Membrane potential and current responses to neurotensin in the longitudinal muscle of the rectum of the fowl

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1 The effects of neurotensin (NT) on membrane potential and membrane current of the longitudinal smooth muscle of chicken rectum were investigated by intracellular recording and whole-cell voltage clamp.

2 NT (3 nM–1.2 μ M), when applied via the bathing medium, produced a concentration-dependent membrane depolarization with an EC_{50} of 18 ± 2 nM ($n = 7$) which was accompanied by an increase in the membrane conductance. The effect was biphasic: an initial, rapid depolarization reached a peak within 2–3 min and then declined to a lower but still elevated level which was sustained until washout.

3 Excitatory junction potentials (e.j.ps), which were non-adrenergic non-cholinergic (NANC) in nature, were decreased in amplitude and total duration in the presence of NT (0.6 μ M). The depression of the e.j.p. was due mainly to the reduction of the membrane resistance.

4 When NT was applied locally by means of pressure ejection from a micropipette containing NT, some cells responded with a membrane depolarization and some failed to respond, whereas e.j.ps could invariably be elicited from all of them.

5 In single muscle cells enzymatically isolated from the muscle and dialyzed under voltage clamp at –50 mV with a CsCl-rich solution, NT (5 or 10 μ M) produced an inward current. NT-induced inward currents were obtained with inclusion of 10 mM EGTA in the pipette solution and their reversal potential was around 0 mV. In cells dialyzed under voltage clamp at 0 mV with a KCl-rich solution, NT (5 μ M) produced a brief outward current followed by abolition of spontaneous transient outward currents.

6 The present results suggest that the membrane depolarization, which may arise from activation of non-selective cation channels, and release of calcium from internal stores produced by neurotensin are responsible for its contractile activity in the longitudinal smooth muscle of chicken rectum. Further, the depolarizing effect may provide support for the involvement of NT in the NANC transmission in this preparation.

Keywords: Neurotensin; smooth muscle; fowl; membrane potential; membrane current; voltage clamp; rectum

Introduction

In the search for the transmitter of non-adrenergic, non-cholinergic (NANC) excitatory nerves in the rectum of the chicken (Komori *et al.*, 1986a,b), a neurotensin-like peptide was isolated from chicken rectum which has different amino acid composition from bovine neurotensin but indistinguishable biological activities (Iwabuchi *et al.*, 1987). This peptide is identical to chicken neurotensin isolated by Caraway & Bhatnagar (1980a,b), from chicken small intestine. Neurotensin has been proposed as a candidate for the excitatory neurotransmitter of the NANC nerves in chicken rectum (Komori *et al.*, 1986a). This proposal rests on the following findings: neurotensin has a high potency in contracting the rectal muscle and the ED_{50} is about 2 nM; its presence in Remak ganglia and the rectum in which cell bodies and axon terminals of the NANC neurones are located, respectively, and its ability to abolish the contractile responses of the rectal muscle to stimulation of NANC neurones as well as the peptide itself. However, membrane responses to neurotensin, changes in membrane potential, membrane resistance and membrane current, have not been measured as yet, although evidence suggests that the NANC e.j.p. is mediated by an increase in membrane permeability to ions, mainly Na ions (Komori & Ohashi, 1982; 1988a).

We have now investigated the effects of neurotensin using microelectrodes and the voltage clamp technique. The pep-

tide was applied locally by pressure ejection from a micropipette or by adding to the bathing medium and its effects on the membrane properties of the longitudinal muscle cells of chicken rectum were studied. A possible involvement of neurotensin in the NANC transmission has also been examined. Throughout the present study, synthetic bovine neurotensin was used, because synthetic chicken neurotensin is not available.

Methods

Preparation of smooth muscle strips and single smooth muscle cells

White Leghorn chickens (*Gallus domesticus*, aged more than 80 days and weighing 0.8–2.0 kg) were stunned and bled to death. The rectal region of the intestine was excised, from which strips (1–2 mm wide and 15–20 mm long) of the longitudinal muscle were cut and used for recording of membrane potential changes. Single smooth muscle cells were obtained enzymatically from small pieces (0.5 $\times$ 0.5 mm) of the longitudinal muscle layer isolated from chicken rectum: tissue pieces were incubated in a physiological salt solution (PSS, composition given below) with 30 μ M added Ca^{2+} plus collagenase (1.25 mg ml⁻¹), papain (4.5 mg ml⁻¹) and bovine serum albumin (5 mg ml⁻¹) for 30–50 min at 37°C. The tissue pieces were then transferred to a PSS with 120 μ M added Ca^{2+} but no added enzymes, and gently agitated to disperse single smooth muscle cells. Single cells were collected by

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centrifuging at 700 r.p.m. for 2 min, suspended in a PSS with 0.8 mM added Ca^{2+} , and stored in a small aliquot on glass cover-slips in a moist atmosphere at 4°C until use. The cells were used in experiments on the day of preparation.

Intracellular recordings of membrane potential

The muscle strip preparation was mounted, mucosal side up, in a 2 ml organ bath with two chambers, one for recording and the other for stimulation, as described by Abe & Tomita (1968). The part in the recording chamber was pinned to a black rubber piece. The two chambers were filled and irrigated at a flow rate of 2–3 ml min⁻¹ with Tyrode solution (composition given below) preheated to 33 ± 1°C and bubbled with air to keep the pH at 7.4. A small hole (about 1.5 mm in diameter) in the partition between the two chambers through which the muscle strip had been passed also served to allow Tyrode solution to flow from the recording chamber to the stimulating chamber. Overflowing solution from the stimulating chamber was sucked away with an aspirator.

Membrane potential changes were recorded intracellularly with microelectrodes filled with 3 M KCl solution and having a resistance of 40–80 MΩ. Electrotonic potentials were evoked by applying current pulses of varied durations to the tissue through the stimulating plates. Voltage gradients in the solution of the stimulating chamber produced by passing current pulses were monitored by one silver electrode. Excitatory junction potentials were elicited by electrical field stimulation of intramural nerves of the tissue with square-wave pulses of 0.1 ms duration at an appropriate intensity (10–50 V). The stimulus pulses were delivered by a stimulator (Nihon Kohden, SEN-3013) through a pair of Ag-AgCl electrodes (1 mm in diameter). One electrode, which was insulated with Araldite (Nagase) except for the tip, was placed in the centre of the tissue 1.5–2.0 mm away from the stimulating partition in contact with it. The other electrode, which was uninsulated, was placed in the bathing solution.

For local application of neurotensin to the tissue, glass micropipettes (3–10 μm in tip diameter) filled with neurotensin (0.3 mM) made up in Tyrode solution were used. The drug-containing pipette was connected by polyethylene tubing to a nitrogen tank via a three-way solenoid valve (CKD corp., UMGI-T1) so that the drug solution could be ejected by pressure (2.5–3.5 kg cm⁻²). Ejected amounts of the drug solution can be controlled by varying the duration of current pulses (8–1000 ms) delivered from a stimulator (Nihon Kohden, MES-3) to turn on the solenoid valve. The relationship between the pulse duration and the drug amount was determined as described by Komori & Ohashi (1988b). The drug-containing pipette was manipulated to place the tip in a position as close as possible to the tissue surface and 50 μm away from the recording microelectrode.

This experimental apparatus permitted electrotonic potentials, excitatory junction potentials and responses to pressure ejection of neurotensin to be recorded from the same cell (Komori & Ohashi, 1988b). The potential changes were displayed on an oscilloscope and photographed.

To Tyrode solution were added atropine (0.5 μM), methysergide (2 μM), pyrilamine (1.3 μM), isoprenaline (1.3 μM) and methoxyverapamil (D 600, 10 μM) and if necessary, guanethidine (2 μM) throughout the course of the experiments in which changes in membrane potential were recorded. Atropine, methysergide and pyrilamine served to block effects of acetylcholine, 5-hydroxytryptamine and histamine, respectively, possibly released from the tissue; guanethidine to block noradrenaline release and isoprenaline and D 600 served to suppress electrical spike activity and development of tension in the smooth muscle. The concentrated solutions of these drugs were made up and stored at -20°C. Final dilutions of the stock solution were made in Tyrode solution just before use and the solutions were frequently renewed.

Recordings of membrane current

A glass cover-slip with cells was placed in a small organ bath (0.6 ml) and then irrigated with PSS. Whole-cell membrane currents were recorded by standard patch-clamp techniques (Hamill *et al.*, 1981) and patch pipettes filled with a solution (composition given below) with a resistance of 4–7 MΩ. Membrane currents were amplified by an amplifier (List, EPC-7), stored on FM tape by a recorder (Sony, FR-3215W) and replayed onto a thermal array recorder (Nihon Kohden, RTA-1100M) for illustration and analysis.

Drugs and solutions

Drugs used were atropine sulphate (Tanabe), methysergide hydrogen maleate (Sandoz), pyrilamine maleate (Sigma), (-)-isoprenaline sulphate (Merck), bovine neurotensin (Peptide Institute Inc., Osaka), methoxyverapamil (D 600, Knoll), adenosine 5'-triphosphate (ATP, Sigma), tetrodotoxin (Sankyo) and guanethidine sulphate (Ciba-Geigy). The stock solutions of all drugs were dissolved in distilled water, made up at 1000 or more times higher concentrations than those used for the experiments, and stored at -20°C. A certain amount of the concentrated drug solution was added to the solution in the organ bath or the reservoir to give the final desired concentration, and the drug was washed away by replacing the bathing solution with fresh solution.

PSS contained (in mM): NaCl 126, KCl 6, CaCl_2 2, MgCl_2 1.2, glucose 14 and HEPES 10.5 (titrated to pH 7.2 with NaOH). Tyrode solution contained (in mM): NaCl 137, KCl 2.7, NaH_2PO_4 0.4, NaHCO_3 12, MgCl_2 1, CaCl_2 1.8 and glucose 5.6. The solution in the patch pipette contained (in mM): CsCl 134, MgCl_2 1.2, MgATP 1, EGTA 0.05, glucose 14 and HEPES 10.5 (titrated to pH 7.2 with NaOH). To record outward K^+ current, the CsCl in the pipette solution was replaced with KCl.

Means are expressed with ± the standard error of the mean. The statistical significance was evaluated by Student's *t* test for paired samples and *P* values of 0.05 or less were considered significant.

Results

Membrane potential response to neurotensin

Bath application The average resting membrane potential of the smooth muscle cells was -54 ± 0.4 mV (mean ± s.e.mean, range -40 to -65 mV, *n* = 140). Bath application of neurotensin (NT) resulted in a concentration-dependent membrane depolarization with EC_{50} of 18 ± 2 nM (*n* = 7). When 0.6 μM NT was applied, the concentration required to produce a nearly maximal response, it produced a biphasic membrane depolarization: the initial depolarization reached a maximum of more than 20 mV within 2–3 min and then it declined to a lower but still elevated level (about 10 mV more positive than the resting membrane potential) which was sustained throughout the period of NT application (Figure 1). The effect of NT was reversible and tetrodotoxin (0.25 μM)-insensitive, indicating a direct action of the peptide on the muscle membrane.

Electrotonic potentials were evoked by applying inward current pulses and recorded from smooth muscle cells at a distance of less than one fifth of the length constant (1.6 mm) measured by spatial decay of electrotonic potentials (Komori & Ohashi, 1982) from the stimulating site. After application of 0.6 μM NT they were decreased in amplitude to $56 \pm 10\%$ (*n* = 10) at the peak depolarization and to $63 \pm 5\%$ (*n* = 10) during the sustained membrane potential (10 min after NT application) of the amplitude of electrotonic potentials recorded immediately before application of the peptide (Figure 2a). As the tissue has cable-like properties (Komori & Ohashi, 1982), a decrease in membrane resistance to about

Figure 1 Membrane potential change recorded intracellularly from smooth muscle cells of the longitudinal muscle layer of chicken rectum induced by $0.6 \mu\text{M}$ neurotensin (NT). Each point represents the mean of measurements from 4–17 cells in different preparations and is plotted against time after NT application. C, the membrane potential before exposure to NT. Vertical bars indicate s.e.mean. Bathing solution containing atropine ($0.5 \mu\text{M}$), methysergide ($2 \mu\text{M}$), pyrilamine ($3 \mu\text{M}$), isoprenaline ($1.3 \mu\text{M}$) and D 600 ($10 \mu\text{M}$) was used throughout the experiment.

Figure 2 The effect of neurotensin (NT) on electrotonic potentials. (a) Electrotonic potentials evoked by applying inward current pulses of 1 s duration using partition stimulation and recorded intracellularly. All responses were from the same cell: (i) control; (ii) and (iii), 4 and 15 min after application of $0.6 \mu\text{M}$ NT; and (iv) 6 min after its washout. Upper trace, applied current strength in terms of V cm^{-1} ; lower trace, potential records. (b) Plots of electrotonic potential amplitude (mV) against applied current strength (I , arbitrary unit) before (●, solid line) and after (○, dashed line) application of $0.6 \mu\text{M}$ NT. (i) and (ii), 5 and 15 min after application of NT. Lines fitted to the data points by eye. The bathing solution contained the same drugs as described in Figure 1.

31% and 40%, respectively can be calculated from the reduction of electrotonic potential amplitude. Figure 2b shows the result of an experiment in which current-voltage relationships were studied before and 5 and 15 min after application of $0.6 \mu\text{M}$ NT. The current-voltage relationship was linear at the three different times and the slopes were clearly less steep in

the presence of NT, also indicating a decrease in the membrane resistance.

Excitatory junction potentials (e.j.ps) were elicited by electrical stimulation of the intramural nerves, which were non-adrenergic, non-cholinergic (NANC) in nature, as previously reported (Takewaki & Ohashi, 1977; Komori & Ohashi, 1982). When e.j.ps were recorded from muscle cells 2 mm away from the electrode for nerve stimulation, their amplitude was $5.4 \pm 0.2 \text{ mV}$ ($n = 34$). Application of $0.6 \mu\text{M}$ NT resulted in a decrease of the amplitude of e.j.ps to $48 \pm 8\%$ ($n = 9$) and $66 \pm 7\%$ ($n = 10$) at the two respective levels of the peak and sustained depolarization produced by NT. The total duration of the e.j.ps was also significantly reduced from the control value of $840 \pm 30 \text{ ms}$ ($n = 10$) to $687 \pm 56 \text{ ms}$ ($n = 10$) at the peak depolarization and to $720 \pm 54 \text{ ms}$ ($n = 10$) during the sustained depolarization (10–15 min later). When the sustained membrane depolarization was eliminated artificially by applying inward current, the e.j.p. remained unchanged. In the absence of NT, displacement of the membrane potential from the resting level to a similar level to the sustained membrane potential produced by NT resulted in a decrease in the amplitude of the e.j.p. only by less than 10%, and no or a very slight decrease in total duration. To investigate the precise relationship between the effects of NT on the e.j.p. and on the membrane resistance, e.j.ps and electrotonic potentials were recorded from the same cell at varied intervals after application of $0.6 \mu\text{M}$ NT. As shown in Figure 3, e.j.ps and electrotonic potentials were decreased in amplitude by NT. The effects of NT on both potentials gradually faded in a parallel manner during early the period of NT application, 20 min from the start of NT application, the electrotonic potential amplitude had returned to the control level but the e.j.p. amplitude was still reduced. After that, electrotonic potentials continued to increase in amplitude and reached a level greater than the control whereas the e.j.p. amplitude did not change any more.

Figure 3 The effects of neurotensin (NT) on e.j.ps and electrotonic potentials. (a) E.j.ps and electrotonic potentials recorded intracellularly from the same cell before and after application of $0.6 \mu\text{M}$ NT. E.j.ps were evoked by electrical field stimulation of intramural nerves with a train of 4 square-wave pulses of 0.1 ms duration at 1 Hz and electrotonic potentials were evoked by applying inward current pulses of 1 s duration. (i) Control; (ii)–(v) 4, 8, 15 and 20 min after application of NT. Upper trace, applied current strength in terms of V cm^{-1} ; lower trace, potential records. (b) Changes in e.j.p. amplitude (○, solid line, e.j.p.), electrotonic potential amplitude (●, solid line, EP) and membrane potential (dashed line, Em) in the continued presence of $0.6 \mu\text{M}$ NT. E.j.p., EP and Em are plotted against time after NT-induced peak depolarization; e.j.p. and EP are expressed as percentages of those measured immediately before NT application. The bathing solution contained guanethidine in addition to the drugs listed for Figure 1.

Local application Local application of NT by means of pressure ejection from a micropipette filled with solution containing NT (0.3 mM) caused either no detectable membrane responses (less than 2 mV) or membrane responses with different features from smooth muscle cells in the area exposed to NT. Further, in some records, small and irregular fluctuations of membrane potential preceded the response, which was occasionally followed by dislodgement of the microelectrode. Cells responding to NT include those showing a membrane depolarization with an amplitude of 2–34 mV, a latency of 0.07–0.3 s and a time taken to reach a peak (time to peak) of 0.1–1.5 s (NT-sensitive cells) (Figures 4, 5 and 6), and those showing a membrane depolarization with a smaller amplitude of 2–5 mV, a longer latency of 1–3 s and a longer time to peak of 1.0–2.5 s (less NT-sensitive cells) (Figure 4b). The former cells were much smaller in number than the latter cells. In addition, the responses in NT-sensitive cells were evoked with a pulse duration of 8–200 ms, but those in less NT-sensitive cells needed a longer pulse duration of 200–1000 ms. As the pulse duration was prolonged to increase the amount of ejected NT, the response increased in amplitude and duration, but not in latency and time to peak. Figure 5a shows membrane responses to NT ejected with varied pulse durations in a NT-sensitive cell. The relationship between the pulse duration and the response amplitude is shown in Figure 5b, suggesting that the upper limit of the response amplitude may be approx. 35 mV. A time to peak of 0.1–0.2 s was measured in some NT-sensitive cells, which was as short as in the e.j.p. elicited by stimulation of intramural nerves, as shown in Figure 4a. However, their latency of 0.07–0.18 s was much longer than the latency of about 0.01 s for the e.j.p. Figure 6 shows successive NT responses when NT was repeatedly applied by pressure pulses of 10 ms duration. It can be seen that the amplitude and time course of the response is little affected by repetitive application at up to 0.8 s interval. However, on careful comparison of two successive responses, the later response was very slightly

Figure 4 Comparison of membrane responses to local application of neurotensin (NT) and e.j.ps recorded from the same cell. (a), (b) and (c) Records from different preparations. E.j.ps were evoked by electrical field stimulation of intramural nerves with square-wave pulses of 0.1 ms duration and NT-induced responses were evoked by pressure ejection at triangles with varied durations from a 0.3 mM NT-filled micropipette. (a) A large depolarization was evoked with a short latency developing as rapidly as does an e.j.p.; (b) a slow depolarization was evoked with a long latency; (c) there was no response to NT. Pressure pulses of a 200 ms duration were used in (a) and (b) and those of 50 and 1000 ms for (i) and (ii), respectively, in (c). The bathing solution contained the same drugs as listed for Figure 3. Note that cells which were less or insensitive to NT could sustain e.j.ps.

Figure 5 Dose-related membrane depolarizations in response to local application of neurotensin (NT). (a) Potential records from a cell in response to NT ejected from a 0.3 mM NT-filled micropipette by pressure pulses of varied durations ((i)–(vi), 8, 9, 10, 20, 30 and 40 ms, respectively). (b) A plot of the amplitude of NT responses against pressure pulse duration (logarithmic scale). Atropine (0.5 μ M), isoprenaline (1.3 μ M) and D 600 (10 μ M) were present in the bathing solution.

Figure 6 Membrane responses to repeated local applications of neurotensin (NT). NT was ejected at varied intervals by pressure pulses of 10 ms duration from a 0.3 mM NT-filled micropipette. (a) Five pulses at 0.2 Hz; (b) 8 pulses at 0.4 Hz; (c) 10 pulses at 0.8 Hz; (d) 12 pulses at 2 Hz. Potential records from the same cell.

smaller in amplitude than the preceding one. At application intervals shorter than 0.8 s, successive responses summed up to a sustained membrane depolarization.

In all of NT-insensitive and less NT-sensitive cells, e.j.ps could be elicited by electrical stimulation of the intramural nerves and their amplitude and time course were similar to those recorded from NT-sensitive cells. Thus, clearly the low sensitivity to the local application of NT is not attributable to any damage to the cells.

Membrane current response to neurotensin

Single smooth muscle cells were held under voltage clamp at -50 mV which was close to the mean resting potential of -54 ± 0.4 mV (see above), and patched with pipettes filled with a CsCl-rich solution to block outward K^+ current. Bath application of NT (5 or 10 μ M) produced an inward current (11 cells) (Figure 7a) or no detectable current (58 cells). NT-induced inward currents declined with time in the continued presence of the peptide. The peak amplitude of the

Figure 7 Inward current responses to neurotensin (NT) and ATP in single muscle cells enzymatically isolated from the longitudinal muscle layer of chicken rectum. The cells were bathed in PSS and dialyzed under voltage clamp at -50 mV with a CsCl-rich solution. (a) A large inward current was evoked in response to $5 \mu\text{M}$ NT; (b) a small inward current was evoked in response to $5 \mu\text{M}$ NT; (c) no current in response to NT occurred but a large inward current response was evoked by $10 \mu\text{M}$ ATP. See text for details.

inward currents varied from 20 pA up to 400 pA in the 11 cells. Similar current responses to NT were obtained with inclusion of 10 mM EGTA in the pipette solution (2 cells), suggesting that the current response may result from activation of calcium-independent ionic channels. In all of the cells, whether they responded to NT or not, a brief inward current with an amplitude of 100–800 pA was recorded when ATP ($10 \mu\text{M}$) was applied (Figure 7c). It is therefore unlikely that variations in sensitivity to NT of different cells is due solely to some damage arising during the enzymatic dispersion.

In order to estimate the reversal potential of the NT-induced inward current, the membrane potential was stepped from the holding potential of -50 mV to test potentials of -10 mV and 10 mV during application of the peptide (Figure 8a). Figure 8b shows a plot of the amplitude of the NT-induced current against clamp potential. It can be seen that the NT-induced current is inward at -50 mV and -10 mV but outward at 10 mV, giving a reversal potential of about -1.5 mV. In three other cells, reversal potentials of NT-induced currents were estimated to be between -4 and 3 mV. The mean reversal potential was -1.0 ± 1.3 mV ($n = 4$).

As calcium-activated K^+ current can be employed as a probe for monitoring a rise in calcium concentration in the cytosol (Komori & Bolton, 1990), cells were patched with pipettes containing a KCl-rich solution, held at 0 mV and exposed to NT. Under these conditions, 3 cells discharged spontaneous transient outward currents (STOCs, Benham & Bolton, 1986) and responded to NT (5 – $10 \mu\text{M}$) with a brief outward current followed by abolition or reduction of STOC discharges (Figure 9a). The effects of NT resembled those of caffeine and carbachol in other types of intestinal cell in which these drugs cause a massive release of calcium from internal stores (Komori & Bolton, 1990; Komori *et al.*, 1992). However, in five other cells, NT was without effect or, if anything, it caused only an increase of STOCs in amplitude and discharge rate (Figure 9b).

Figure 8 Estimation of the reversal potential of the inward current response to neurotensin (NT) in a single muscle cell. The cell was bathed in PSS and dialyzed under voltage clamp at -50 mV with a CsCl-rich solution. (a) Current response to $5 \mu\text{M}$ NT recorded during stepping from -50 mV to test potentials of -10 and 10 mV. Upper trace, voltage step protocol; lower trace, current record. (b) A plot of NT-induced current response amplitude against clamp potential. The calculated regression line is given as $y = 1.3x + 1.5$ ($r = 0.997$, $P < 0.01$), where y is current response amplitude (pA) and x is clamp potential (mV).

Figure 9 Outward current responses to $5 \mu\text{M}$ neurotensin (NT) in single muscle cells dialyzed under voltage clamp at 0 mV with a KCl-rich solution. (a) A brief outward current was evoked followed by abolition of spontaneous transient outward currents (STOCs); (b) an increase in amplitude and discharge rate of STOCs was evoked and then abolished. (a) and (b) are records from different cells.

Discussion

The present results demonstrated that NT, applied by addition to the bathing solution or locally by pressure ejection from a micropipette, can cause the membrane of the longitudinal muscle of chicken rectum to depolarize. A similar

effect of NT on the membrane potential has been observed on the smooth muscle of the guinea-pig taenia coli (Kitabgi *et al.*, 1979). The membrane response to NT in chicken rectum smooth muscle was found to be biphasic on prolonged exposure to NT. The first phase of depolarization was shorter in duration and larger in amplitude than the second steady-state phase of depolarization. The depolarization was accompanied by an increase in membrane conductance, indicating that NT opens ion channels present in the cell membrane. This is strongly supported by the fact that in single smooth muscle cells held under voltage clamp at -10 or -50 mV, NT induced an inward current which consisted of an initial, peak phase followed by a gradually declining phase and was accompanied by a decrease in input resistance and an increase in current noise. The reversal potential of the NT-induced current of about 0 mV suggests that NT may activate ion channels through which either Cl^- alone (distributed equally on both sides of the cell membrane) or more than one kind of ion can flow. It is very likely that the inward current is carried by a mixture of cations such as Na^+ , K^+ and Ca^{2+} rather than a calcium-activated Cl^- current, as it was generated when EGTA (10 mM) was included in the patch pipette.

The whole-cell patch clamp experiments also revealed that NT elicited a brief outward K^+ current followed by inhibition of STOCs when the cell was held under voltage clamp at 0 mV and the patch pipette contained a KCl-rich solution. The outward current response was substantially similar to current responses to caffeine and carbachol (Komori & Ohashi, unpublished data). In many types of smooth muscle cell, such current responses to caffeine, muscarinic receptor agonists and adrenoceptor agonists are believed to result from a massive release of calcium from internal stores (Benham & Bolton, 1986; Komori *et al.*, 1992). Therefore, it is highly probable that NT, like caffeine and carbachol, can also release calcium from internal stores.

The present results provide evidence in favour of NT as a possible neurotransmitter of non-adrenergic, non-cholinergic (NANC) nerves in chicken rectum (Komori *et al.*, 1986a). NT can cause the membrane of the longitudinal muscle of the chicken rectum to depolarize. Membrane depolarizations evoked by locally applied NT in some cells were very similar in property to e.j.ps evoked by NANC nerve stimulation. The rising phase of NT responses was as rapid as that of e.j.ps recorded from the same cell. Trains of membrane depolarizations evoked by repetitive jets of locally ejected NT were comparable with trains of e.j.ps evoked by repetitive NANC nerve stimulation. The finding that cells, which failed to respond to locally applied NT, exhibited e.j.ps following NANC nerve stimulation does not preclude the possibility that NT is a transmitter for the NANC nerves. Cells in the smooth muscle of the chicken rectum are electrically coupled to each other (Komori & Ohashi, 1982), and e.j.ps evoked in cells in which the transmitter acts may spread electrotonically to other cells. Depression of e.j.p. amplitude produced by NT in a relatively high concentration is mainly attributed to reduction of membrane resistance but not to desensitization of NT receptors, since effects of NT on both e.j.ps and electrotonic potentials faded almost in parallel. NT-induced membrane depolarization results in a decrease in driving force for the e.j.p. which has a reversal potential of -15 mV (Komori & Ohashi, 1988a) and this also contributes to the depression of e.j.p. amplitude.

Prolonged exposure to NT revealed that NT may have a

dual action on the membrane conductance, as an activator and as an inhibitor of ion channels in the longitudinal muscle of chicken rectum. The membrane conductance was first increased and then gradually declined to normal approx. 20 min after the start of NT exposure. After that, the membrane conductance continued to reduce to beyond control. The change in membrane conductance occurred even after the membrane depolarization reached a steady level. As previously reported (Kitabgi & Freychet, 1978; 1979; Kitabgi, 1982), if NT acts indirectly through release of active substances other than acetylcholine, 5-hydroxytryptamine and histamine from neural and/or non-neural elements, the dual effect on the membrane conductance may be not merely the result of activation of NT receptors located on the smooth muscle. Another possibility is that multiple subtypes of NT receptor mediating different membrane responses (Quirion *et al.*, 1980) are present in the smooth muscle, since the peptide has been reported to act directly on the smooth muscle to relax or contract in a variety of gastrointestinal preparations (Carraway & Leeman, 1973; Rökäus *et al.*, 1977; Kitabgi & Freychet, 1978; Quirion *et al.*, 1980; Kitabgi & Vincent, 1981). More strategic tools such as specific antagonists are required for confirmation of the dual action of NT on the membrane conductance. Thus, there is scope for further study on the hypothesis that NT acts as a transmitter of NANC neurones in the chicken rectum (Komori *et al.*, 1986a).

Following bath application of NT, a decreased membrane potential could be recorded from all the cells tested. When NT was locally applied, only a fraction of the cell population of the muscle was sensitive to NT. Similar results were obtained in enzymatically dispersed single muscle cells. However, in the case of ATP, all the cells in the muscle respond to its local application with a membrane depolarization (Komori & Ohashi, 1988b) and in the present experiments, most of the enzymatically dispersed single cells showed a good sensitivity to ATP. If one makes allowance for some limitations imposed by pressure ejection and enzyme treatment, the present results suggest that NT-sensitive cells, cells possessing NT receptors, may be a fraction of the cell population and distributed in a tessellated manner in the longitudinal muscle layer of chicken rectum. The muscle appears to have highly developed intercellular coupling because of its length constant of 1.6 mm measured by spatial decay of electrotonic potentials (Komori & Ohashi, 1982). The presence of electrical coupling between cells possessing NT receptors and cells lacking NT receptors serves to elicit a modest membrane depolarization, and may account for a large part of the consistency of membrane responses, when NT was applied by addition to the bathing solution.

The longitudinal muscle of chicken rectum belongs to unitary muscles according to Bozler's classification (Bozler, 1948) and is spontaneously active. In this type of smooth muscle, membrane depolarization can increase the discharge rate of propagated action potentials leading to Ca entry through voltage-dependent Ca channels without causing depolarization block and thus it is effective in producing tension. The contractile activity of neurotensin in the rat fundus is sensitive to nifedipine and related calcium channel blockers, and it has been suggested that it may be coupled to voltage-dependent calcium channels (Donoso *et al.*, 1986). Thus, it can be well understood that NT, which causes a modest membrane depolarization, is a very potent agent in contracting the longitudinal muscle of chicken rectum (Komori *et al.*, 1986a).

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