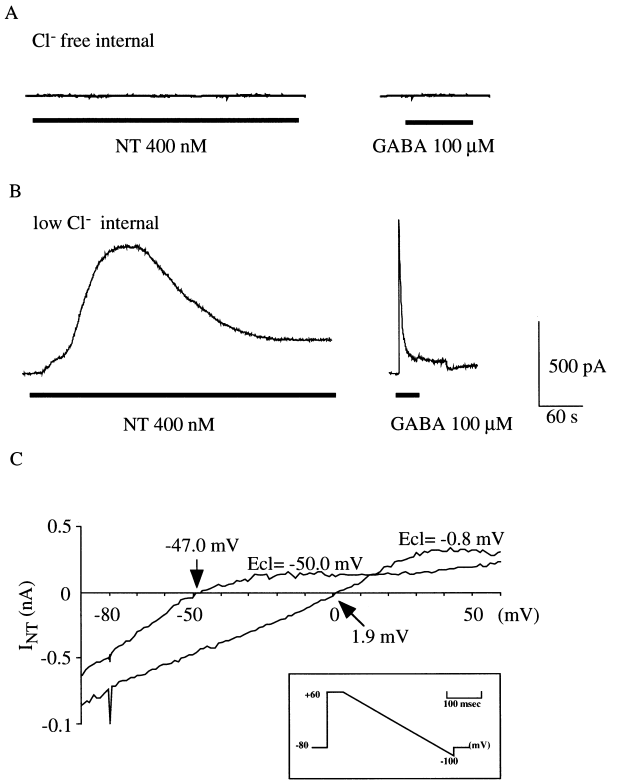

Fig. 4. Dependence of neurotensin-induced current (I_{NT}) on Cl^-. Cells were bathed in normal PSS, and neurotensin (NT; 400 nM) or GABA (100 μM) was applied as indicated by solid lines below current traces. (A) Current traces from one cell held at −80 mV and dialysed with a Cl^--free pipette solution (substituted glutamate for Cl^-). (B) Current traces from another cell held at 0 mV and dialysed with a 5-mM Cl^--containing pipette solution to give the Cl^- equilibrium potential as −84 mV. In (A) and (B), application of NT and GABA was performed at 5-min intervals, respectively. (C) Current–voltage (I–V) relationships for I_{NT} obtained by applying a ramp pulse (see the inset) in two different cells in which the Cl^- equilibrium potential (E_{Cl}) was given as −0.8 and −50.0 mV, respectively. The arrows indicate the reversal potentials of I_{NT} in those cells. See text for details.

To determine if Cl^- channels are activated by neurotensin, three different series of experiments were performed. First, cells were intracellularly dialysed with a Cl^--free solution and then exposed to neurotensin as well as GABA. As shown in Fig. 4A, neither neurotensin nor GABA elicited any appreciable current in the virtual absence of intracellular Cl^- ($n = 5$). Second, cells were intracellularly dialysed with a solution in which Cl^- concentration was reduced to 5 mM and then voltage-clamped at 0 mV. In these cells, the equilibrium potential for Cl^- (E_{Cl}) across the cell membrane was estimated to be −84 mV, and following activation of Cl^- channels, the membrane current would be expected to flow outward. In fact, neurotensin as well as GABA elicited an outward current, as shown in Fig. 4B ($n = 5$). Finally, the reversal potential for I_{NT} was determined in cells intracellularly dialysed either with a KCl-based solution to give an E_{Cl} value of −0.8 mV or with a 19-mM Cl^- solution to give a different E_{Cl} value of −50.0 mV. In these cells, a ramp pulse from 60 to −100 mV over 350 ms (see the inset in Fig. 4C) was applied before and during application of neurotensin, and current–voltage (I–V) relationships for I_{NT} were plotted by subtracting the I–V curve obtained before neurotensin application from that obtained near the peak of I_{NT}. As shown in Fig. 4C, the I–V relationship in a cell with an $E_{Cl} = -0.8$ mV gave a value of 1.9 mV as the reversal potential for I_{NT} and the corresponding relationship in another cell with an $E_{Cl} = -50.0$ mV gave a value of −47.0 mV. This similarity of the I_{NT} reversal potential to the E_{Cl} was obtained in three other cells.

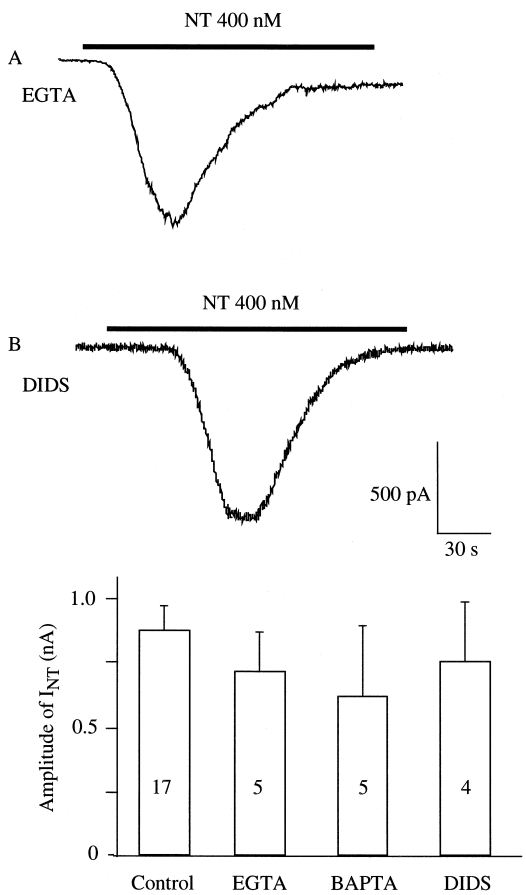

Fig. 5. Neurotensin-induced current (I_{NT}) in the virtual absence of intracellular Ca^{2+} and after blockage of Ca^{2+}-activated Cl^- channels. Cells were dialysed with a pipette solution containing 20 mM EGTA or 20 mM BAPTA to keep the intracellular Ca^{2+} concentration virtually zero, or exposed to an extracellular solution containing 100 μM DIDS to block Ca^{2+}-activated Cl^- channels, I_{NT} was recorded at a holding potential of −80 mV. (A and B) Recording traces of I_{NT} from one cell treated with 20 mM EGTA and from another cell treated with 100 μM DIDS, respectively. Neurotensin (NT; 400 nM) was applied as indicated by the solid lines above current traces. (C) The mean peak amplitudes of I_{NT} in the intracellular presence of 20 mM EGTA and 20 mM BAPTA and in the extracellular presence of 100 μM DIDS. Each column indicates the mean ± S.E.M. (vertical bars) of measurements in attached numbers of cells. None of the mean values for EGTA, BAPTA and DIDS was significantly different from the control value (control).

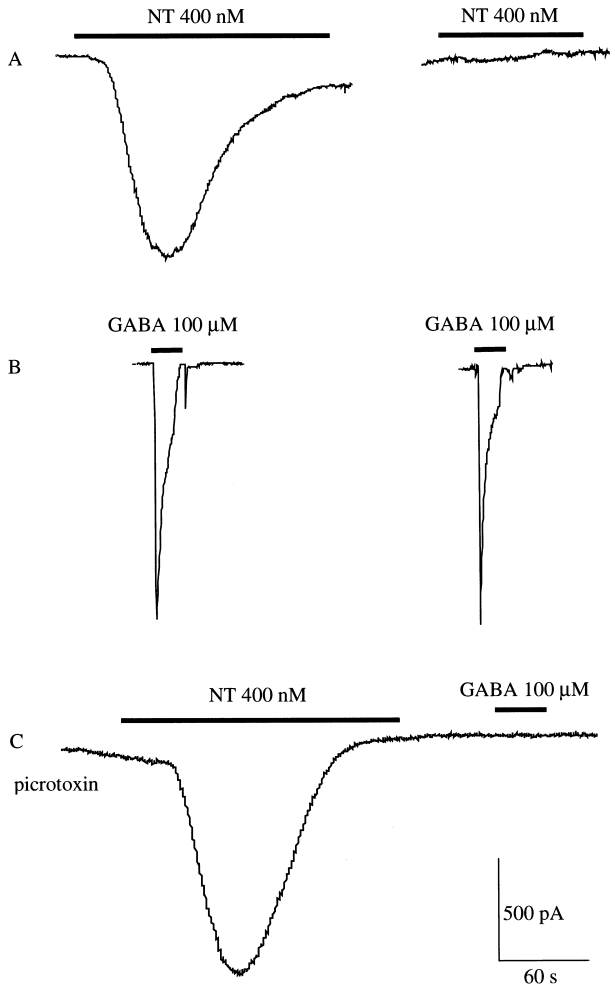

Fig. 6. Different profiles of neurotensin-induced current (I_{NT}) and GABA-induced current (I_{GABA}). Cells were bathed in normal PSS and held at −80 mV using patch pipettes filled with KCl-based solution. Neurotensin (NT; 400 nM) and GABA (100 μM) were applied as indicated by solid lines above current traces. (A and B) Current traces from two different cells in which application of NT (A) or GABA (B) was repeated after 5 min. Note that the second application of NT produced no detectable current. (C) Current traces from a cell to which NT and then GABA were applied in the presence of 100 μM picrotoxin, a blocker of GABA-gated Cl^- channel. Note that NT, but not GABA, was effective in eliciting an inward current.

These results indicate that neurotensin exerts an effect to produce activation of Cl^- channels in dorsal root ganglion cells from the guinea-pig.

3.3. Dependence of I_{NT} on intracellular Ca^{2+}

Two types of Cl^- channels have been demonstrated to be present in dorsal root ganglion cells, one which requires intracellular Ca^{2+} to be activated, and another which does not.

In cells dialysed intracellularly with 20 mM of EGTA or BAPTA to keep the intracellular Ca^{2+} concentration virtually zero, I_{NT} was elicited (Fig. 5A). The mean peak amplitudes of I_{NT} in the presence of intracellular EGTA and BAPTA were 725 ± 167 pA ($n = 5$) and 627 ± 336 pA ($n = 5$), respectively, neither of which significantly differed from the control value (886 ± 119 pA; $n = 17$) (Fig. 5C). In cells exposed extracellularly to 100 μM DIDS, a blocker of Ca^{2+}-activated Cl^- channel, I_{NT} was elicited with a mean peak amplitude of 743 ± 231 pA ($n = 4$). The mean value was also not significantly different from the control (Fig. 5C). These properties of I_{NT} resembled those of I_{GABA} (data not shown).

The results show that Cl^- channels responsible for I_{NT} are distinct from Ca^{2+}-activated Cl^- channels.

3.4. Comparison between neurotensin-induced Cl^- current and GABA-induced Cl^- current

Neurotensin, like GABA, was found to cause activation of Ca^{2+}-independent Cl^- channels. However, the Cl^- current produced by neurotensin (I_{NT}) was different in some aspects from the Cl^- current produced by GABA (I_{GABA}).

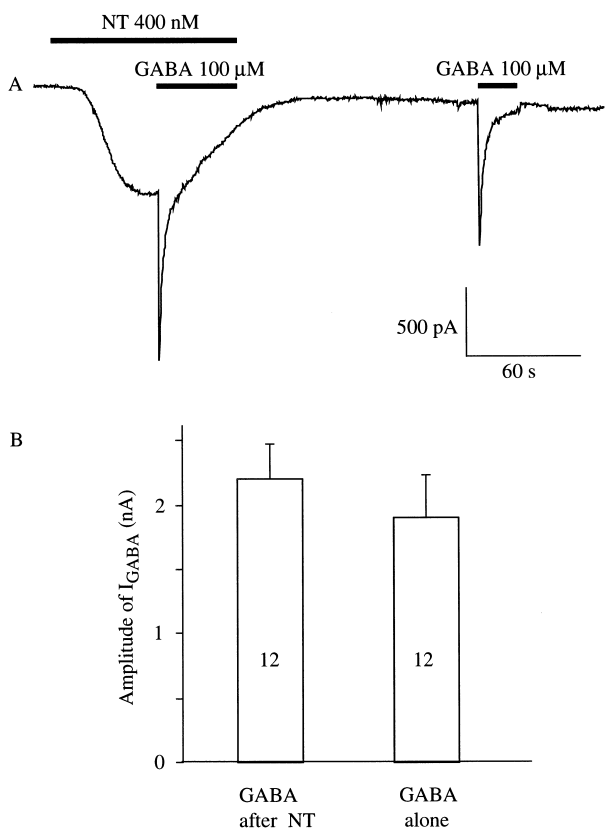

Fig. 7. GABA-induced current (I_{GABA}) during and after neurotensin-induced current (I_{NT}). Cells were bathed in normal PSS and held at −80 mV using patch pipettes filled with KCl-based solution. (A) Current responses of a cell to GABA (100 μM) applied in the presence of neurotensin (NT; 400 nM) and then in its absence. GABA and NT were applied as indicated by solid lines above current traces. (B) Comparison of the peak amplitudes of I_{GABA} elicited during activation of I_{NT} and after removal of neurotensin. Each column indicates the mean ± S.E.M. (vertical bars) of measurements in 12 cells.

I_{NT}, which was recorded from 12 cells and matched I_{GABA} in the peak amplitude, occurred with time-to-peak of 43.9 ± 3.4 s and half-decay time of 46.0 ± 4.2 s. Both parameters of I_{GABA} were within a few seconds. When neurotensin was applied again after a 5-min interval, the second application produced a very small or no detectable current response (Fig. 6A). In case of GABA, successive GABA responses were obtained and their peak amplitude and time course were little changed (Fig. 6B).

As shown in Fig. 6C, treatment with 100 μM picrotoxin, a blocker of the GABA-gated Cl^- channel, allowed neurotensin to induce an inward current. The mean peak amplitude of I_{NT} of 962 ± 124 pA ($n = 4$) in picrotoxin-treated cells was not different from that of I_{NT} in control cells (see above).

When GABA was applied during activation of I_{NT}, it produced an additional current, as shown in Fig. 7A. The mean peak amplitude of I_{GABA} elicited at near peak of I_{NT} was 2210 ± 328 pA ($n = 12$), which was not significantly different from the corresponding value (1910 ± 378 pA; $n = 12$) obtained after removal of neurotensin (Fig. 7B). In addition, I_{GABA} during I_{NT} had much the same time course as that of I_{GABA} in the absence of neurotensin (Fig. 7A). The additive effects of neurotensin and GABA suggest that they may activate separate types of Cl^- channels.

3.5. Involvement of G-proteins in generation of I_{NT}

In cells dialysed intracellularly with 2 mM GDPβS to block activation of G-proteins, neurotensin induced an inward current (Fig. 8A). However, the peak amplitude of the inward current varied from some 20 up to 200 pA in different cells, giving a mean value of 106 ± 22 pA ($n = 10$), which was significantly smaller ($P < 0.01$) than the control value (886 ± 119 pA, see above). In GDPβS-treated cells, current responses to GABA were comparable with those in control cells (Fig. 8A).

Intracellular application of 0.5 mM GTPγS, an activator of G-proteins, via the patch pipette resulted in the generation of an inward current with a peak amplitude of 871 ± 93 pA ($n = 5$), as shown in Fig. 8B. The GTPγS-induced inward current began to occur within 4 min after breakthrough of the patch membrane (see the arrow in Fig. 8B) to reach a peak in 3 to 8 min, and then gradually declined to a sustained level. Neurotensin, when applied during the generation of the GTPγS-induced current, was without effect ($n = 3$, data not shown).

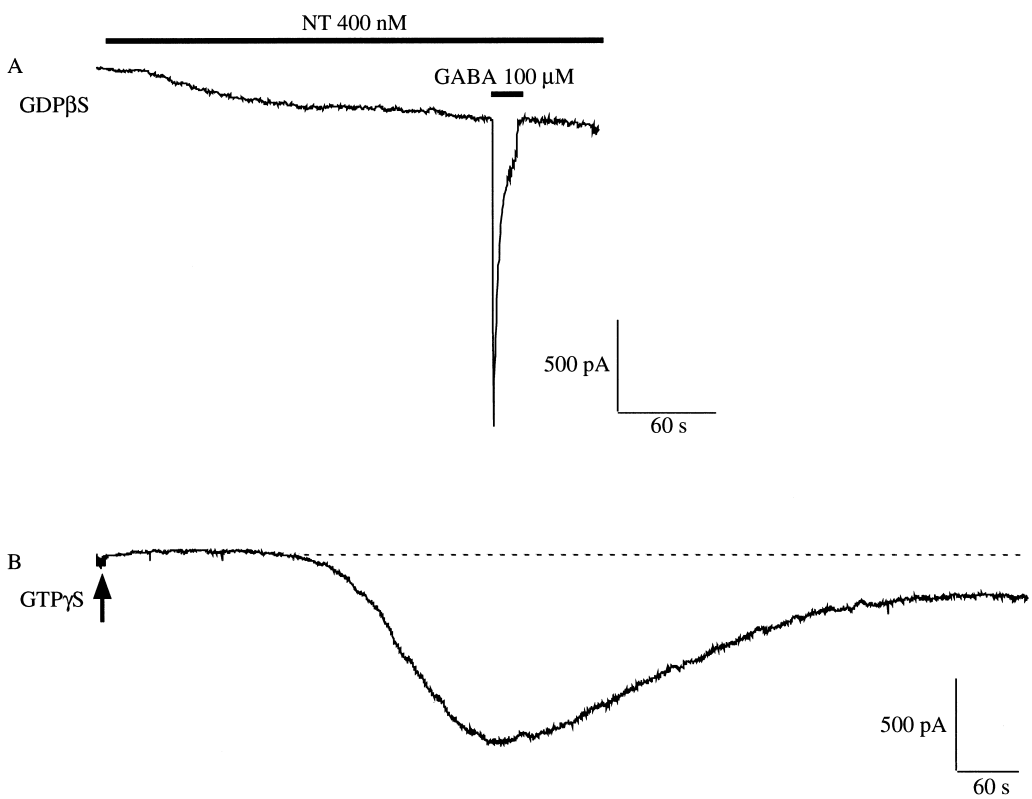

Fig. 8. Involvement of G-proteins in neurotensin-induced current (I_{NT}). Cells were bathed in normal PSS and voltage-clamped at −80 mV. (A) Current responses of a GDPβS-treated cell to neurotensin (NT; 400 nM) and subsequent GABA (100 μM), applied as indicated by the solid lines. GDPβS (2 mM) was intracellularly applied via the patch pipette. Note that NT did not produce a typical pattern of inward current as seen in control cells (e.g., see Fig. 6A). (B) A current response of a cell to 0.5 mM GTPγS applied intracellularly via the patch pipette. The GTPγS application was started at the arrow, indicating the time when the patch membrane was ruptured. Approximately 4 min later, an inward current was elicited without application of NT.

The results suggest that G-proteins are involved in the generation of I_{NT}.

4. Discussion

Neurotensin elicited an inward current (I_{NT}) in guinea-pig dorsal root ganglion cells. I_{NT} remained almost unaltered after elimination of Na^+ from the extracellular fluid but disappeared after elimination of intracellular Cl^-, its flow direction could be changed by reversal of the driving force for Cl^-, the difference between the Cl^- equilibrium potential (E_{Cl}) across the cell membrane and the holding potential, and its I–V relationship obtained with a ramp pulse protocol indicated a reversal potential close to E_{Cl} (see Fig. 4C). In the ramp pulse experiments, a net I_{NT} was obtained by subtracting the I–V curve obtained before from that after application of neurotensin. Thus, we considered that the I_{NT} was little contaminated by Na^+ and Ca^{2+} currents. In addition, Na^+ channels were inactivated for the initial period of the ramp pulse during which the membrane potential was stepped to +60 mV for 50 ms. Thus, the above results provide evidence that neurotensin produces activation of Cl^- channels.

I_{NT} was blocked by SR 48692 (100 nM), but I_{GABA} remained almost unchanged after being rendered insensitive to neurotensin with this drug. It is, therefore, apparent that I_{NT} is mediated via activation of specific receptors for neurotensin. I_{NT}, like I_{GABA}, is due to activation of Ca^{2+}-independent Cl^- channels, since the current could be elicited in the virtual absence of intracellular Ca^{2+} and was resistant to DIDS, a blocker of Ca^{2+}-activated Cl^- channels. However, I_{NT} was recorded from cells where GABA-gated Cl^- channels were blocked by picrotoxin. Moreover, I_{GABA} elicited at around the peak of I_{NT} showed no significant change in amplitude, indicating that I_{NT} and I_{GABA} are additive. Therefore, it is suggested that I_{NT} may be the result of activation of Cl^- channels distinct from those activated by GABA.

Recently, Valeyev et al. (1996) have reported that I_{GABA} in human dorsal root ganglion cells is resistant to picrotoxin and is not mediated by authentic GABA receptors. It will be necessary to characterize the Cl^- channels carrying this type of I_{GABA} and those carrying I_{NT} observed in the present study.

Neurotensin receptors belong to a family of receptors, which regulate distinct effector proteins through mediation of a group of G-proteins (Tanaka et al., 1990; Jiang et al., 1994). The present observations of the inhibition of I_{NT} with GDPβS, and the induction of I_{NT}-like currents and the disappearance of I_{NT} with GTPγS are compatible with the idea that the neurotensin receptor is a G-protein coupled receptor. In neurons other than dorsal root ganglion neurons, it has previously been suggested that neurotensin receptors are linked via $G_{q/11}$-type G-protein to activation of the protein kinase C pathway, leading to phosphorylation of ion channels (Farkas et al., 1994; Wang and Wu, 1996; Cathala and Paupardin-Tritsch, 1997). In dorsal root ganglion cells, the same signal transduction pathway might be involved in the activation of Cl^- channels responsible for I_{NT}.

In the present experiments, a second application of GABA (100 μM) 5 min later caused no desensitization of I_{GABA}. However, we found in preliminary experiments that if GABA was applied again after a 3-min interval, the size of I_{GABA} was reduced to a similar extent as observed by Martina et al. (1996). This is the reason why GABA as well as neurotensin was added after a 5-min interval, to compare desensitization of I_{NT} and I_{GABA}. This comparison revealed that the effect of neurotensin on Cl^- channels was readily desensitized, and this may result from desensitization of the neurotensin receptor itself, as previously reported (Audinat et al., 1989; Tanaka et al., 1990). The depolarizing effect of neurotensin on the membrane of the frontal pyramidal neurons in slices of rat cerebral cortex was found to disappear in a short time and failed to appear when application of neurotensin was repeated within 20 min of the first application (Audinat et al., 1989).

The dorsal root ganglion seems unlikely to be a simple relay station, which conducts peripherally induced sensory inputs, such as pain to the central nervous system, since neurotensin as well as GABA is present in the dorsal root ganglion (Zhang et al., 1996) and produces activation of Cl^- channels in the dorsal root ganglion cell membrane, leading to stabilization of the membrane. Neurotensin and GABA, whether they are secreted as a neurotransmitter or a neuromodulator (Nicoll and Alger, 1979; Snyder, 1980; Goedert et al., 1984; Senba et al., 1982; Kalivas et al., 1983; Komori et al., 1986; Audinat et al., 1989; Seutin et al., 1989; Stapelfeldt and Szurszewski, 1989), may have an inhibitory function in the dorsal root ganglion. In fact, neurotensin has an antinociceptive effect (Clineschmidt et al., 1979; Yaksh et al., 1982; Spampinato et al., 1988). Neurotensin would act in a very different way from GABA, as the action is slower in onset and development, and more readily desensitized. The existence of neurotensin- and GABA-mediated inhibitory systems, which have such different properties, in the dorsal root ganglion must be related to a unidentified function. A fine and complicated modulation of information from the peripheral apparatus could be brought about by the operation of two different systems. The release of neurotensin in the periaqueductal gray in rat midbrain has been demonstrated to be mediated by μ-type opioid receptors (Stiller et al., 1997). Yaksh et al. (1982) reported that cells containing neurotensin are present in a close contact with cells containing enkephalin in the substantia gelatinosa in rat spinal cord, and they suggested that neurotensin acts to release enkephalin from those cells and that the action of neurotensin is related to antinociception. The present results do not exclude the

possibility that the antinociceptive effect of neurotensin is indirectly brought about by opioid systems. Recently, Smith et al. (1997) reported that neurotensin had a dual effect on pain sensation, an inhibitory effect at low concentrations and a facilitatory effect at high concentrations. Apart from the mechanism by which the dual effect is exerted, neurotensin seems to act as a pain modulator.

Dubuc et al. (1994) reported that SR 48692 failed to block analgesia produced by intracerebral injection of neurotensin and suggested that the antinociceptive effect of neurotensin is mediated by SR 48692-insensitive neurotensin receptors in the central nervous system. If so, two types of neurotensin receptor might be involved in the regulation of antinociception, one is SR 48692-insensitive, and the other is SR 48692-sensitive.

In summary, neurotensin exerts an effect to activate Cl^- channels distinct from those activated by GABA in guinea-pig dorsal root ganglion cells, and the effect is mediated by SR 48692-sensitive neurotensin receptors and is readily desensitized. Neurotensin may have an inhibitory function in the dorsal root ganglion.

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