Effects of neurotensin on rat supraoptic nucleus neurones *in vitro*

Karen Kirkpatrick and Charles W. Bourque*

Centre for Research in Neuroscience, Montreal General Hospital and McGill University, 1650 Cedar Avenue, Montreal, PQ, Canada H3G 1A4

1. The electrophysiological actions of neurotensin on magnocellular neurosecretory cells (MNCs) were examined during intracellular recording from seventy-three supraoptic nucleus neurones in superfused explants of rat hypothalamus.

2. Application of neurotensin tridecapeptide (NT(1–13); 1 nM to 3 μM) caused a membrane depolarization and reversibly attenuated the after-hyperpolarization (AHP) which followed current-evoked spike trains. This effect was accompanied by increased firing frequency during depolarizing current pulses evoked from a fixed potential.

3. The effects of neurotensin could be mimicked by the *C*-terminal fragment, NT(8–13), but not by the *N*-terminal fragment, NT(1–8).

4. Depolarizing responses to NT(1–13) or NT(8–13), retained during K^+ channel blockade with internal Cs^+, were accompanied by increased membrane conductance. Current- and voltage-clamp analyses revealed that neurotensin-evoked depolarizations result partly from the activation of a non-selective cationic conductance reversing near −34 mV.

5. Depolarizing responses to neurotensin were retained in the presence of TTX or in Ca^{2+}-free solutions, indicating the involvement of receptors located on the plasma membrane of MNCs themselves.

6. Through these effects endogenously released neurotensin may modulate excitability, activity patterns and secretion from the hypothalamo-neurohypophysial axis.

Magnocellular neurosecretory cells (MNCs) located in the supraoptic nucleus of the hypothalamus project axons to the neural lobe of the pituitary where systemic release of oxytocin and vasopressin is regulated by the arrival of action potentials generated at the soma (Poulain & Wakerley, 1982). In response to physiological requirements for neurohypophysial hormone release, MNCs increase their firing frequency or adopt various stereotypical patterns of bursting activity (Poulain & Wakerley, 1982; Poulain, Brown & Wakerley, 1988). The latter have been shown to optimize excitation–secretion coupling at the axon terminals of MNCs and to adapt hormone release to the specific requirements of individual target organs (Bicknell, 1988). Since discharge patterns in MNCs are determined by intrinsic ionic conductances (Bourque & Renaud, 1990), their modulation by neurotransmitters may be a key mechanism in the central regulation of neurohypophysial hormone release. Previous studies have demonstrated that an apamin-sensitive Ca^{2+}-dependent K^+ conductance ($g_{K(Ca)}$) underlying the after-hyperpolarization (Bourque & Brown, 1987) can participate in spike frequency adaptation (Bourque & Brown, 1987), and in the generation of phasic (Andrew & Dudek, 1984) and clustered firing (Hu & Bourque, 1992). In view of these multiple contributions to electrical activity, the apamin-sensitive $g_{K(Ca)}$ represents a potential target for neurotransmitter-mediated regulation of discharge patterns in MNCs.

Neurotensin is a putative peptidergic neurotransmitter (Stowe & Nemeroff, 1991), expressed both in the brain (Carraway & Leeman, 1973) and in the gastrointestinal tract (Carraway, Kitabgi & Leeman, 1978). In the rat, neurotensin application has been found to excite various neurones through the suppression of K^+ conductances (Audinat, Hermel & Crépel, 1989; Keegan, Woodruff & Pinnock, 1992; Farkas, Nakajima & Nakajima, 1994; Jiang, Pessia & North, 1994) and possibly attenuation of the after-hyperpolarization (AHP; Audinat *et al.* 1989). Interestingly, neurotensin actions in the intestine

* To whom correspondence should be addressed at: Division of Neurology, Montreal General Hospital, 1650 Cedar Avenue, Montreal, PQ, Canada H3G 1A4.

specifically involve apamin-sensitive K^+ channels (Huidboro-Toro & Zhu, 1984; Kullak, Donosa & Huidboro-Toro, 1987; Mulè, Posterino, Geraci & Serio, 1992; Christinck, Daniel & Fox-Threlkeld, 1992). This suggests that channels underlying the AHP in neurones may be a target for neurotensin. The presence of neurotensin-like immunoreactive material in fibres within and surrounding the rat supraoptic nucleus (Jennes, Stumpf & Kalivas, 1982), therefore, suggests that this peptide may serve as an endogenous modulator of K^+ channels in the hypothalamo-neurohypophysial system. This study was undertaken to examine the possibility that neurotensin might specifically modulate the apamin-sensitive $g_{K(Ca)}$ of MNCs and thereby regulate the electrical activity of these cells in the supraoptic nucleus of the rat. Our results indicate that neurotensin not only inhibits this conductance, but that it also provokes depolarization by activating a non-selective cationic current. Abstracts of this work have appeared (Kirkpatrick & Bourque, 1991*a*, *b*, 1993).

METHODS

Preparation of hypothalamic explants

Rat basal hypothalamic explants (8 × 8 × 2 mm) were obtained from 180–300 g male Long–Evans rats killed by decapitation with a small animal guillotine (Stoelting; No. 51330) and pinned to a Sylgard (Dow Corning Corporation, USA) base in a temperature-regulated (33–35 °C) perfusion chamber (Yang, Bourque & Renaud, 1991). There, they were superfused (0·6–1·0 ml min^{-1}) with an oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF) delivered via a Tygon tube placed over the median eminence. Cotton wicks were positioned so as to reduce the height of surface ACSF thereby minimizing electrode capacitance. Electrophysiological recordings were obtained between 3 and 10 h post-decapitation.

Solutions and drugs

The composition of ACSF (pH 7·4) was (mM): NaCl, 115; $CaCl_2$, 2–3; $MgCl_2$, 1·3; KCl, 3; $NaHCO_3$, 26; glucose, 10 (Fisher Scientific Company, Canada). The osmolality of the ACSF was measured using a freezing point microosmometer (Advanced Instruments Inc., USA) and was adjusted to 295 ± 3 mosmol kg^{-1} by the addition of distilled water. Stock solutions (10^{-4}–10^{-5} M) of neurotensin (NT(1–13)), neurotensin(8–13) (NT(8–13)), neurotensin(1–8) (NT(1–8)) or apamin (all obtained from Sigma Chemical Company, USA) were prepared by dissolving the peptide in ACSF lacking glucose and $CaCl_2$. At the time of the experiments these were either diluted in ACSF and bath-applied at the concentrations indicated in the text, or they were slowly injected as a 10–50 μl bolus into the superfusion line. The latter procedure produced transient (10–30 s) and reproducible peptide applications at peak concentrations estimated as described elsewhere (Yang *et al.* 1991). Nominally Ca^{2+}-free ACSF was prepared by replacing $CaCl_2$ with an equimolar amount of $MnCl_2$ (Fisher Scientific Company). When required, caesium chloride (Sigma Chemical Company; 1–3 mM) was directly dissolved into the ACSF.

Microelectrodes and cellular impalement

Cells were impaled with microelectrodes prepared from glass capillary tubes (1·2 mm i.d., A-M Systems Inc., USA) on a P87 Flaming-Brown micropipette puller (Sutter Instruments Inc., USA). The pipettes were filled with either potassium acetate (1·5–3 M; Fisher Scientific Company) or a mixture of potassium acetate (1·5 M) and KCl (0·5 M; Fisher Scientific Company). In some experiments, caesium acetate (1–3 M in H_2O; Sigma Chemical Company) was used as the electrode filling solution. Collectively, the tip resistances of these electrodes ranged between 50 and 120 MΩ. The reference electrode consisted of either a chlorided silver wire immersed in ACSF, or a chlorided silver wire embedded in a tube containing 10% agar (in 1 M KCl) that was in contact with the ACSF. Cells were impaled by advancing the electrode in rapid 4 μm steps using a piezoelectric device (Scientific Precision Instruments, USA) mounted on a micromanipulator (David Kopf Instruments, USA).

Data acquisition and analysis

An axoclamp 2A amplifier (Axon Instruments Inc., USA) was used for intracellular recordings of membrane voltage (DC to 10 kHz) and current (DC to 0·5 kHz). Signals acquired were monitored on a chart recorder (Gould Ltd, USA) and stored on videotape. Current or voltage commands were either delivered through an external stimulation unit, or via an AT-compatible computer equipped with a Labmaster interface driven by pCLAMP software (Axon Instruments Inc.). The latter was also used for data acquisition and subsequent analysis. Voltage-clamp recordings were obtained in the discontinuous mode with the switching frequency adjusted to allow complete settling of the input voltage between cycles (0·9–3·2 kHz).

RESULTS

The data analysed below were obtained from intracellular recordings in neurones of the rat supraoptic nucleus. The cells were characterized as MNCs because they displayed transient outward rectification during depolarization from negative potentials (Bourque & Renaud, 1990) and frequency-dependent spike broadening (Andrew & Dudek, 1985; Bourque & Renaud, 1985). These properties are regarded as being specific to MNCs *in vivo* (Bourque & Renaud, 1991; Dyball, Tasker, Wuarin & Dudek, 1991) and *in vitro* (Bourque & Renaud, 1990) but not to neighbouring non-neurosecretory cells. The cells retained for analysis ($n = 73$) showed input resistances greater than 130 MΩ and resting membrane potentials between −50 and −65 mV. Action potential amplitudes measured from baseline were greater than 70 mV.

Effects of neurotensin on the AHP

Trains of action potentials evoked by depolarizing current pulses were consistently followed by an AHP when elicited at intervals greater than 10 s. Application of neurotensin tridecapeptide (NT(1–13); 1 nM to 3 μM) caused a reversible decrease in the amplitude of the AHP (measured from the initial baseline) following constant spike trains in twelve of thirteen cells tested (Fig. 1). In eleven of these cells, the response was associated with membrane depolarization, an effect which could enhance or induce the firing of action potentials (Fig. 1). One cell failed to depolarize despite a consistent inhibition of the AHP upon

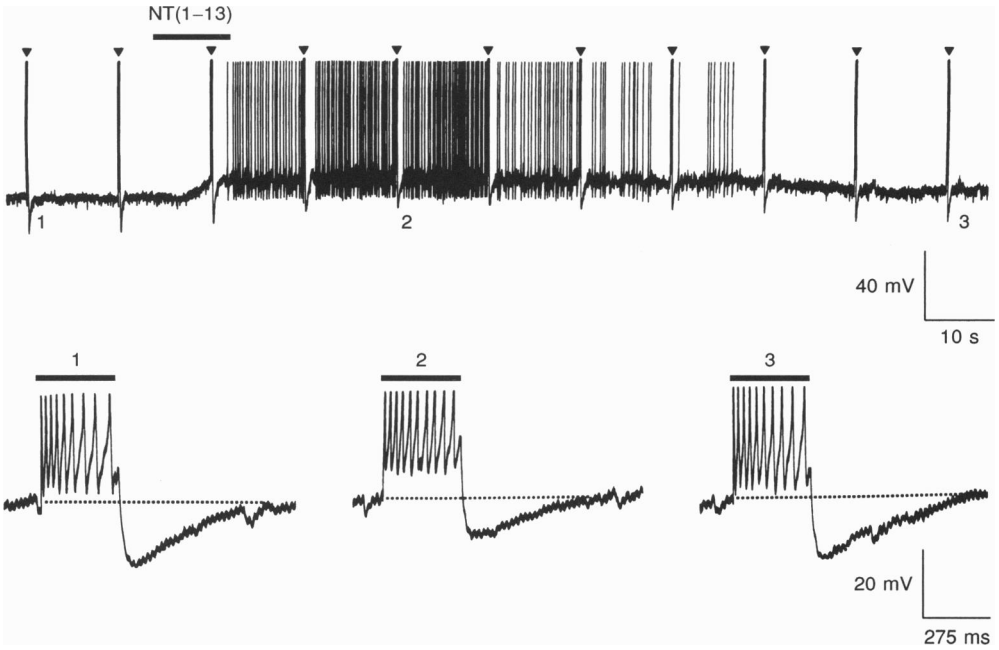

Figure 1. Effects of neurotensin on a supraoptic nucleus neurone recorded in a superfused rat hypothalamic explant

Application of neurotensin (NT(1–13); 3 μM; bar) induced a reversible depolarization and action potential discharge (upper trace). The triangles indicate where trains of action potentials were evoked by depolarizing current pulses (300 ms). Traces expanded below reveal that neurotensin reversibly attenuated the amplitude of the AHP following current-evoked spike trains (bars).

NT(1–13) application. In fifteen of seventeen MNCs, attenuation of the AHP could also be induced by addition of the *C*-terminal hexapeptide fragment of neurotensin (NT(8–13); Fig. 2, upper traces). In contrast, application of the *N*-terminal octapeptide (NT(1–8)) was without effect on five cells which were otherwise responsive to either NT(1–13) or to NT(8–13) (Fig. 2, lower traces). At the maximum dose tested (3 μM), the amplitude of the AHP in the presence of NT(1–13) or NT(8–13) was reduced to $64 \pm 4\%$ (mean $\pm$ S.E.M.; $n = 19$) of control. In three cells whose initial membrane potential was fixed to maintain a constant driving force, and where pulse amplitude was

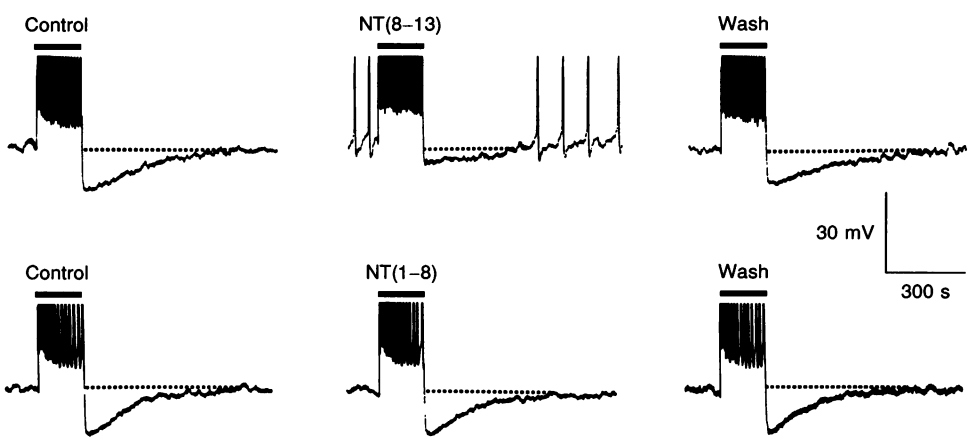

Figure 2. Effects of neurotensin fragments on AHPs evoked by current pulses of fixed amplitude

Upper traces show that exogenous application of 3 μM NT(8–13) reversibly attenuated the AHP evoked by current pulses of fixed amplitude (bar, 300 pA). Initial membrane potential under control and wash conditions was −65 mV. In the presence of NT(8–13) the cell was depolarized to −57 mV. Application of 3 μM NT(1–8) to the same cell was without effect.

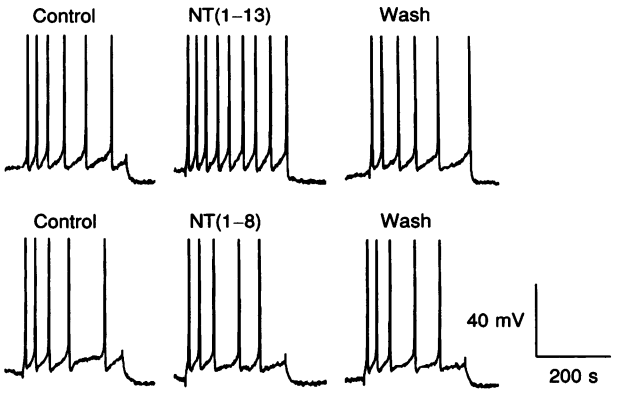

Figure 3. Effects of neurotensin on spike frequency adaptation in a supraoptic neurone
Upper traces show that application of 3 μM NT(1–13) reversibly increased the number of action potentials evoked by a depolarizing current pulse of constant amplitude (280 ms; 150 pA). In this experiment the initial membrane potential of the cell was maintained constant by current injection. Lower traces show that application of 3 μM NT(1–8) was without effect.

adjusted to elicit a constant number of spikes, application of 3 μM neurotensin reduced the AHP amplitude to 47 $\pm$ 6% of control.

Effects of neurotensin on spike frequency adaptation

As previously reported (Andrew & Dudek, 1984; Bourque, Randle & Renaud, 1985), trains of action potentials evoked by constant current pulses featured spike frequency adaptation, a progressive but saturating increase in consecutive interspike intervals. Since this phenomenon is partly mediated through the apamin-sensitive $g_{K(Ca)}$ (Bourque & Brown, 1987), we examined whether neurotensin could also modulate the frequency of action potential discharge recorded in response to depolarizing current pulses. Firing frequencies during brief (< 1 s) current pulses were quantified as the reciprocal of the mean interspike interval in five cells. Application of NT(1–13) or NT(8–13) increased the mean frequency of firing from 38 $\pm$ 5 to 65 $\pm$ 16 Hz during such pulses (Fig. 3).

Effects of neurotensin on membrane potential

In explants perfused with standard ACSF neurotensin fragments were either without effect (NT(1–8); $n = 7$), or mimicked (NT(8–13)) the actions of NT(1–13), inducing reversible depolarization and increased firing in thirty-six of thirty-nine cells tested (Fig. 4). Addition of 0·5 μM TTX to the ACSF abolished Na^+-dependent action potentials and associated synaptic transmission. Under these conditions depolarizations evoked by NT(1–13) and NT(8–13) persisted (Fig. 5, $n = 3$), suggesting that this effect was mediated by activation of receptors located on the plasma membrane of MNCs.

Neurotensin effects on AHP and membrane potential involve distinct mechanisms

Previous studies have shown that the AHP following trains of action potentials in MNCs results from the activation of a $g_{K(Ca)}$ (Andrew & Dukek, 1984; Bourque *et al.* 1985). Since suppression of K^+ conductances can result in membrane depolarization (e.g. Adams, Brown & Constanti, 1982), it is conceivable that the inhibition of $g_{K(Ca)}$, or other K^+ conductances, could be responsible for the neurotensin-evoked depolarization of MNCs. In each of two cells, bath application of a Ca^{2+}-free ACSF abolished the AHP following current-evoked trains of action potentials. Under these conditions application of NT(1–13) produced large depolarizations and induced action potential discharge (Fig. 6*A*), suggesting that inhibition of $g_{K(Ca)}$ is not essential for the generation of these responses. To examine the possible involvement of other K^+ conductances the effects of neurotensin were also examined following intracellular Cs^+ injection. In each of six MNCs, depolarizing current pulses applied following Cs^+ loading evoked trains of prolonged action potentials (> 50 ms) which were marked by the absence of an AHP. Under these conditions application of either NT(8–13) or NT(1–13) still elicited reversible depolarization and enhanced firing either in the presence ($n = 5$) or absence of extracellular Ca^{2+} (Fig. 6*B*). These results indicate that depolarizing responses to neurotensin involve the activation of a

Figure 4. Effects of neurotensin fragments on membrane potential in a supraoptic nucleus neurone
The top trace illustrates that application of NT(1–8) (3 μM; bar) had no effect on the membrane potential ($V_m = -67$ mV). In the lower panel, application of NT(8–13) (3 μM; bar) elicits a reversible 10 mV depolarization accompanied by action potential discharge.

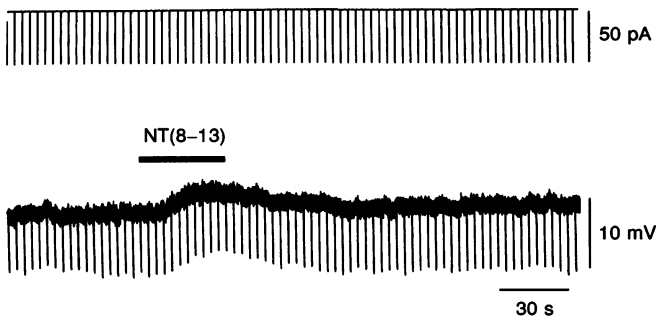

Figure 5. Intracellular recording of membrane potential (bottom trace) obtained from a supraoptic nucleus neurone in a hypothalamic explant superfused with ACSF containing 0·5 μM TTX

Downward deflections are electrotonic potential responses to hyperpolarizing current pulses (upper trace). Application of NT(8–13) (3 μM; bar) under these conditions elicited a reversible depolarization. Note that this effect is not accompanied by obvious changes in input resistance.

distinct conductance. In agreement, depolarizing responses to NT(8–13) were retained, and isolated from effects on the conductance underlying the AHP, when recorded in the presence of 100 nM apamin (Fig. 7*A*; $n = 7$), a concentration sufficient to block the AHP in MNCs (Bourque & Brown, 1987).

Ionic basis of the neurotensin-evoked depolarization

Under conditions blocking the AHP (see above), neurotensin-evoked depolarization was associated with a 18 ± 2% decrease in input resistance in nine of eleven cells tested (e.g. Figs 6*B* and 7*B*). Increased input resistance following neurotensin application was seen in two of the cells superfused with ACSF containing 100 nM apamin. Extrapolation of voltage–current relations measured in cells displaying decreased resistance upon exposure to neurotensin agonists revealed a mean apparent reversal potential of −34 ± 3 mV ($n = 9$). Since current clamp estimates of reversal potential can be biased by voltage-dependent conductances, neurotensin-evoked responses were also examined under voltage clamp conditions. In six neurones held at membrane potentials near rest,

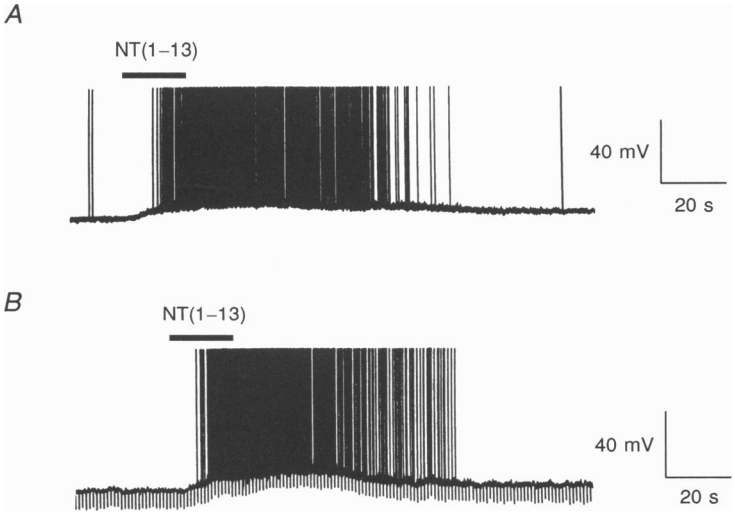

Figure 6. Intracellular recordings obtained from supraoptic nucleus MNCs impaled in explants superfused with Ca^{2+}-free ACSF containing 2 mM Mn^{2+}

The cell in *A* was impaled with a micropipette filled with 2 M potassium acetate. Under these conditions (holding potential (V_h) = −65 mV) application of NT(1–13) (3 μM; bar) evoked reversible depolarization and firing. The trace shown in *B* was recorded from a cell impaled with a pipette containing 1·5 M caesium acetate. Under these conditions (V_h = −68 mV) application of NT(1–13) (3 μM; bar) evoked reversible depolarization and firing. Downward deflections in *B* are electrotonic voltage responses to constant current pulses (50 pA; 80 ms) delivered at 0·83 Hz. Note that the depolarization recorded in *B* is associated with a small decrease in input resistance.

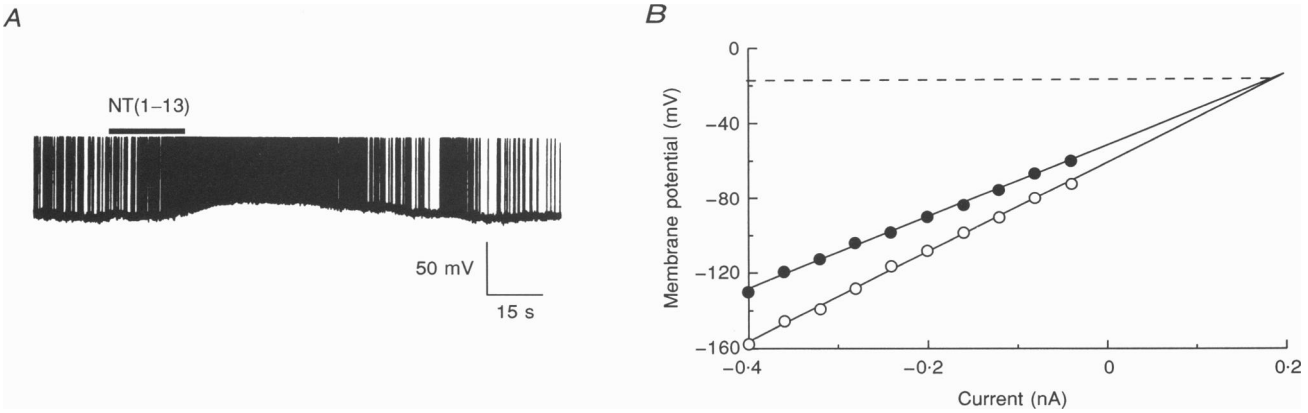

Figure 7. Effects of neurotensin on a supraoptic neurone impaled in a hypothalamic explant superfused with 100 nM apamin to block the AHP

Panel *A* shows that under such conditions application of NT(8–13) (3 μM; bar) evoked reversible depolarization and increased firing frequency. The graph in *B* plots steady-state voltage–current relations measured before (○) and during (●) a subsequent application of 3 μM NT(8–13) to the same cell. Slope resistance in NT(8–13) was decreased to 80% of control (238 MΩ). Note that the data extrapolate to approximately −20 mV.

application of NT(8–13) evoked an inward current associated with increased membrane conductance (Fig. 8). As illustrated in Fig. 9, the amplitude of this response varied linearly over the range of potentials tested (−90 to −50 mV). Extrapolation of current–voltage relations confirmed a mean reversal potential of -34 ± 7 mV ($n = 6$).

Chloride ions are unlikely to have participated in the neurotensin-mediated depolarizations since E_{Cl} (chloride reversal potential) in MNCs lies near −75 mV (Randle, Bourque & Renaud, 1986). Moreover, responses to NT(1–13) and NT(8–13) were unaffected by intracellular Cl^- injection ($n = 16$). These results suggest that neurotensin elicits the activation of a population of non-selective cation-permeable channels. Previous studies have shown that MNCs in the guinea-pig express a hyperpolarization-activated, cationic conductance (I_h) which can be blocked by extracellular Cs^+ (Erickson, Ronnekleiv & Kelly, 1993). However, the present results indicate that the inward current evoked by neurotensin shows little voltage dependence near resting potentials (Fig. 9).

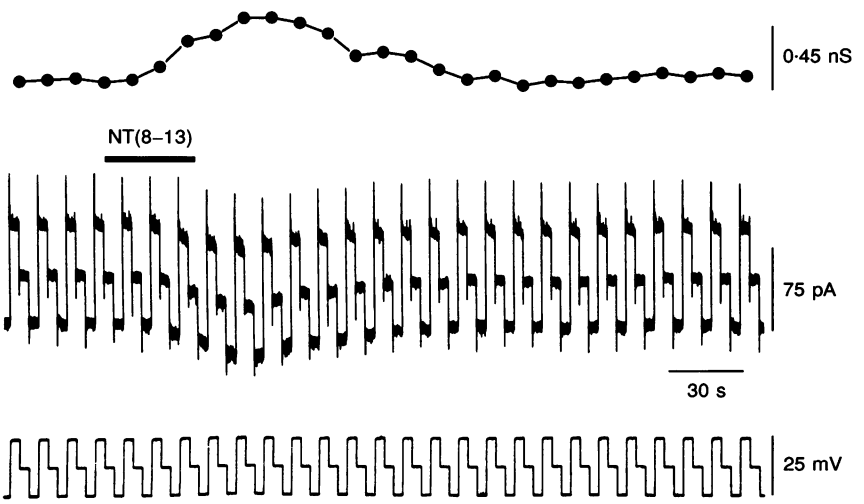

Figure 8. Voltage clamp analysis of changes in membrane conductance associated with neurotensin effects in a supraoptic neurone

The membrane potential (V_m; bottom trace) of the cell was repeatedly and sequentially stepped to −59, −72 and −84 mV. The middle trace shows that application of 3 μM NT(8–13) induced reversible inward currents (I) at each of the test potentials. Note that NT(8–13) elicits a reversible increase of membrane conductance ($\Delta g = \Delta I / \Delta V_m$; top trace).

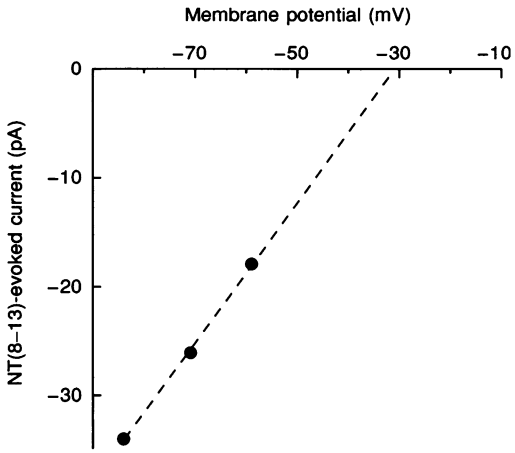

Figure 9. Voltage dependence of the current evoked by NT(8–13) in the cell shown in Fig. 8

Current amplitude at each test potential was measured by subtracting steady-state currents observed before and during application of the peptide. The straight line through the data (fitted by eye) reveals an extrapolated reversal potential of approximately −30 mV.

Additionally, depolarization in response to NT(8–13) was unaffected by the addition of 1–3 mM Cs^+ to the ACSF ($n = 3$; not shown), suggesting the involvement of a cationic conductance distinct from that underlying I_h.

DISCUSSION

The results of this study suggest an excitatory role for neurotensin in the regulation of the hypothalamo-neurohypophysial system of the rat. Since more than 94% of the neurones recorded in our experiments were excited by neurotensin receptor agonists, it appears that both oxytocin- and vasopressin-releasing MNCs are potentially subject to the modulatory effects of this peptide. Electrophysiologically, the actions of neurotensin were mimicked by NT(8–13) but not by the *N*-terminal fragment NT(1–8). This observation is consistent with the evident conservation of the peptide's *C*-terminal region across many species (Carraway, Ruane & Kim, 1982) and corresponds to the activity profiles these fragments have been reported to exert in binding (Tanaka, Masu & Nakanishi, 1990) and biological assays (Carraway & Leeman, 1975). Neurotensin-evoked membrane depolarizations persisted in the presence of tetrodotoxin, to block Na^+-dependent action potentials, or in Ca^{2+}-free, Mn^{2+}-containing solutions to block synaptic transmission. These observations indicate that at least some of the receptors involved were intrinsic to the magnocellular neurones themselves.

While their origin is at present unknown, fibres containing neurotensin-like immunoreactivity have been noticed both within and surrounding the rat supraoptic nucleus (Jennes *et al.* 1982). Consistent with the latter observation, binding sites for radiolabelled neurotensin have been observed in areas immediately dorsal and lateral to the supraoptic nucleus (Moyse *et al.* 1987). The area dorsal to the nucleus is believed to contain interneurones projecting into the supraoptic nucleus where they may influence the activity of MNCs (Renaud, Cunningham, Nissen & Yang, 1993; Way & Dyball, 1993). This region therefore represents a site for possible presynaptic actions of neurotensin. In contrast, the ventral and lateral borders of the nucleus contain dendrites of magnocellular neurones (Armstrong, Schöler & McNeill, 1982). The lateral border of the supraoptic nucleus therefore represents a possible site for the postsynaptic regulation of MNCs by endogenously released neurotensin.

The results of our study indicate that occupation of neurotensin receptors in MNCs can induce membrane depolarization and attenuate the AHP. Consistent with a proposed role in spike frequency adaptation (Bourque & Brown, 1987), attenuation of the AHP by neurotensin agonists was associated with enhanced neuronal firing during constant depolarizing stimuli (Fig. 3). Consequently, the responsiveness of supraoptic nucleus MNCs to excitatory stimuli sufficient to drive repetitive firing would presumably be enhanced in the presence of neurotensin. Interestingly, episodes of milk ejection in the rat have been found to result from the occurrence of synchronous high-frequency bursts in oxytocinergic MNCs (Poulain & Wakerley, 1982). The frequencies at which action potentials are discharged during such events typically exceed the maximal firing rates recorded in either vasopressin- or oxytocin-releasing cells between episodes of milk ejection (Dyball & Leng, 1986). Dyball & Leng (1986) have therefore suggested that a temporary change in the membrane properties of oxytocin cells may enable them to fire at the higher frequencies associated with milk ejection. Although there is no evidence to indicate that neurotensin plays a role in milk ejection, the actions of this peptide represent a mechanism by which endogenous neurotransmitters may increase the excitability of these cells during lactation.

Inhibition of the conductance underlying the AHP by apamin has been recently demonstrated to abolish rhythmic spike clustering induced by NMDA receptor activation (Hu & Bourque, 1992). Moreover, attenuation of the AHP is known to enhance the depolarizing after-potential (Bourque & Brown, 1987) that is responsible for

the onset and maintenance of phasic bursting activity by MNCs (Andrew & Dudek, 1983). Modulation of the apamin-sensitive $g_{K(Ca)}$ by endogenously released neurotensin, therefore, may contribute to the regulation of these activity patterns.

In addition to effects on the AHP, our recordings revealed that neurotensin causes a separately mediated membrane depolarization that can elicit action potential firing when spike threshold is reached. Interestingly, neurotensin-evoked depolarizations observed in other cells have been reported to result from the suppression of potassium conductance (e.g. Audinat *et al.* 1989; Jiang *et al.* 1992; Keegan *et al.* 1992). Although the existence of such a mechanism was not ruled out in the present experiments, neurotensin-evoked depolarization in supraoptic MNCs persisted following blockade of K^+ channels with apamin or intracellular Cs^+ injection, implying that an additional ionic conductance is subject to modulation following the activation of neurotensin receptors. In agreement, voltage-clamp analysis revealed that neurotensin-evoked depolarization was associated with the activation of a non-selective cationic conductance reversing at a potential near −34 mV.

In summary, neurotensin receptor occupation in MNCs may have direct and indirect effects. By causing membrane depolarization, this peptide could directly enhance firing in spontaneously active cells, or increase the responsiveness of near-threshold neurones to excitatory synaptic input. Through it's effect on the apamin-sensitive $g_{K(Ca)}$, neurotensin will increase cell excitability and may modulate the expression of various forms of bursting activity. These results imply an important role for endogenously released neurotensin in the regulation of the hypothalamo-neurohypophysial axis of the rat.

Adams, P. R., Brown, D. A. & Constanti, A. (1982). Pharmacological inhibition of the M-current. *Journal of Physiology* **332**, 223–262.

Andrew, R. D. & Dudek, F. E. (1983). Burst discharge in mammalian neuroendocrine cells involves an intrinsic regenerative mechanism. *Science* **221**, 1050–1052.

Andrew, R. D. & Dudek, F. E. (1984). Intrinsic inhibition in magnocellular neuroendocrine cells of rat hypothalamus. *Journal of Physiology* **353**, 171–185.

Andrew, R. D. & Dudek, F. E. (1985). Spike broadening in magnocellular neuroendocrine cells of rat hypothalamus. *Brain Research* **334**, 176–179.

Armstrong, W. E., Schöler, J. & McNeill, T. H. (1982). Immunocytochemical, golgi and electron microscopic characterization of putative dendrites in the ventral glial lamina of the rat supraoptic nucleus. *Neuroscience* **7**, 679–694.

Audinat, E., Hermel, J.-M. & Crépel, F. (1989). Neurotensin induced excitation of neurons of the rat's frontal cortex studied intracellularly *in vitro*. *Experimental Brain Research* **78**, 358–368.

Bicknell, R. J. (1988). Optimizing release from peptide hormone secretory nerve terminals. *Journal of Experimental Biology* **139**, 51–65.

Bourque, C. W. & Brown, D. A. (1987). Apamin and d-tubocurarine block the afterhyperpolarization of rat supraoptic neurosecretory neurons. *Neuroscience Letters* **82**, 185–190.

Bourque, C. W., Randle, J. C. R. & Renaud, L. P. (1985). Ca^{2+}-dependent K^+ conductance in rat supraoptic nucleus neurosecretory neurons. *Journal of Neurophysiology* **54**, 1375–1382.

Bourque, C. W. & Renaud, L. P. (1985). Activity dependence of action potential duration in rat supraoptic neurones recorded *in vitro*. *Journal of Physiology* **363**, 429–439.

Bourque, C. W. & Renaud, L. P. (1990). Electrophysiology of mammalian magnocellular vasopressin and oxytocin secreting neurons. *Frontiers in Neuroendocrinology* **11**, 183–212.

Bourque, C. W. & Renaud, L. P. (1991). Membrane properties of rat magnocellular neuroendocrine cells *in vivo*. *Brain Research* **540**, 349–352.

Carraway, R., Kitabgi, P. & Leeman, S. (1978). The amino acid sequence of radioimmunoassayable neurotensin from bovine intestine. *Journal of Biological Chemistry* **253**, 7996–7998.

Carraway, R. & Leeman, S. E. (1973). The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. *Journal of Biological Chemistry* **248**, 6854–6861.

Carraway, R. & Leeman, S. (1975). Structural requirements for the biologicl activity of neurotensin, a new vasoactive peptide. In *Peptides: Chemistry, Structure and Function*, ed. Walter, R. & Meienhofer, J., pp. 679–685. Ann Arbor Publishers Inc., Ann Arbor, MI, USA.

Carraway, R., Ruane, S. E. & Kim, H.-R. (1982). Distribution and immunochemical character of neurotensin-like material in representative vertebrates and invertebrates: Apparent conservation of the COOH-terminal region during evolution. *Peptides* **1**, 115–123.

Christinck, F., Daniel, E. E. & Fox-Threlkeld, J. E. T. (1992). Inhibitory and excitatory mechanisms of neurotensin action in canine intestinal circular muscle *in vitro*. *Canadian Journal of Physiology and Pharmacology* **70**, 1423–1431.

Dyball, R. E. J. & Leng, G. (1986). Regulation of the milk ejection reflex in the rat. *Journal of Physiology* **380**, 239–256.

Dyball, R. E. J., Tasker, J.-G., Wuarin, J.-P. & Dudek, F. E. (1991). *In vivo* intracellular recording of neurons in the supraoptic nucleus of the rat hypothalamus. *Journal of Neuroendocrinology* **3**, 383–386.

Erickson, K. R., Ronnekleiv, O. K. & Kelly, M. J. (1993). Electrophysiology of guinea-pig supraoptic neurones: role of a hyperpolarization-activated cation current in phasic firing. *Journal of Physiology* **460**, 407–425.

Farkas, R. H., Nakajima, S. & Nakajima, Y. (1994). Neurotensin excites basal forebraain cholinergic neurons: Ionic and signal-transduction mechanisms. *Proceedings of the National Academy of Sciences of the USA* **91**, 2853–2857.

Hu, B. & Bourque, C. W. (1992). NMDA receptor-mediated bursting activity in rat supraoptic nucleus neurones *in vitro*. *Journal of Physiology* **458**, 667–687.

Huidboro-Toro, J. P. & Zhu, Y.-X. (1984). Neurotensin receptors on the ileum of the guinea-pig: evidence for the coexistence of inhibitory and excitatory receptors. *European Journal of Pharmacology* **102**, 237–250.

JENNES, L., STUMPF, W. E. & KALIVAS, P. W. (1982). Neurotensin: topographical distribution in the rat brain by immunohistochemistry. *Journal of Comparative Neurology* **210**, 211–224.

JIANG, Z. G., PESSIA, M. & NORTH, R. A. (1994). Neurotensin excitation of rat ventral tegmental neurones. *Journal of Physiology* **474**, 119–129.

KEEGAN, K. D., WOODRUFF, G. N. & PINNOCK, R. D. (1992). Sensitivity of the neurotensin response of rat substantia nigra neurones *in vitro* to toxins and ion channel blockers. *Society for Neuroscience Abstracts* **18**, 763.

KIRKPATRICK, K. & BOURQUE, C. W. (1991*a*). Neurotensin depolarizes rat vasopressin neurons. *Third IBRO World Congress of Neuroscience Abstracts*, 379.

KIRKPATRICK, K. & BOURQUE, C. W. (1991*b*). Effects of neurotensin on rat magnocellular neurosecretory cells (MNCs) *in vitro*. *Society for Neuroscience Abstracts* **17**, 1188.

KIRKPATRICK, K. & BOURQUE, C. W. (1993). Neurotensin modulates two ionic conductances in rat supraoptic magnocellular neuroendocrine cells. *Canadian Journal of Physiology and Pharmacology* **71**, Aix.

KULLAK, A., DONOSA, M. V. & HUIDBORO-TORO, J. P. (1987). Extracellular Ca^{2+} dependence of the neurotensin-induced relaxation of intestinal smooth muscles: studies with Ca^{2+} channel blockers and Bay K-8644. *European Journal of Pharmacology* **135**, 297–305.

MOYSE, E., ROSTENE, W., VIAL, M., LEONARD, K., MAZELLA, J., KITABGI, P., VINCENT, J.-P. & BEAUDET, A. (1987). Distribution of neurotensin binding sites in rat brain: a light microscopic radioautographic study using monoiodo [^{125}I] neurotensin. *Neuroscience* **22**, 525–536.

MULÈ, F., POSTORINO, A., GERACI, A. & SERIO, R. (1992). Neurotensin: dual effect on the motor activity of rat duodenum. *European Journal of Pharmacology* **212**, 215–224.

POULAIN, D. A., BROWN, D. & WAKERLEY, J. B. (1988). Statistical analysis of patterns of electrical activity in vasopressin and oxytocin secreting neurones. In *Pulsatility in Neuroendocrine Systems*, ed. LENG, G., pp. 119–154. CRC Press, Boca Raton, FL, USA.

POULAIN, D. A. & WAKERLEY, J. B. (1982). Electrophysiology of hypothalamic magnocellular neurones secreting oxytocin and vasopressin. *Neuroscience* **7**, 773–808.

RANDLE, J. C. R., BOURQUE, C. W. & RENAUD, L. P. (1986). Characterization of spontaneous and evoked inhibitory postsynaptic potentials in rat supraoptic neurosecretory neurons *in vitro*. *Journal of Neurophysiology* **56**, 1703–1717.

RENAUD, L. P., CUNNINGHAM, J. T., NISSEN, R. & YANG, C. R. (1993). Electrophysiology of central pathways controlling release of neurohypophysial hormones: focus on the lamina terminalis and diagonal band inputs to the supraoptic nucleus. *Annals of the New York Academy of Sciences* **689**, 122–132.

STOWE, Z. N. & NEMEROFF, C. B. (1991). The electrophysiological actions of neurotensin in the central nervous system. *Life Sciences* **49**, 987–1002.

TANAKA, K., MASU, K. & NAKANISHI, S. (1990). Structure and functional expression of the cloned rat neurotensin receptor. *Neuron* **4**, 847–854.

WAY, S. A. & DYBALL, R. E. J. (1993). Interaction between magnocellular neurons and cells of the perinuclear zone involves NMDA receptors. *Annals of the New York Academy of Sciences* **689**, 683–684.

YANG, C. R., BOURQUE, C. W. & RENAUD, L. P. (1991). Dopamine D_2 receptor activation depolarizes rat supraoptic neurones in rat hypothalamic explants. *Journal of Physiology* **443**, 405–419.

Acknowledgements

We thank S. Papas and D. Richard for their helpful comments on the manuscript. This work was supported by the Medical Research Council of Canada. K. K. is recipient of a Studentship from Le Fonds FCAR and C. W. B. is an MRC Scientist.

Received 14 February 1994; accepted 21 June 1994.