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NEUROTENSIN MODULATES SYNAPTIC TRANSMISSION IN THE NUCLEUS OF THE SOLITARY TRACT OF THE RAT

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Abstract—Whole-cell patch clamp recordings were made from neurons of the rat subpostremal region of the nucleus tractus solitarius (NTS) in transverse brainstem slices. Neurotensin (NT) enhanced the firing rate of action potentials from 0.8±0.4 Hz in control to 1.9±1.3 Hz (n=9) and increased their decay time. The peak amplitude of the after-hyperpolarization was decreased by 34±5% (n=9). These effects were associated with a depolarization of 4±1 mV (n=10) in the resting membrane potential and an increase in the input resistance (from 768±220 MΩ to 986±220 MΩ; n=5) and were compensated by manually hyperpolarizing the cell to control values. In voltage clamp experiments NT decreased an outward current (from 488±161 to 340±96 pA at +40 mV; n=5) which reversed near the potassium equilibrium potential. In addition, NT increased the frequency of both excitatory and inhibitory spontaneous synaptic currents, an effect blocked by tetrodotoxin, and did not change the evoked excitatory or inhibitory postsynaptic currents. The selective NTR1 receptor antagonist SR48692 reversibly blocked the effects of NT on both action potentials and spontaneous synaptic currents. Our results suggest that NTR1 receptors can modulate post-synaptic responses in neurons of the subpostremal NTS by increasing cell excitability as a result of blockade of a potassium conductance.

Key words: electrophysiology, slice, post-synaptic currents, excitability, depolarization.

The nucleus tractus solitarius (NTS) has a strategic position in relation to the brainstem neuronal networks due to the multiple modalities of visceral sensory afferents that reaches it (Loewy, 1990). This variety of peripheral neural afferents and its extensive central connections, make the NTS a site containing heterogeneity and a vast assortment of neurotransmitters/modulators and their receptors (Van Giersbergen et al., 1992). Although a large number of neuroactive substances seem to be involved in the neurohumoral integration within the NTS, *in vitro* electrophysiological recordings have suggested that glutamate and GABA are the principal neurotransmitters released in response to electrical stimulation of afferent sensory inputs in several NTS subdivisions (Mifflin and Felder, 1990; Brooks et al., 1992; Fortin and Champagnat, 1993; Andresen and Yang, 1995; Wang and Bradley, 1995; Smith et al., 1998). A considerable amount of experimental data suggests that the tridecapeptide neurotensin (NT) may play at least a modulator role in the processing of the viscerosensory information reaching the NTS (Kubo and Kihara, 1990; Ciriello and Zhang, 1997; Seagard et al., 2000). NT is a putative peptidergic neurotransmitter (Stowe and Nemeroff, 1991; Hermans and Maloteaux, 1998), first isolated and chemically characterized by Carraway and Leeman (1973) from hypothalamic extracts. NT receptors are present in neurons intrinsic to the NTS and in those sending projections to other CNS nuclei (Riche et al., 1990; Wang et al., 1992). Microinjections of NT intracerebroventricularly or directly into the NTS lead to a cardiovascular response involving components of the baroreflex (Rioux et al., 1981; Quirion et al., 1981; Sumners et al., 1981; Shido and Nagasaka, 1985; Kubo and Kihara, 1990; Ciriello and Zhang, 1997; Seagard et al., 2000).

In this study we examine the electrophysiological consequences of activating NT receptors in neurons of the subpostremal NTS, in transverse brainstem slices using the whole cell patch clamp technique. We have found that NT leads to a depolarization of the neurons, by suppressing a potassium conductance, thereby increasing excitability of the system.

EXPERIMENTAL PROCEDURES

Brain slices

Brainsteim slices were prepared from 20 to 25 days-old Wistar rats of either sex. Following decapitation and craniotomy, the brain and upper cervical spinal cord were removed and submerged in ice-cold (2–3 °C) artificial cerebrospinal fluid (ACSF) pH 7.35–7.4, equilibrated with 95% O_2–5% CO_2 (carbogen). The ACSF contained (mM): 120 NaCl; 2.5 KCl; 1.0 $MgCl_2$; 2.0 $CaCl_2$; 25 $NaHCO_3$; 1.25 NaH_2PO_4; 25 glucose and the osmolality was 305–310 mOsm/kg H_2O. After the brain stem (approximately 8 mm) was dissected, it was glued with cyanoacrylate glue, through its ventral surface, to the vertical arm of an L-shaped agar block (4% agar in ACSF) and taken to the stage of a Vibratome (MA756; Campden Instruments, Leicester, UK). Routinely, two transverse slices (300 μm thick) containing the subpostremal NTS were obtained from each animal. After cutting, the slices were incubated for 60 min at 32 °C with ACSF constantly gassed with carbogen. A single slice was transferred to the recording chamber (0.4 ml) on the stage of an upright microscope (E600; Nikon Inc.,

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Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, after-hyperpolarization; AP5, D(−)-2-amino-5-phosphonopentanoic acid; DNQX, 6,7-dinitroquinolixaline-2,3 dione; NMDA, *N*-methyl-D-aspartate acid; NT, neurotensin; NTS, nucleus tractus solitarius; NTS1, high affinity neurotensin receptor; sEPSC, spontaneous excitatory postsynaptic currents; sIPSCs, spontaneous inhibitory postsynaptic currents; SR49682, non-peptide antagonist of the high affinity neurotensin receptor; ST, solitary tract; TTX, tetrodotoxin.

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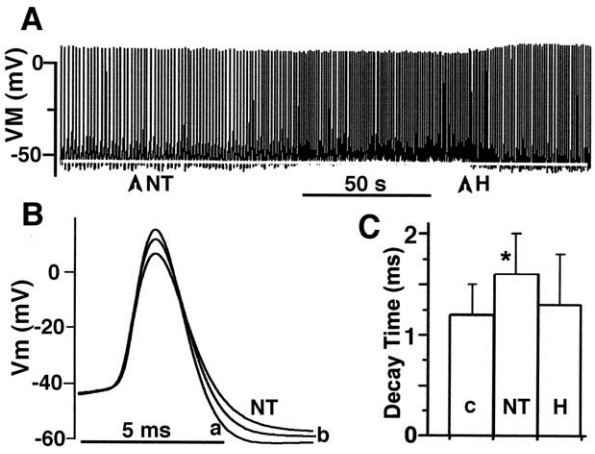

Fig. 1. NT increases the frequency of firing of action potentials. (A) NT (500 nM, arrowhead marked NT) leads to a depolarization of the cell and to an increase in the frequency of action potentials discharge. At the arrowhead marked H hyperpolarizing current was injected until the membrane potential returned to approximately the same level as in the control situation. (B) Averaged action potentials, aligned by their peak, under control (trace a; 116 events), after 500 nM NT (trace NT; 86 events) and after hyperpolarizing the cell (trace b; 106 events) superimposed on time (same cell as in A). In the presence of NT the decay time is slower and the hyperpolarizing after potential amplitude is smaller than in the control situation. Hyperpolarizing the cell returns the system to near control conditions. (C) Shows the average of the decay times ($n=9$), in the conditions described above. NT increased the decay time from 1.2 ± 0.4 ms to 1.5 ± 0.4 ms. * Indicates statistically significant difference in relation to control. The decay time was measured as the time between 10 and 90% of the peak value. Action potentials were recorded with a patch pipette filled with potassium gluconate.

Tokyo, Japan), held in place with a nylon net mounted on a platinum wire, and continuously superfused with ACSF (saturated with carbogen) at a rate of 2–3 ml/min, driven by gravity. All drugs were added to the perfusion solution at known concentrations and applied for the times indicated under the particular experiment. Experiments were performed at room temperature (23–25 °C). NT and tetrodotoxin (TTX) were purchased from Sigma Chemical Co (St. Louis, MO, USA), AP5 (D(−)-2-amino-5-phosphonopentanoic acid), DNQX (6,7-dinitroquinolixaline-2,3 dione) and bicuculline methochloride were from Tocris Cookson Inc. (Ellisville, MO, USA). SR48692 (batch no. 97–01292), was a generous gift of Sanofi Recherche (Toulouse, France). All other salts were reagent grade from Sigma. Efforts were made to minimize the number of animals used and their suffering in accordance with the Ethical Guidelines for the Use of Laboratory Animals of the Faculty of Medicine of Ribeirão Preto and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Electrophysiology

Patch pipettes were pulled from borosilicate glass tubing (Sutter Instrument Co., Novato, CA, USA) on a P-97 puller (Sutter Instrument Co.) and had the tip fire polished on a microforge (Narishige MF-83, Tokyo, Japan). The internal solutions were (mM): 130 CsF; 10 NaCl; 2 $MgCl_2$; 3 K-ATP; 0.2 Na-GTP; 10 EGTA; 10 HEPES; pH adjusted to 7.3 with CsOH and osmolality of 295–305 mOsm/kg H_2O or 140 potassium gluconate; 10 NaCl; 2 $MgCl_2$; 3 K-ATP; 0.2 NA-GTP; 10 EGTA; 10 HEPES; pH 7.3 adjusted with KOH and osmolality 295–305 mOsm/kg H_2O, as indicated under the particular experiments. The indifferent electrode was a Ag/AgCl wire connected to the extracellular solution via an agar bridge (2.5% in the internal solutions). When filled with the above solutions the pipettes had resistance between 4 and 8 MΩ. Junction potentials were calculated using the Axoscope 1.0 program and the results corrected accordingly. Cells were approached by the 'blind patch' method and seal resistances in excess of 2–5 GΩ were obtained prior to entering the whole-cell configuration. Access resistances averaged 13.4 ± 7.1 MΩ ($n=40$) and were corrected between 70 and 80%. Cell capacitance, evaluated from settings of the capacitance cancellation circuitry of the amplifier, was in the range of 7–15 pF. Recordings were made with an EPC-7 (List Medical, Darmstadt, Germany) patch clamp amplifier. Whole-cell currents and voltages were low-pass filtered at 3 kHz (eight pole Bessel filter, LPF8; Warner Instruments Corp., Hamden, USA) digitized at 10 kHz (except where indicated) by a computer driven A/D converter (Digidata 1200B; Axon Instruments, Foster City, USA), and stored on hard disk using the pClamp6 software (Axon Instruments). Data were analyzed off-line using the MiniAnalysis program (Synaptosoft, NJ, USA), Clampfit or Axoscope (Axon Instruments). Synaptic responses of the NTS neurons were evoked by electrical stimulation (7–15 V, 50–100 μs, 0.2–0.5 Hz, stimulus isolation unit-DS2A; Digitimer Ltd., Garden City, UK) of the ipsilateral solitary tract (ST) through a twisted bipolar platinum electrode with 70 μm diameter. The pooled data are expressed as the mean±S.D. and statistical significance of differences between values ($P<0.05$) were determined by the Student's *t*-test.

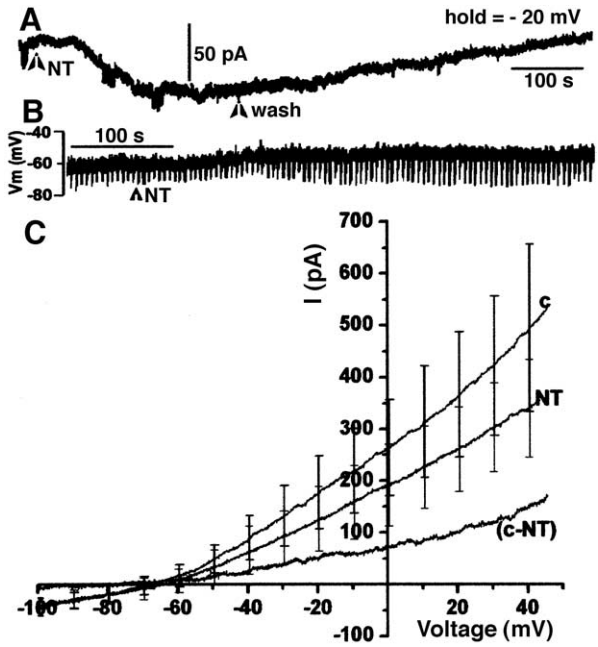

Fig. 2. NT blocks an outward ionic current. (A) A trace were the cell was clamped at −20 mV and NT applied at the indicated time. An inward current is clearly seen. Washing out NT (arrowhead marked wash) slowly brings the holding current to its control level. (B) A recording of the resting membrane potential in another cell. NT (500 nM, arrowhead) not only depolarizes the cell but also increases the input resistance (from 768 ± 220–986 ± 240 MΩ, $n=5$). Downward deflections are voltage responses to hyperpolarizing current pulses (15 pA; 50 ms). (C) Averaged I–V curves ($n=5$) in control conditions (c), in the presence of 500 nM NT (NT) and the NT blocked portion of the current, obtained by subtracting trace NT from trace c. All recordings were made in the continuous presence of 1 μM TTX. The pipettes were filled with a potassium gluconate solution. Records A and B were filtered at 1 kHz and sampled at 0.5 kHz. IxV curves were constructed by applying voltage ramps (−100 to +50 mV, 400 ms) to the cell. Current responses were filtered at 2 kHz and sampled at 10 kHz.