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Cardio-respiratory effects of systemic neurotensin injection are mediated through activation of neurotensin NTS_1 receptors

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ABSTRACT

The purpose of our study was to determine the cardio-respiratory pattern exerted by the systemic injection of neurotensin, contribution of neurotensin NTS_1 receptors and the neural pathways mediating the responses. The effects of an intravenous injection (i.v.) of neurotensin were investigated in anaesthetized, spontaneously breathing rats in following experimental schemes: (i) control animals before and after midcervical vagotomy; (ii) in three separate subgroups of rats: neurally intact, vagotomized at supranodosal level and initially midcervically vagotomized exposed to section of the carotid sinus nerves (CSNs); (iii) in the intact rats 2 minutes after blockade of neurotensin NTS_1 receptors with SR 142948. Intravenous injection of 10 μg/kg of neurotensin in the intact rats evoked prompt increase in the respiratory rate followed by a prolonged slowing down coupled with augmented tidal volume. Midcervical vagotomy precluded the effects of neurotensin on the frequency of breathing, while CSNs section reduced the increase in tidal volume. In all the neural states neurotensin caused significant fall in mean arterial blood pressure preceded by prompt hypertensive response. The cardio-respiratory effects of neurotensin were blocked by pre-treatment with NTS_1 receptor antagonist. The results of this study showed that neurotensin acting through NTS_1 receptors augments the tidal component of the breathing pattern in a large portion via carotid body afferentation whereas the respiratory timing response to neurotensin depends entirely on the intact midcervical vagi. Blood pressure effects evoked by an intravenous neurotensin occur outside vagal and CSNs pathways and might result from activation of the peripheral vascular NTS_1 receptors.

1. Introduction

Neurotensin, 13-amino-acid peptide, acts in the mammalian brain as a primary neurotransmitter or neuromodulator of classical neurotransmitters (Ferraro et al., 2008). This tridecapeptide contributes to a variety of physiological functions and pathologic processes. It modulates dopamine signaling in the central nervous system (CNS), is involved in an opioid-independent antinociception, hypothermia, muscle relaxation, and pituitary hormone secretion (Boules et al., 2006; Mustain et al., 2011). The effects of neurotensin are mediated mainly via the activation of two G protein-coupled receptor subtypes: NTS_1 and NTS_2, while the physiological role of the third neurotensin receptor, NTS_3, remains undefined (Boules et al., 2006; Mazella and Vincent, 2006).

Neurotensin-immunoreactive cell bodies, fibers and nerve terminals have been found in the brainstem areas involved in the control of respiration, i.e. nucleus tractus solitarii, nucleus ambiguous, nucleus parabrachialis lateralis, and nucleus of Kolliker-Fuse (Uhl et al., 1979; Jennes et al., 1982).

Several experimental data suggested that neurotensin may be involved in the central pathways controlling respiration. Tridecapeptide neurotensin injected into cerebral ventricles (i.c.v.) of rats provoked hypotension and respiratory depression in a dose-dependent manner, leading occasionally to apnea (Pazos et al., 1984). In cats, local application of this neuropeptide on ventrolateral nucleus tractus solitarii triggered off an apneustic pattern of breathing recorded in phrenic motor output (Morin-Surun et al., 1986).

At the periphery neurotensin was shown to be localized within the lungs (Wood et al., 1981; Robbins et al., 1995; Moody, 2006) and neurotensin receptors were identified in the rat bronchi (Aas and Helle, 1982). Martin et al. (1994) showed that neurotensin exerts an inhibitory action on neurotransmission in guinea-pig airways. Moreover, there exists a direct evidence of NTS1 protein expression in the human and rat carotid bodies (Porzionato et al., 2009).

While no research on the respiratory effects of systemic administration of neurotensin in experimental animals has ever

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been conducted, there were reports on hypotension induced by the neuropeptide in guinea-pigs and rats (Rioux et al., 1989; Oishi et al., 1981; Gully et al., 1996). Therefore the potential role of neurotensin and the contribution of reflex pathways needs to be explained. In view of the fact that drugs targeting neurotensin signaling are being considered in the therapies of schizophrenia, chronic pain and cancer such knowledge should be valid (Tyler-McMahon et al., 2000; Moody, 2006; Mustain et al., 2011). Si RNA techniques to reduce neurotensin NTS_2 receptor expression were successfully applied in the pain research experiments, as well (Dore-Savard et al., 2008).

In the present study we are the first to determine the cardio-respiratory pattern exerted by intravenous (i.v.) neurotensin injection. The present experiments were conducted to determine potential neural pathways: vagus nerves, nodose ganglia and carotid chemoreceptor afferents that mediate neurotensin-induced cardio-respiratory responses. Finally the involvement of neurotensin NTS_1 receptor was assessed.

2. Materials and methods

2.1. Animals and surgical procedures

Ethical approval for the experimental procedures used in this study was obtained from the local institutional review committee. All animal procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Adult male Wistar rats weighing 180–220 g were anesthetized with an intraperitoneal (i.p.) injection of 600 mg/kg urethane (Sigma) and 120 mg/kg alpha-chloralose (Fluka AG). The level of anesthesia was assessed by monitoring any responsiveness of the blood pressure to noxious pinch of the hind paw; supplemental doses of urethane and chloralose were administered i.v. as required. The animals were placed in the supine position and they breathed spontaneously room air. The trachea was exposed in the neck, sectioned below the larynx and cannulated. Catheters were inserted into the femoral vein for drug administration and supplemental doses of anesthetic, and to the femoral artery for blood pressure monitoring and blood-gas sampling. Rectal temperature was maintained close to 37–38 °C by a heating pad.

The midcervical vagi were bluntly dissected and prepared for midcervical bilateral vagotomy prior to measuring the studied variables in neurally intact rats. In the second experimental paradigm, the rostral parts of the midcervical vagi were separated from the superior cervical ganglia. The nodose ganglia were bluntly dissected from the surrounding tissue; their blood supply was preserved intact. The supranodose vagi were transected 2 mm distal to the rostral poles of the ganglia. In another group of initially midcervically vagotomized rats both carotid sinus/body nerves were identified under an operating microscope, freed from the surrounding tissue, and prepared for a bilateral section at their junction with the glossopharyngeal nerves later during the experiment. The carotid denervation was confirmed by the absence of any response to i.v. injection of 50 μg of NaCN (sodium cyanide), which dose elicits a brisk respiratory response in rats with intact carotid sinus nerves (CSNs).

2.2. Apparatus and measurements

Tidal volume signals were recorded with a pneumotachograph head attached to the tracheal cannula linked to a Research Pneumotach System (RSS 100h, Hans Rudolph Inc., Kansas City, USA) and a computerized recording system (Windows software version 3.07.02, KORR Medical Technologies Inc., Salt Lake City, USA) for measuring and recording respiratory frequency (f), tidal volume (V_T), respiratory minute volume (V_E), inspiratory (T_I) and expiratory (T_E) times. The electromyogram of the costal diaphragm was recorded with bipolar electrodes, amplified (NL 104, Digitimer), filtered and measured with a model AS 101 (Asbit) leaky integrator (time constant = 100 ms). Arterial blood pressure and heart rate were measured with a BP-2 blood pressure monitor (Columbus Instruments, OH, USA). The recordings were registered on an Omnilight 8 M 36 apparatus (Honeywell, Tokyo, Japan).

Blood gases (PaO_2 and $PaCO_2$) were measured in the blood samples of intact animals before and ten minutes after neurotensin injection with VetStat Electrolyte and Blood-Gas Analyzer (IDEXX Laboratories, Warsaw, Poland).

2.3. Drugs

The drugs were prepared freshly from powder prior to each experiment. Neurotensin (Tocris Bioscience, Bristol UK) was dissolved in isotonic saline (0.9% w/v aqueous sodium chloride) and injected at a dose of 10 μg/kg as a bolus to the femoral vein. The dose of the drug used in our study was obtained from our preliminary dose-response experiments (Table 1), which showed that this dose caused maximum and uniform changes in tidal volume, respiratory rate and blood pressure in neurally intact rats (n=15). To the best of our knowledge selective antagonist for NTS_2 receptor has not been synthesized, as yet. SR 142948 (2-[[[5-(2,6-Dimethoxyphenyl)-1-[4-[[[3-(dimethylamino)propyl]methylamino]carbonyl]-2-(1-methylethyl)phenyl]-1*H*-pyrazol-3-yl]carbonyl]amino]-tricyclo[3.3.1.13,7]-decane-2-carboxylic acid), (Tocris Bioscience, Bristol UK)-neurotensin NTS_1 receptor antagonist was dissolved in physiological saline and injected i.v. at a dose of 100 and 500 μg/kg. The optimal doses of the antagonists were derived from preliminary experiments (data not shown). Each drug bolus was injected in volumes ranging from 0.18 to 0.22 ml and was immediately flushed with a 0.2 ml aliquots of saline.

Table 1
Dose dependent effect of neurotensin on tidal volume (V_T), frequency of breathing (f) and mean arterial blood pressure. Maximum post-neurotensin change in cardio-respiratory parameters expressed as means and as a percentage of increase in V_T and decrease in f and blood pressure from the basal means.

Dose μg/kg	V_T (ml)			f (breaths/min)			Blood pressure (mmHg)		
	Control	Max change	Increase in V_T (%)	Control	Max change	Decrease in f (%)	Control	Max change	Decrease in blood pressure (%)
1	1.88 ± 0.84	2.03 ± 0.88 [a]	7.9	66.0 ± 14	62.7 ± 14	5.03	89.2 ± 20	66.9 ± 20	24.9
5	1.63 ± 0.05	1.91 ± 0.15 [b]	17.1	73.5 ± 7.2	62.2 ± 7.4 [c]	15.33	90.6 ± 12	53.5 ± 6.0 [b]	40.9
10	1.54 ± 0.10	1.91 ± 0.13 [b]	24	76.2 ± 9.0	54.7 ± 6.5[b]	28.1	95.9 ± 11	47.9 ± 5.9[b]	49.9

Note: All values are means ± S.D., (n=15; 5 rats per each dose).

[a] $P<0.05$.

[b] $P<0.01$.

[c] $P<0.001$, compared to the respective control value.