Ionic Mechanisms of Action of Neurotensin in Acutely Dissociated Neurons From the Diagonal Band of Broca of the Rat

BALVINDER S. JASSAR, KIM H. HARRIS, PAULA M. OSTASHEWSKI, AND JACK H. JHAMANDAS

Department of Medicine (Neurology) and Division of Neuroscience, University of Alberta, Edmonton, Alberta T6G 2B7, Canada

Jassar, Balvinder S., Kim H. Harris, Paula M. Ostashewski, and Jack H. Jhamandas. Ionic mechanisms of action of neurotensin in acutely dissociated neurons from the diagonal band of Broca of the rat. J. Neurophysiol. 81: 234–246, 1999. Whole cell recordings were performed on acutely dissociated neurons from the horizontal limb of the diagonal band of Broca (hDBB) from rats to elucidate the ionic mechanisms of action of neurotensin. Neurotensin caused a decrease in whole cell voltage-activated outward currents and failed to elicit a response when Ca\(^{2+}\) influx was blocked by changing the external solution to the one containing 0 mM Ca\(^{2+}\) and 50 μM Cd\(^{2+}\), suggesting the involvement of Ca\(^{2+}\)-dependent conductances. Charybdotoxin, a specific blocker of voltage-sensitive calcium-activated K\(^{+}\) channels (I\(_{\text{Ca}}\)), caused a decrease in outward currents comparable with that caused by blocking calcium influx and occluded the neurotensin-induced decrease in outward currents. Similarly, 50 μM tetrodynammonium ions also blocked the neurotensin response. Also neurotensin reduced whole cell barium currents (I\(_{\text{Ba}}\)) and calcium currents (I\(_{\text{Ca}}\)). Amiloride and ω-conotoxin GVIA, but not nimodipine, were able to eliminate the neurotensin-induced decrease in I\(_{\text{Ba}}\). Thus T- and N- but not L-type calcium channels are subject to modulation by neurotensin, and this may account for its effects on I\(_{\text{Ca}}\). The predicted changes in action potential as a result of the blockade of currents through calcium channels culminating into changes in I\(_{\text{Ca}}\) were confirmed in the bridge current-clamp recordings. Specifically, neurotensin application led to depolarization of the resting membrane potential, broadening of spike and a decrease in afterhyperpolarization and accommodation. These alterations in action potential characteristics that resulted in increased firing rate and excitability of the hDBB neurons also were produced by application of charybdotoxin. Neurotensin effects on these properties were occluded by 2-[(1–7-chloro-4-quinolonyl)-5-(2,6-di-methoxyphenyl) pyrazol-3-yl] carbonylamino] tricyclo (3.3.1.1.3) decan-2-carboxylic acid, a nonpeptide high-affinity neurotensin receptor antagonist. Neurotensin blockade of I\(_{\text{Ca}}\) possibly through I\(_{\text{Ca}}\), is a potential physiological mechanism whereby this peptide may evoke alterations in the cortical arousal, sleep-wake cycle, and theta rhythm.

INTRODUCTION

Diagonal band of Broca (DBB) is a basal forebrain area implicated in important physiological functions such as learning and memory, the generation of hippocampal theta rhythm, and central cardiovascular regulation (Jakab and Leranth 1995). The neurons in this area constitute a heterogeneous population consisting, mainly, of two chemical phenotypes: cholinergic and GABAergic neurons (Panula et al. 1984; Rye et al. 1984). These two cell types are distinct not only chemomorphologically but also with respect to their electrophysiological properties (Griffith 1988).

The horizontal limb of DBB (hDBB) neurons have been shown to respond to putative peptidergic neurotransmitters such as vasopressin and neurotensin (Alonso et al. 1994; Easaw et al. 1997a; Jassar et al. 1996). Neurotensin, a tridecapeptide, is taken up and internalized selectively within cholinergic neurons of the DBB (Faure et al. 1992). The source for the neurotensin input to cholinergic neurons is unknown at present. Neurotensin excites neurons in a number of areas within the CNS such as spinal cord (Miletic and Randic 1979), hypothalamus (Baldino and Wolfson 1985), cortex (Audinat et al. 1989), ventral tegmental region (Jiang et al. 1994), and supraoptic nucleus (Kirkpatrick and Bourque 1995). This peptide also enhances excitatory neurotransmission in the parabrachial nucleus (Saleh et al. 1997). The ionic mechanisms responsible for these excitatory effects in different neuronal types are diverse. Neurotensin has been shown to decrease inward rectifier potassium currents and activate cation or nonselective conductances in cultured rat basal forebrain cholinergic neurons (Farkas et al. 1994); reduce a potassium conductance in ventral tegmental neurons (Jiang et al. 1994); and attenuate afterhyperpolarization in supraoptic nucleus neurons (Kirkpatrick and Bourque 1995); and block T- and N- but not L-type calcium currents in cultured newborn rat nucleus basalis neurons (Margret-Mitrovic et al. 1997). In the DBB neurons, neurotensin promotes oscillatory bursting behavior (Alonso et al. 1994). However, the ionic mechanisms by which neurotensin elicits this response is unknown.

In the context of investigating the basis for oscillatory behavior evoked by neurotensin in the basal forebrain neurons in the brain slice, we performed a whole cell patch-clamp study on the actions of neurotensin on acutely dissociated neurons from this area. Our data show that attenuation of voltage-sensitive calcium-activated potassium conductances (I\(_{\text{Ca}}\)), which might be a consequence of the blockade of calcium currents, is a potential mechanism for the action of neurotensin on hDBB neurons and can explain the effects of neurotensin in regulating the excitability of basal forebrain neurons. A preliminary account of this work has appeared in abstract form (Jassar and Jhamandas 1995).
METHODS

Dissociation procedures

Details of the procedure for acute dissociation of neurons from the diagonal band of Broca are described in Easaw et al. (1997a). Briefly, brains were removed quickly from decapitated male Sprague Dawley rats (15–21 day postnatal) and placed in cold artificial cerebrospinal fluid (ACSF), which contained (in mM) 140 NaCl, 2.5 KCl, 1.5 CaCl₂, 5 MgCl₂, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and 33 n-glucose (pH 7.4). Brain slices (350 μm thick) were cut on a vibratome, and the area containing the hDBB was dissected out. Acutely dissociated neurons were prepared by enzymatic treatment of slice with trypsin (0.65 mg/ml) at 30°C, followed by mechanical triturating for dispersion of individual cells. Cell were then plated on poly-L-lysine (0.005% wt/vol)-coated coverslips and viewed under an inverted microscope (Zeiss Axiosvert 35). All solutions were kept oxygenated by continuous bubbling with pure oxygen.

Electrophysiological recordings

Whole cell patch-clamp recordings were performed at room temperature (usually 20°C) using an Axopatch-1D amplifier. Series resistance compensation was monitored continuously at >80% and readjusted during the course of each experiment. Junction potential was nulled with the pipette tip immersed in the bath. Internal patch pipette solution contained (in mM) 140 K-methylsulfate, 10 ethylene glycol-bis(β-aminooethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), 5 MgCl₂, 1 CaCl₂, 10 HEPES, 2.2 Na₂-ATP, and 0.3 Na-GTP (pH 7.2). Filter was set at 50 kHz during data acquisition. Data were acquired and analyzed using pCLAMP software (version 6.0.3). Cells were held at −80 mV, which was close to the resting membrane potential (RMP) measured in earlier studies on neurons from the basal forebrain (Casaw et al. 1997a; Griffith 1988; Segal 1986). A 1-s hyperpolarizing command to −110 mV was applied to remove inactivation of K⁺ channels so that the maximum current could be activated during the subsequent voltage ramp to +30 mV (10 or 20 mV/s) that followed it. No obvious tail currents were observed at the end of the ramp when the command potential was returned to −80 mV. Because the neurotensin effects were similar in magnitude with both kinds of voltage ramps (i.e., 10 and 20 mV/s) throughout the voltage range examined, the data were pooled together.

Whole cell recordings also were done under bridge current-clamp mode using Axoclamp-2B amplifier to examine the effects of neurotensin on action potential characteristics of the acutely dissociated hDBB neurons. Action potentials were evoked by brief current injection through the patch pipette. The resting membrane potential and voltages attained at peak of the spike and the afterhyperpolarization were recorded for comparison under different experimental conditions. Action potential duration was measured at threshold. Afterhyperpolarization duration was measured from the point where the spike crosses the baseline during its descent to the point where the trajectory of the afterhyperpolarization returns to baseline. The hold currents (patch-clamp experiments) and the membrane voltages (current-clamp experiments) were recorded on a chart recorder (Gould 22005S). Because the action potentials were truncated in chart recordings due to slow response of the pen and the high gain settings, we also recorded the evoked or spontaneous events on computer using pCLAMP software (version 6.0.3).

Currents through calcium channels were recorded using either Ba²⁺ or Ca²⁺ as charge carrier. The external solution contained (in mM) 150 tetraethylammonium chloride, 2 BaCl₂ or CaCl₂, 10 HEPES 10, and 30 glucose (pH to 7.4 with TEA-OH). The internal patch pipette solution consisted of (in mM) 130 Cs-methanesulfonate, 2 MgCl₂, 10 HEPES, 10 bis-(o-aminophenoxy)-N,N,N’,N’-tetraacetate acid, 4 Mg-ATP, 0.3 Na-GTP, and 0.1 leupeptin (pH to 7.2 with CsOH). Because leak currents were minimal under our recording conditions, did not change during the recordings, and were not affected by application of neurotensin, these were not subtracted in subsequent measurements of steady-state barium or calcium currents. The capacitance transients have been omitted in the raw current traces shown in Figs. 4 and 5 for aesthetic purposes only.

Drugs and solutions

Neurotensin, charybotoxin, ω-conotoxin GVIA, amiloride, apamin, and tetraethylammonium chloride were obtained from Sigma Chemical (St. Louis, MO). Nimodipine and BayK 8644 were gifts from Dr. Susan Dunn (University of Alberta) and 2-[1-(7-chloro-4-quinoliny1)-5-(2,6-dimethoxyphenyl)pyrazol-3-yl]carbonylamino]tricyclo(3.3.1.1)decan-2-carboxylic acid (SR 48692) was a gift from the Sanofi, Rescher. Except for nimodipine, and SR 48692, all the agents were dissolved in distilled water to make 1,000× stock solution. Nimodipine and SR 48692 were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was 1:1,000 to 1:10,000. Stock solutions were diluted in external perfusing medium just before the time of application. All drugs and chemicals were applied via bath perfusion at the rate of 3–5 ml/min, which allowed complete exchange in about 0.5 min. Student’s t-test was used to test significance of effects. Results are presented as means ± SE.

RESULTS

Most of the acutely dissociated neurons from the hDBB had neuron like morphology (i.e., large cells with a conspicuous nucleus, nucleolus and few blunt processes which were truncated axon/dendrites). Because the larger cholinergic neurons take up and internalize neurotensin selectively in a neurotensin-dependent manner (Alonso et al. 1994), we selected to record only from cells that appeared large on visual inspection. The average membrane capacitance estimated electronically on the Axopatch-1D amplifier was 17.2 ± 30 mV, beyond which the currents Ba²⁺ or Ca²⁺ were similar in magnitude with both kinds of voltage ramps (i.e., 10 and 20 mV/s). Under our recording conditions, the average input conductance measured from the slope of the I-V relationships between −60 and −110 mV was 1.20 ± 0.11 nS (n = 100).

Initially, currents were evoked by applying voltage step commands from −140 to +80 mV. The currents at voltage steps to +40 mV or more positive were usually >10 nA. When cells with such large currents are recorded using an Axopatch-1D amplifier, voltage-clamp error is quite significant because of uncompensated series resistance. However, the currents evoked by applying voltage ramps (10 or 20 mV/s) in the same voltage range were of smaller amplitudes. Figure 1A shows the currents evoked by applying a voltage ramp from −140 to +120 mV in a hDBB neuron. The voltage dependence of activation of the currents during a ramp (−140 to +120 mV) between the voltage range −40 and +40 mV was steep, and the peak currents were observed at about +30 mV. Depolarization beyond this voltage resulted in a plateau followed by a decrease in the amplitude of currents up to +70 to +80 mV, beyond which the currents started to increase again. This typical shape of the J-V plot with a maximum current between +30 to +50 mV is indicative of the presence of calcium-activated currents (Hille 1992; Lancaster and Pennefather 1987).
Figure 1B shows the typical voltage-activated currents in a hDBB neuron evoked by applying a voltage ramp from −140 to +30 mV under control conditions ([K⁺]₀ = 2.5 mM) and under conditions when the external solution contained 25 mM K⁺. The estimated Eₖ from the Nernst equation was −102 mV under control conditions and −44 mV in the presence of 25 mM K⁺. If these cells express inwardly rectifying K⁺ channels, one would predict a considerable increase in inward currents below −44 mV in the presence of 25 mM K⁺. However, very little current was generated in the voltage range from −140 to −50 mV, and the currents began to activate at about −50 mV. This suggests the lack of voltage-activated inwardly rectifying type K⁺ currents in the acutely dissociated hDBB neurons. Thus to avoid the unnecessary hyperpolarization to −140 mV, which usually impaired viability of the cells, the lower limit of the voltage ramp was set at −110 mV. This was sufficient to remove inactivation of the K⁺ currents that are present in these neurons.

Based on these observations, we used a ramp-clamp protocol where the cells were held at −80 mV and subjected to voltage ramps from −110 to +30 mV at the rate of 10 or 20 mV/s after conditioning at −110 mV for 1 s.

Effects of neurotensin on whole cell potassium currents in hDBB neurons

Responses were obtained with either 1 µM or 100 nM neurotensin. When the concentration was 1 µM, the responses desensitized and recovery was invariably impossible to obtain. However, with 100 nM the response was maximum, repeatable, and reversible within ~10 min (Jiang et al. 1994). Therefore for all the experiments mentioned under RESULTS, 100 nM neurotensin was used. Figure 1C shows the voltage-activated currents recorded from a hDBB neuron under control conditions, in the presence of neurotensin, and recovery after washout. Application of neurotensin affected the whole cell currents in 84 of the 100 cells tested. The amplitude of the currents generated at potentials below −60 mV was minuscule (maximum <30 pA). The average holding current at −80 mV was 0.017 ± 0.004 nA under control conditions and 0.001 ± 0.005 nA in the presence of neuro-
Neurotensin ($n = 84; P < 0.001$; Fig. 1D). Thus although there was a small but significant change in the holding currents at $-80$ mV (Fig. 1, C and D), it was impossible to obtain a correct measurement of reversal potentials from the records of raw currents under different conditions. However, neurotensin produced an obvious decrement in the voltage-activated outward currents at more depolarized potentials, especially at $+30$ mV (Fig. 1, C and D). The amplitude of the currents at $+30$ mV was significantly reduced from $6.4 \pm 0.3$ nA under control conditions to $4.9 \pm 0.2$ nA ($n = 84; P < 0.001$) in the presence of neurotensin; this represents a decrease of $23.2 \pm 1.2\%$ ($n = 84$) at this potential. Input conductance measured from the slope of the $I-V$ relationships in the voltage range from $-110$ to $-60$ mV was not significantly affected by neurotensin (control: $1.21 \pm 0.10$ nS; neurotensin: $1.10 \pm 0.08$ nS; $n = 84; P > 0.2$).

Under our recording conditions, currents that are activated in the voltage range in which neurotensin exerts its actions include the classical Hodgkin-Huxley type delayed rectifier ($I_{K}$) and the calcium-activated potassium currents ($I_{KCa}$). The fast inactivation kinetics of the transient outward potassium current ($I_{K}$) (Belluzzi et al. 1985; Cooper and Shrier 1985; Griffith and Sim 1990) makes it unlikely that these channels are activated during the relatively slow voltage ramps utilized in our experiments. This suggests that the conductances affected by neurotensin include $I_{K}$ and/or $I_{KCa}$.

### Effects of the blockade of calcium influx

Because the $I_{KCa}$ are activated by $Ca^{2+}$, which flows into the cell during the depolarizing phase of the action potential, their contribution to the voltage-activated conductances can be assessed by blocking $Ca^{2+}$ influx (Lancaster et al. 1991). This was achieved by replacing the extracellular $Ca^{2+}$ with $Mg^{2+}$. $Cd^{2+}$ (50 $\mu M$) was included in the external perfusing solution to ensure complete blockade of the $Ca^{2+}$ influx. Figure 2 shows the $I-V$ relationship of currents recorded in a hDBB neuron evoked by applying voltage ramps under different conditions as follows. When the normal external solution was changed to the one containing 0 mM $Ca^{2+}$ and 50 $\mu M$ $Cd^{2+}$, the outward currents elicited in the range from $-40$ to $+30$ mV were reduced as compared with those under control conditions (Fig. 2A). The amplitude of the currents at $+30$ mV decreased significantly from $5.2 \pm 0.6$ nA under control conditions to $4.0 \pm 0.5$ nA ($n = 9; P < 0.05$) in the presence of 0 mM $Ca^{2+}$ and 50 $\mu M$ $Cd^{2+}$. The exposure of the cells to 0 mM $Ca^{2+}$ and 50 $\mu M$ $Cd^{2+}$ thus resulted in 24.1 $\pm 4.5\%$ reduction in the outward currents. Neurotensin application in the presence of 0 mM $Ca^{2+}$ and 50 $\mu M$ $Cd^{2+}$ $27.9 \pm 4.4\%$; $n = 9; P > 0.2$). The occlusion of the neurotensin response by blocking the $Ca^{2+}$ influx suggests that neurotensin blocks $I_{KCa}$ and/or $I_{Ca}$.

### Currents evoked by voltage ramps do not contain apamin-sensitive currents

There are two major families of biophysically and structurally distinct ion channels that underlie the $I_{KCa}$—one being the voltage-insensitive type and the other voltage-sensitive. The former includes the apamin-sensitive conductance, $I_{AHP}$, which is known to determine the amplitude and duration of the afterhyperpolarization of an action potential; and the latter comprises the larger conductance $I_{Ca}$ channels, which are responsible for repolarization of the action potential (Lancaster and Pennefather 1987; Lancaster et al. 1991; but see also Belluzzi and Sacchi 1991). There can be a substantial amount of calcium influx through the voltage-activated calcium channels during the relatively slow ramps employed in these experiments; this then can lead to activation of $I_{AHP}$. Thus although $I_{AHP}$ is a voltage-insensitive con-
ductance, a portion of the currents generated by the voltage ramps may contain $I_{\text{AMP}}$ because its activation is also dependent on the influx of Ca$^{2+}$.

Apamin has been shown to selectively and specifically block the slow afterhyperpolarization that follows an action potential in supraoptic neurosecretory neurons and cat spinal motoneurons ($IC_{50} \sim 1.3$ nM) (Bourque and Brown 1987; Zhang and Krnjevic 1987). Therefore we applied 50–500 nM apamin to assess the contribution of apamin-sensitive currents to the total voltage-activated currents. Figure 2B shows the voltage-activated currents evoked in a hDBB neuron by applying voltage ramp from −110 to +30 mV under control conditions and in the presence of apamin. Application of apamin had very little effect on the currents ($n = 5$), suggesting that the apamin-sensitive currents ($I_{\text{AMP}}$) do not appear to make any significant contribution to the voltage-activated currents evoked during the voltage ramps. Also neurotensin still evoked a decrease in the outward currents comparable with that observed under control conditions (see Fig. 1D). $I_{\text{AMP}}$ also was evoked by applying the calcium influx induced by applying a depolarizing pulse to +30 mV ($V_{\text{th}} = −80$ mV; 80- to 400-ms duration) and observing tail currents flowing at −40 mV. The tails were not affected by application of 100 nM neurotensin (data not shown). These results suggest that the neurotensin-sensitive currents are not a part of the voltage-insensitive, apamin-sensitive component of $I_{K,\text{Ca}}$. By corollary, the currents that are blocked by neurotensin are likely to be the voltage-sensitive component of $I_{K,\text{Ca}}$, i.e., $I_C$.

$I_C$ underlies the neurotensin-induced decrease in voltage-activated outward currents

The contribution of $I_C$ to the voltage-activated currents can be determined by using the selective blockers of this family of voltage-sensitive calcium-activated channels. These include the charybdotoxin (CTX; $IC_{50} 0.2–5$ nM) (Lancaster and Pennefather 1987; Storm 1990), a scorpion toxin obtained from Leirius quinquestriatus var. herbeus, and low millimolar concentrations of tetraethylammonium ions (TEA) (Storm 1990).

CHARYBDOXIN OCCLUDES NEUROTENSIN RESPONSE IN hDBB NEURONS. Figure 2C shows the $I-V$ relationship of currents evoked under control conditions, in the presence of 25 nM CTX, and neurotensin (100 nM) in the presence of CTX (25 nM). CTX caused a reduction in outward currents in the same voltage range (−40 to +30 mV) as neurotensin. Also the CTX-induced decrease in the currents occurred in the same voltage range and was of a similar magnitude as that obtained by blocking the depolarization-induced calcium influx. Taken together these data strongly support the specificity and efficacy of 25 nM CTX in blocking voltage-sensitive calcium-activated currents in our preparation.

The amplitude of the outward currents at +30 mV was reduced significantly from 7.0 ± 0.4 nA under control conditions to 5.2 ± 0.5 nA in the presence of neurotensin ($n = 19$; $P < 0.001$). After recovery from neurotensin effects to almost control levels ($6.8 ± 0.5$ nA; $n = 17$), subsequent application of CTX reduced the currents to 5.2 ± 0.6 nA, and then the application of CTX and neurotensin together reduced the currents to 5.1 ± 0.5 nA ($n = 17$). Thus the currents were decreased $24.2 ± 2.1\%$ ($n = 19$) by neurotensin alone, $25.2 ± 2.1\%$ ($n = 17$) by CTX alone, and $25.4 ± 2.1\%$ ($n = 17$) when CTX and neurotensin were applied together. The total percent decrease in the outward currents caused by neurotensin was not significantly different from that caused by CTX alone or when neurotensin and CTX were applied together ($P > 0.2$). This abolition of neurotensin response in the presence of $I_C$ blockade with CTX appears consistent with the notion that neurotensin blocks outward voltage-sensitive Ca$^{2+}$-activated K$^+$ channels.

TETRAETHYLAMMONIUM IONS ALSO BLOCK THE NEUROTENSIN RESPONSE. TEA blocks several kinds of voltage-activated potassium channels; however, low millimolar concentrations of TEA has been shown to selectively block $I_C$ (Storm 1990). Therefore we used micro- to millimolar concentrations of TEA to examine the concentration-dependent effects of this agent on blocking $I_C$. The bar diagram in Fig. 3A shows the comparison of average currents observed at +30 mV from five cells under control conditions and in the presence of 10 and 100 μM and 1 mM TEA. The average outward currents at +30 mV were 7.2 ± 1.1 nA under control conditions and were reduced to 5.5 ± 1.1 nA, 5.0 ± 0.8 nA, and 3.2 ± 0.5 nA in the presence of 10 μM, 100 μM, and 1 mM TEA, respectively. Thus the reduction in the currents was $23.8 ± 5.5\%$, $28.2 ± 5.6\%$, and $54.5 ± 4.5\%$, respectively. These data illustrate a dose-dependent sensitivity of voltage-activated outward currents, to application of TEA in hDBB neurons. Furthermore it suggests that the concentration of TEA required to block the amount of outward current attributable to $I_C$ in these cells is between 10 and 100 μM. Therefore we used 50 μM TEA to investigate whether it could occlude the neurotensin-induced decrease in the outward currents.

Figure 3B shows the reversible decrease in the voltage-activated outward currents caused by neurotensin. Exposure of the same neuron to 50 μM TEA caused a similar decrease (Fig. 3C). Subsequent application of neurotensin in the presence of TEA did not significantly change the currents (Fig. 3D). Similar results were obtained with five other neurons. TEA (50 μM) reduced the currents significantly at +30 mV from $6.9 ± 1.1$ to $5.5 ± 0.5$ nA ($n = 8$; $P < 0.01$). Thus TEA alone reduced the currents by $20.9 ± 2.5\%$ ($n = 8$). With subsequent application of neurotensin in the presence of TEA, the currents changed to $5.3 ± 0.7$ nA ($n = 8$). The total decrease in the whole cell currents when neurotensin and TEA were applied together was $23.2 ± 1.3\%$ ($n = 8$). The decrement in current in the presence of TEA alone was not significantly different from that when TEA and neurotensin were applied together ($P > 0.2$). Thus these data show that the response to neurotensin could be occluded by 50 μM TEA.

Effects of neurotensin on currents through calcium channels

Because neurotensin blocks $I_C$, one possible target of action can be the voltage-gated calcium channels through which the calcium influx responsible for the activation of $I_C$ occurs. Three different kinds of voltage-gated calcium channels have been shown to be present in the guinea pig magnocellular cholinergic basal forebrain neurons: the low-
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FIG. 3. Tetraethylammonium (TEA) and neurotensin response in hDBB neurons. A: bar diagram of the average data on the concentration-dependent effects of TEA showing exquisite sensitivity of the outward K⁺ currents. B–D: comparison of effect of TEA, neurotensin, and neurotensin in the presence of TEA in a hDBB neuron. B: I-V plots of currents evoked in a hDBB neuron illustrating the neurotensin-induced decrease in outward currents in the voltage range from −30 to +30 mV followed by partial recovery. C: I-V relationships of currents evoked in the same neuron as above showing the decrease in outward current caused due to blockade by 50 μM TEA and recovery on washout of TEA. D: I-V relationships of currents evoked under control conditions and in the presence of 50 μM TEA and 100 nM neurotensin. Ramps @ 20 mV/s.

These data confirm the previously described advantages of using Ba²⁺ for recording the currents through calcium channels because it is a better charge carrier (Fox et al. 1987; Griffith et al. 1994; Hille 1991) and avoids calcium-dependent inactivation of calcium channels (Chad and Eckert 1986).

The amount of currents evoked in the voltage range from −80 to −50 mV was very small, and the fast activating and inactivating classical T-type currents were not observed even with longer pulses of ±500-ms duration with either Ba²⁺ or Ca²⁺ (see Allen et al. 1993; Fox et al. 1987; Griffith et al. 1994). The biophysical fingerprints of T-type channels were thus not any more obvious in Ca²⁺ than in Ba²⁺ either in raw data records or in I-V relationships.

Application of neurotensin decreased peak and end $I_{Ca}$ at 0 mV by 28.4 ± 7.3% and 31.9 ± 6.9%, respectively ($n = 11$). Peak and end $I_{Ba}$ at −20 mV were reduced by 31.4 ± 5.0% and 30.5 ± 6.6%, respectively ($n = 10$). Neurotensin-induced decreases in peak or end $I_{Ca}$ and $I_{Ba}$ were not significantly different ($P > 0.2$). Thus neurotensin effects were similar in the presence of either divalent cation. In light of these observations, we selected 20-ms pulses using Ba²⁺ as a charge carrier as optimal conditions to study the effects of neurotensin on specific calcium channels.

Figure 5A (top) shows the barium currents through calcium channels evoked in a hDBB neuron by applying a 20-ms step command to −10 mV from a holding potential of

voltage-activated (LVA) T type and the high-voltage-activated (HVA) L and N types (Allen et al. 1993; Griffith et al. 1994; Margeta-Mitrovic et al. 1997). We used biophysical and pharmacological approaches to examine their possible individual involvement in neurotensin-evoked response.

Currents through calcium channels were recorded using Ba²⁺ ($I_{Ba}$) or Ca²⁺ ($I_{Ca}$) as the charge carrier. Figure 4, A and C, shows $I_{Ca}$ and $I_{Ba}$, respectively, generated by applying 500-ms depolarizing pulses ($V_H = −90$ mV) under control conditions and in the presence of neurotensin. Substitution of Ca²⁺ with Ba²⁺ resulted in larger and more slowly inactivating currents. Because the inactivation profile of both the $I_{Ba}$ and the $I_{Ca}$ showed apparent current dependence (Jassar et al. 1993; Jones and Marks 1989), we compared the peak and end currents at maximum amplitudes rather than at the same voltages. The fraction of currents remaining at the end of the 500-ms command pulse was 0.79 ± 0.03 ($n = 11$) at −20 mV for $I_{Ba}$ compared with 0.60 ± 0.03 ($n = 20$) for $I_{Ca}$ at 0 mV ($V_H = −90$ mV). Thus the currents inactivated by 20.7 ± 2.5% in Ba²⁺ as opposed to 39.4 ± 3.4% in Ca²⁺. In addition, the $V$-I relationships of $I_{Ba}$ were shifted to the left by 10 or 20 mV in each cell compared with $I_{Ca}$ (Fig. 4, B and D). The maximum peak current amplitude for $I_{Ca}$ was at 0 mV ($−1.38 ± 0.18$, $n = 20$) and for $I_{Ba}$ at −20 mV ($−3.58 ± 0.36$, $n = 11$). The maximum end current amplitude for $I_{Ca}$ was 0.83 ± 0.09 nA ($n = 20$) at 0 mV and 2.84 ± 0.29 nA ($n = 11$) for $I_{Ba}$ at −20 mV.

The concentration-dependent effects of TEA showed exquisite sensitivity of the outward K⁺ currents.
−80 mV under control conditions and subsequently in the presence of neurotensin. The effects of neurotensin on \( I_{\text{n}} \) are illustrated in Fig. 5A, bottom. The maximum current-density was 80.7 ± 10.5 pA/pF at −10 mV under control conditions (\( n = 15 \)) in this group of cells. Neurotensin reduced the whole cell \( I_{\text{n}} \) at voltages between −40 and +30 mV. The whole cell \( I_{\text{n}} \) at −10 mV were significantly reduced (25.9 ± 2.7%; \( P < 0.001 \)) from 1.26 ± 0.17 nA (\( n = 15 \)) under control conditions to 0.96 ± 0.13 nA (\( n = 15 \)) in the presence of neurotensin.

Figure 5B (top) shows typical response of a hDBB neuron to a voltage command step from −80 to −30 mV under control conditions, in the presence of amiloride, and neurotensin in the presence of amiloride. Figure 5B, bottom, illustrates \( I-V \) relationships of the averaged data under control conditions, in the presence of amiloride and neurotensin in the presence of amiloride. The currents that are activated by stepping to command potentials of up to −15 mV have been shown to include the LVA T-type currents in addition to the HVA calcium currents (Allen et al. 1993). The raw current traces in Fig. 5B, top, demonstrate the presence of amiloride-sensitive T-type channels and the occlusion of the neurotensin response when T-type calcium channels are blocked by this agent. Examination of Fig. 5B (bottom) shows the rightward shift in the \( I-V \) relationship caused by amiloride in the voltage range from −40 to −10 mV. \( I_{\text{n},\text{s}} \) were significantly decreased in the voltage range between −40 and −20 mV in the presence of amiloride (control: −1.67 ± 0.32 nA; amiloride: −1.21 ± 0.23 nA; \( n = 8 \); \( P < 0.002 \) at −20 mV). Neurotensin did elicit a decrease in \( I_{\text{n},\text{s}} \) in this voltage range in the presence of amiloride (amiloride: −1.21 ± 0.23 nA; amiloride + neurotensin: −1.10 ± 0.021 nA; \( n = 8 \); \( P > 0.1 \) at −20 mV). Neurotensin evoked reduction in the \( I_{\text{n},\text{s}} \) at -20 mV (11.9 ± 3.5%, \( n = 8 \)) was significantly less in the presence of amiloride than that under control conditions at the same voltage (26.5 ± 2.9, \( n = 15 \), \( P < 0.001 \)).

In contrast, amiloride had no significant effect on \( I_{\text{n},\text{s}} \) in the voltage range positive to −10 mV and the effect of neurotensin was still observed in its presence (see Fig. 5B, bottom). For example, the amount of current evoked at −10 mV was 1.86 ± 0.29 nA under control conditions, −1.91 ± 0.31 nA in the presence of amiloride, and −1.44 ± 0.23 nA when neurotensin was applied in the presence of amiloride (\( n = 8 \)). The neurotensin-evoked decrease of \( I_{\text{n},\text{s}} \) at −10 mV thus was unaffected by the presence of amiloride (26.7 ± 5.2%; \( P < 0.01 \)). These data on the specific voltage range where amiloride exerts its effects confirm the specificity of the effects of amiloride on T-type channels at concentrations used in our study. These data show that neurotensin-induced decrease in \( I_{\text{n},\text{s}} \) involves blockade of T-type calcium channels.

Another type of calcium channels present in the hDBB neurons is the dihydropyridine-sensitive L-type calcium channels. \( I_{\text{l}} \) evoked in these neurons could be enhanced by BayK 8644 (data not shown) and blocked partially by nimodipine, a dihydropyridine-type calcium channel antagonist. Figure 5C (top) shows the effects of application of nimodipine alone and also of neurotensin in the presence of nimodipine on the \( I_{\text{n},\text{s}} \) at −10 mV in a hDBB neuron. The averaged data for \( I-V \) relationship are shown in Fig. 5C, bottom. The amount of currents at −10 mV was −1.29 ± 0.18 nA under control conditions, −0.81 ± 0.11 nA in the presence of 10 µM nimodipine, and −0.42 ± 0.11 nA when the same cells were exposed to neurotensin in the presence of nimodipine (\( n = 9 \)). Thus nimodipine reduced the currents by 35.8 ± 4.5%, and in the presence of neurotensin,
the remaining currents were further decreased by 39.4 ± 6.3%. In the presence of nimodipine, neurotensin evoked 25.3 ± 5.4% decrease in I_{Ba}s compared with control. This decrease in I_{Ba}s was thus comparable with that observed when neurotensin was applied alone under control conditions (25.9 ± 2.7%; P < 0.01). This clearly shows the lack of involvement of nimodipine-sensitive L-type calcium channels in neurotensin response.

We also tested for the presence of ω-conotoxin-sensitive N-type calcium channels and their modulation by neurotensin in hDBB neurons. The maximum irreversible blockade of I_{Ba}s on application of 100 nM ω-conotoxin GVIA was obtained in <5 min (n = 21). Typical recordings of the I_{Ba} evoked at −10 mV in a hDBB neuron under control conditions, in the presence of ω-conotoxin, and neurotensin in the presence of ω-conotoxin are shown in Fig. 5D (top). Figure 5D, bottom, illustrates the I-V relationships of the averaged data from eight cells. The average I_{Ba} at −10 mV was −1.65 ± 0.13 nA under control conditions; this average was reduced to −0.89 ± 0.09 nA in the presence of 100 nM ω-conotoxin, and neurotensin did not cause any further significant decrease (−0.75 ± 0.09 nA; P > 0.2). Thus ω-conotoxin blocked 48.4 ± 2.7% of the control I_{Ba} at −10 mV and the total blockade by neurotensin and ω-conotoxin applied together was 52.5 ± 2.9% (n = 8; P > 0.2).

**Effects of neurotensin on action potentials and excitability**

Neurotensin has been shown to promote oscillatory behavior in basal forebrain neurons in the brain slice preparation (Alonso et al. 1994). Because our study was conducted on acutely dissociated neurons, we verified if the neurotensin-evoked changes in excitability also could occur under these conditions. Therefore we performed bridge current-clamp recordings in whole cell mode to examine the effects of neurotensin on hDBB neurons. In addition, we also determined whether the neurotensin induced changes in excitability that could be predicted from the observed decrease in currents through the calcium and I_{C} channels.

Figure 6 illustrates the effects of neurotensin on action potential waveform and excitability recorded in a hDBB neuron. Figure 6A shows the neurotensin evoked reversible depolarization of the membrane potential resulting in an increased firing rate (approximately ≈4 Hz). In a majority of
cells, this depolarization was sufficient to bring the cell to threshold with resultant generation of action potentials that led to spontaneous firing. In some cells (9/25) that were resting at membrane potential close to −80 mV, this depolarization was not enough to bring them to threshold. Application of neurotensin resulted in depolarization of the membrane potential (RMP = −69.7 ± 1.0 mV) in 25 of 28 neurons tested, the magnitude of which varied from 15 to 25 mV (18.7 ± 1.0 mV). However, in both the cases, the changes in action potential waveform and excitability were similar. When the action potentials generated by brief application of currents or the spontaneous action potentials generated during this phase were examined, the action potentials in the presence of neurotensin attained smaller peaks (decrease = 8.2 ± 2.1 mV, P < 0.005) and longer duration (increase = 1.2 ± 0.2 ms, P < 0.001; Fig. 6B). In Fig. 6C, the recordings from the same neuron further demonstrate these effects and the decrease in the afterhyperpolarization. The afterhyperpolarization peak potentials were reduced by 4.4 ± 0.7 mV (P < 0.001).

Figure 6D shows the trains of action potentials generated by injecting a 600-ms current pulse (1.0 nA) under control conditions and in the presence of neurotensin. The ratio of interspike interval between the first two and the last two action potentials provides a measure of accommodation, with a ratio approaching 1 indicating loss of accommodation. Under control conditions, this ratio was 0.54 ± 0.04 (n = 14), and it increased to 0.71 ± 0.03 (n = 14) in the presence of neurotensin (P < 0.05), indicating attenuation of accommodation in the presence of the peptide. Further, the number of spikes generated by a 600-ms depolarizing current pulse was 6.5 ± 0.9 under control conditions and 9.4 ± 0.9 in the presence of neurotensin (P < 0.005; n = 25). Thus neurotensin decreases accommodation and increases excitability in these neurons. The resting membrane potential was 5–10 mV more negative on recovery from neurotensin-induced depolarization or spontaneous firing than that under control conditions before application of neurotensin.

**Effects of charybdotoxin and ω-conotoxin GVIA**

If the effects of neurotensin were the result of blockade of I_C, then application of charybdotoxin might be expected to have the same effects as neurotensin. Figure 7A shows that application of 25 nM charybdotoxin on a hDBB neuron resulted in a depolarization of the neuron of ~8 mV leading to spontaneous firing of action potentials. Such reversible effects were observed in five other neurons as well. The magnitude of depolarization varied from 7 to 12 mV. The changes in action potential characteristics observed were similar to those with neurotensin.

Application of ω-conotoxin GVIA slowly depolarized neurons (12–15 mV, n = 4) but did not induce spontaneous firing (Fig. 7B). Subsequent application of neurotensin resulted in an additional but very small depolarization.

**Effects of nonpeptide neurotensin receptor antagonist SR 48692 on neurotensin response**

Two G-protein coupled neurotensin receptors (high and low affinity) have been identified and cloned in mammalian brain. Of these, the high-affinity site that is sensitive to the nonpeptide neurotensin receptor antagonist SR 48692 (Gully et al. 1993) is predominantly expressed in neurons (Nouel et al. 1997). We tested the effects of this antagonist on neurotensin response. Typical recordings in a hDBB neuron are shown in Fig. 7, C and D. This cell, which had a resting membrane potential of −70 mV, depolarized to approximately −50 mV with spontaneous firing on application of 100 nM neurotensin and recovered on washout (Fig. 7C). Neurotensin evoked the characteristic changes in action potential and afterhyperpolarization similar to those shown in Fig. 6. After recovery, this cell was exposed to the neurotensin antagonist SR 48692 (100 nM), which had very little effect on resting membrane potential (Fig. 7D). Nor did it have any effects on action potential waveform and afterhyperpolarization. This was followed by application of neuro-
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**FIG. 7.**  
A: effects of charybdotoxin on a hDBB neuron. Chart recording showing the changes in membrane voltage induced by application of charybdotoxin and recovery on washout. B: effects of \( \omega \)-conotoxin GVIA on a hDBB neuron and neurotensin response. Chart recording showing the changes in membrane voltage induced by application of \( \omega \)-conotoxin GVIA. C: effects of neurotensin on a hDBB neuron. Chart recording showing the changes in membrane voltage induced by application of neurotensin and the recovery on washout. D: effects of the nonpeptide neurotensin receptor antagonist \( [2-\{(1-7\text{-chloro}-4\text{-quinolinyl})-5\text{-\(\theta\)-dimethoxyphenyl}\text{-pyrazol-3-yl}\text{-carbonylamino}\text{-tricyclo(3.3.1.1.)}\text{decan-2-carboxylic acid, (SR 48692)}] \) on neurotensin response in a hDBB neuron. Chart recording showing the changes in membrane voltage induced by application of 100 nM SR 48692 (after recovery from neurotensin effects); and subsequent application of neurotensin in the presence of SR 48692. Sharp upward deflection on the traces are the evoked membrane voltage responses to brief or sustained current injections. ± ± ± , resting membrane potential. Calibration bars on A refer to A only, and the ones on C apply to B±D.

tensin (100 nM) in the presence of the antagonist. Under these conditions, neurotensin failed to produce any effects either on the resting membrane potential (Fig. 7D) or on the action potential characteristics and the evoked firing rate (data not shown). The cell was allowed to recover for 15 min and reexposed to neurotensin. This resulted again in a typical neurotensin response similar to that described above. Similar observations were made on five other neurons which were neurotensin responsive. These data illustrate the antagonism of the response by the nonpeptide neurotensin receptor antagonist SR 48692, suggesting that neurotensin exerts its actions through a single kind of high-affinity SR-48692-sensitive receptors in these cells.

**DISCUSSION**

In the present study, we investigated the ionic mechanisms of action of neurotensin on acutely dissociated hDBB neurons. Neurotensin blocks currents through T- and N-type calcium channels and the charybdotoxin-sensitive voltage-dependent calcium-activated potassium channels. The effects observed on the membrane voltage, action potential waveform, and the excitability characteristics of the hDBB neurons on exposure to neurotensin were explicable with the changes observed in these conductances and were antagonized effectively by the nonpeptide neurotensin receptor antagonist, SR 48692. This is the first comprehensive report on the ionic basis of neurotensin actions on basal forebrain neurons.

There are two major types of neurons in DBB that are morphologically distinct. The larger ones are cholinergic and the smaller ones are GABAergic. We selected cells based on this criteria through visual inspection. This criteria seemed adequate to choose cholinergic cells because 84 of the 100 cells we recorded from responded to neurotensin. Here it is important to reiterate that neurotensin is taken up and internalized selectively by a receptor-mediated process by cholinergic cells of the basal forebrain neurons, which include the hDBB (Faure et al. 1992). Moreover fluoresceinylated neurotensin has been used as a marker to identify cholinergic neurons (Alonso et al. 1994). Therefore the cells that responded to neurotensin were presumed to be cholinergic.

**Neurotensin and potassium currents**

Neurotensin caused a decrease in outward currents in acutely dissociated DBB neurons in the voltage-activated currents in the voltage range from \(-30\) to \(+30\) mV by blocking the voltage-sensitive Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductances \( (I_{Ca}) \). The biophysical and pharmacological characterization of the currents underlying neurotensin response strongly support the blockade of \( I_{Ca} \) through its effects on \( I_{Ca} \) as a potential mechanism by which neurotensin excites DBB cholinergic cells. Blockade of Ca\textsuperscript{2+} influx by changing the external solution to the one containing 0 mM Ca\textsuperscript{2+} and 50 \( \mu\)M Cd\textsuperscript{2+} reduces outward currents in the voltage range from \(-30\) to \(+30\) mV and neurotensin failed to elicit any significant response in the presence of this external solution. Furthermore, CTX, a specific blocker of \( I_{Ca} \) channels, almost completely occluded the response to neurotensin. Also 10–50 \( \mu\)M TEA decreased the outward currents by approxi-
mately same amount as that by blocking Ca\(^{2+}\) influx or by CTX application. This shows the exquisite sensitivity of \(I_C\) channels to TEA in the hDBB neurons. Neurotensin (100 nM) blocks the same amount of current in these neurons, and 50 \(\mu\)M TEA also occluded the response. These observations suggest the commonality of the mechanism of action of these agents and indicate that neurotensin blocks or inhibits activation of \(I_C\) channels, thereby causing a decrease in outward currents.

Neurotensin has been shown to affect diverse conductances in different preparations. In the rat, neurotensin excites neurons through the suppression of inwardly rectifying K\(^+\) conductances (Audinat et al. 1989; Farkas et al. 1994; Jiang et al. 1994; Keegan et al. 1992), activation of nonselective cation-permeable channels (Farkas et al. 1994; Jiang et al. 1994; Kirkpatrick and Bourque 1995) and possibly attenuation of the afterhyperpolarization (AHP) (Audinat et al. 1989; Kirkpatrick and Bourque 1995). In our preparation, the resting input resistance was very high in whole cell clamped acutely dissociated hDBB neurons. There was no evidence for the presence of net voltage-activated currents in the voltage range from −60 to −140 mV because the \(I-V\) relationship in this range was almost flat (Fig. 1B). This means that there were no inwardly rectifying conductances present in these cells. Moreover no such conductances were observable even when the external K\(^+\) was raised to 25 mM. This further supports the absence of voltage-gated inwardly rectifying conductances in the acutely dissociated hDBB neurons. Furthermore the resting input conductance as measured from the slope of the \(I-V\) relationship in the voltage range from −60 to −110 mV was not significantly affected by neurotensin. Thus neurotensin does not seem to have obvious effects on inwardly rectifying conductances in the acutely dissociated neurons. This finding is not consistent with those of Audinat et al. (1989), Keegan et al. (1992), Farkas et al. (1994), and Jiang et al. (1994). The differences may be attributed to the fact that the studies cited above were performed on neurons in slice preparation of the different regions of the brain, whereas the present study was conducted on acutely dissociated hDBB neurons. It is possible that the inwardly rectifying conductances did not survive the enzymatic treatment and the mechanical dispersion required to obtain dissociated neurons. Alternatively these conductances might be expressed preferentially on the processes rather than on the neuronal soma in hDBB neurons.

The calcium influx during a long depolarization is usually sufficient to activate \(I_{AHP}\). However, we observed neither any apamin-sensitive currents during the ramp nor any effects of neurotensin on the tail-currents at −40 mV after depolarization to +30 mV (i.e., biophysically evoked \(I_{AHP}\)). This difference in our findings from those of others (Audinat et al. 1989; Kirkpatrick and Bourque 1995) may be explained by the differences in preparation as stated above. An additional reason for the absence of \(I_{AHP}\) might be the recording conditions as \(I_{AHP}\) may be lost with time due to washout of the intracellular components necessary for the survival and/or activation of \(I_{AHP}\) channels. \(I_{AHP}\) would be expected to survive longer under our recording conditions because we used potassium methylsulfate as the major intracellular anion. This agent has been suggested to preserve ion channel function and Ca\(^{2+}\) homeostasis in whole cell experiments and thus maintain ionic conductances such as \(I_{AHP}\) and \(I_C\) that are sensitive to intracellular Ca\(^{2+}\) (Zhang et al. 1994).

**Neurotensin inhibits calcium currents**

The magnocellular cholinergic basal forebrain neurons possess HVA and LVA calcium currents (Allen et al. 1993; Griffith et al. 1994; Margeta-Mitrovic 1997). As previously mentioned, we did not observe classical T-type currents. However a peculiar shift in the \(I_{in}\) \(I-V\) relationship in the voltage range negative to −20 mV and the selective voltage range in which 0.5 mM amiloride exerted its effects argue in favor of the presence of T-type channels in the hDBB neurons and its specificity as a blocker of these channels. Ni\(^{2+}\) (0.5 mM) on the other hand affected barium currents throughout the voltage range of −40 to +30 mV (data not shown). Thus we considered amiloride to be a more specific blocker of LVA than Ni\(^{2+}\).

The HVA barium currents consisted of a dihydropyridine-sensitive component (~35%), a ω-conotoxin-sensitive component (~43%), and another component that was resistant to both dihydropyridine and ω-conotoxin (~22%). Neurotensin-induced decrease in barium currents was blocked almost completely by ω-conotoxin GVIA but not by nimodipine. Thus neurotensin seems to selectively affect T- and N-type calcium channels and blocks currents partially. Neurotensin blocks not only T- and N-type but also L-type calcium channels in cultured newborn rat nucleus basalis neurons (Margeta-Mitrovic et al. 1997). Because neurotensin can block both LVA and HVA calcium channels, it can affect potentially not only the action potential characteristics but also the resting membrane potential through its effects on calcium-activated conductances over a wide voltage range. The neurotensin-induced decrease in \(I_C\) is comparable with that caused by complete blockade of calcium influx by replacing the external perfusing medium with the one containing 0 mM Ca\(^{2+}\) and 50 \(\mu\)M Cd\(^{2+}\). Thus a partial blockade of calcium channels seems to block \(I_C\) completely.

**Neurotensin augments excitability of neurons**

In a current-clamp study on neurons in the forebrain slice preparation, neurotensin has been shown to promote oscillatory bursting behavior as a result of depolarization and reduction in slow afterhyperpolarization (Alonso et al. 1994). There was also an enhancement in the amplitude and duration of the low-threshold spikes induced by neurotensin. However, the above study did not investigate the ionic bases of the neurotensin actions in detail.

\(I_C\) is responsible for repolarization phase of the action potential and also has a role in determining the amplitude and the decay of the first few milliseconds of the afterhyperpolarization (Adams and Galvan 1986; Belluzzi and Sacchi 1990, 1991; Lancaster and Pennefather 1987). A loss of \(I_C\) as a result of blockade of calcium conductances will result in slowing of repolarization leading to spike broadening and attenuation of afterhyperpolarization amplitude and duration. Thus the reduction in slow afterhyperpolarization and the increase in duration of low-threshold spikes can be explained by the pronounced decrease in \(I_C\) observed in our study. This decrease in \(I_C\) thus can culminate into an increase in
excitability, which might underlie the oscillatory bursting induced by neurotensin. The neurotensin-evoked decrease in \( I_c \) seems to be important even at the resting membrane potential as charybdoxin application resulted in a reversible depolarization that culminated into increased firing rate (see Fig. 7A). Given the high-input resistance of these cells at rest of close to 1 GΩ, even a relatively small inward current (20 pA; Fig. 1, C and D) would be enough to produce the degree of depolarization observed under current-clamp conditions (20 mV). Because \( I_c \) channels have been shown to be functionally colocalized with calcium channels, \( I_c \) channels can be activated readily by the influx of calcium ions (Robitaille et al. 1993). This colocalization makes it possible to record \( I_c \) even with high EGTA concentration in the intracellular solution. Assuming an average conductance of 100 pS for the \( I_c \) channels (Moczydlowski and Latorre 1983), one needs to block only 10 of these channels to produce a current of 22 pA ([K\(^+\)]\(_{in}\) = 2.5 mM, [K\(^+\)]\(_{out}\) = 140 mM, RPM = −80 mV, \( G_i = 1.1 \) nS), which will result in a depolarization of 20 mV. Prolonged depolarization by neurotensin and decrement in hyperpolarization during afterhyperpolarization can promote oscillatory bursting behavior when hDBB neurons are driven by excitatory inputs (Easaw et al. 1997b).

Neurotensin-induced partial blockade of calcium channels leaves >50% calcium channels still unblocked. The increased duration of spike may compensate for the decreased number of channels and maintain enough calcium influx to activate calcium-dependent processes. The integrated calcium influx during neurotensin-evoked sustained depolarization and/or increased firing rate can raise intracellular calcium levels well above the resting levels if the buffering capacity of the cell is overwhelmed. Activation of the calcium-dependent potassium conductances (\( I_c \) and \( I_{AMP} \)) by these higher calcium levels may be responsible for the hyperpolarized resting membrane potential observed on recovery (Figs. 6A and 7C) and account for the quiescent phase of the oscillatory behavior.

Neurotensin receptors in hDBB neurons

At least two different types of neurotensin receptors have been identified in the mammalian brain: high and low affinity (Le et al. 1996; Vincent 1995). The high-affinity sites, predominantly expressed in neurons, are sensitive to the non-peptide neurotensin receptor antagonist (SR 48692). The low-affinity sites are levocabastine sensitive and expressed preferentially on neuroglia (Noel et al. 1997). Neurotensin-induced changes in the resting membrane potential, action potential waveform, and excitability characteristics were blocked almost completely by SR 48692 at nanomolar concentrations. Specific reversible blockade of the neurotensin responses by this antagonist at nanomolar concentrations implies that the cholinergic hDBB neurons mainly express high-affinity neurotensin receptors.

Functional implications

A profuse peptidergic innervation has been demonstrated in the area where cholinergic cells have been immunohistochemically localized (Candy et al. 1985; Mai et al. 1987; Smith et al. 1985). The cholinergic neurons from this area project to cerebral cortex (Rye et al. 1984; Shute and Lewis 1967) and play an important role in cortical arousal by influencing sleep-waking cycle (Khatib et al. 1992). Through the septohippocampal pathways these neurons can evoke an atropine-sensitive excitation of hippocampal neurons (Cole and Nicoll 1983; Krnjevic and Ropert 1982; Nicoll 1985). Neurotensin microinjection into the region of the cholinergic basalis neurons produced a decrease in delta activity (1–3 Hz) and an increase in both high-frequency gamma activity (30–60 Hz) and rhythmic theta activity (4–8 Hz) in rats (Cape et al. 1996). This was associated with a behaviorally quiet, waking state that alternated with paradoxical sleep. Because blockade of \( I_c \) and thus \( I_c \) by neurotensin can affect resting membrane potential, action potential and excitability characteristics in hDBB neurons, it is a potential ionic mechanism that may underlie various physiological processes involving this area of the basal forebrain such as cortical arousal, sleep-wake cycle, and theta rhythm.

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Address for reprint requests: J. H. Jhamandas, Dept. of Medicine (Neurology), Division of Neurology, 2E3.17 Walter Mackenzie Centre, University of Alberta, Edmonton, Alberta T6G 2B7, Canada.

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