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Neuromedin U causes biphasic cardiovascular effects and impairs baroreflex function in rostral ventrolateral medulla of spontaneously hypertensive rat

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ABSTRACT

Neuromedin U (NMU) causes biphasic cardiovascular and sympathetic responses and attenuates adaptive reflexes in the rostral ventrolateral medulla (RVLM) and spinal cord in normotensive animal. However, the role of NMU in the pathogenesis of hypertension is unknown. The effect of NMU on baseline cardiorespiratory variables in the RVLM and spinal cord were investigated in urethane-anaesthetized, vagotomized and artificially ventilated male spontaneously hypertensive rats (SHR) and Wistar–Kyoto rats (WKY). Experiments were also conducted to determine the effects of NMU on somatosympathetic and baroreceptor reflexes in the RVLM of SHR and WKY. NMU injected into the RVLM and spinal cord elicited biphasic response, a brief pressor and sympathoexcitatory response followed by a prolonged depressor and sympathoinhibitory response in both hypertensive and normotensive rat models. The pressor, sympathoexcitatory and sympathoinhibitory responses evoked by NMU were exaggerated in SHR. Phrenic nerve amplitude was also increased following intrathecal or microinjection of NMU into the RVLM of both strains. NMU injection into the RVLM attenuated the somatosympathetic reflex in both SHR and WKY. Baroreflex sensitivity was impaired in SHR at baseline and further impaired following NMU injection into the RVLM. NMU did not affect baroreflex activity in WKY. The present study provides functional evidence that NMU can have an important effect on the cardiovascular and reflex responses that are integrated in the RVLM and spinal cord. A role for NMU in the development and maintenance of essential hypertension remains to be determined.

1. Introduction

Neuromedin U (NMU) was originally purified from porcine spinal cord and named because of its potent contractile activity on uterine smooth muscle [19]. NMU is implicated not only in uterine smooth muscle contraction, but also in other functions such as feeding, blood pressure (BP) regulation and regional blood flow, ion transport in the gut, adrenocortical function, anxiety-related behavior, nociception, inflammation, bone-remodeling and, more recently even in cancer, suggesting differential physiological and pharmacological roles of NMU (reviewed in [20]). NMU acts at two GPCRs ($G_{q/11}$ and/or $G_{i/0}$), NMU receptor 1 (NMU1) and NMU receptor 2 (NMU2) [1]. NMU1 is predominantly expressed in peripheral tissues while NMU2 in the central nervous system [12,31,43,48].

The presence of NMU immunoreactive neurons in key cardiovascular areas of the brainstem and spinal cord including the nucleus tractus solitarius (NTS), dorsal motor nucleus of the vagus, inferior olive and area postrema [12], raising the possibility that NMU may participate in the central control of cardiovascular function. NMU increases sympathetic nerve activity (SNA), BP, heart rate (HR) and plasma noradrenaline when given by intracerebroventricular injection [5,44]. Microinjection of NMU into the NTS decreases BP and HR [45]. Recently we demonstrated that NMU delivered intrathecally or microinjected into the RVLM, shows biphasic effects on sympathetic vasomotor tone and differentially modulates sympathetic reflexes [33,34]. However, whether the NMU system in the RVLM and/or in the spinal cord contributes to the pathogenesis of hypertension is unknown. These data, along with those concerning the role of NMU in the modulation of

Abbreviations: AUC, area under the curve; BP, blood pressure; HR, heart rate; i.t., intrathecal; MAP, mean arterial pressure; NMU, neuromedin U; NMU1, neuromedin U receptor 1; NMU2, neuromedin U receptor 2; NTS, nucleus tractus solitarius; PBS, phosphate buffered saline; PE, phenylephrine hydrochloride; PNA, phrenic nerve activity; PNamp, phrenic nerve amplitude; PNf, phrenic nerve frequency; RVLM, rostral ventrolateral medulla; SD, Sprague-Dawley rats; SHR, spontaneously hypertensive rats; SPN, sympathetic preganglionic neurons; sSNA, splanchnic sympathetic nerve activity; WKY, Wistar–Kyoto rats.

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cardiovascular function and sympathetic reflexes in normotensive rats, led us to hypothesize that altered NMU activity in the RVLM and spinal cord contribute to the pathogenesis of hypertension in SHR. The objectives of the present study were 1) to investigate the cardiovascular and respiratory effects of NMU following microinjection into the RVLM, and intrathecal (i.t.) injection at the T5–T7 level of thoracic spinal cord in SHR and normotensive Wistar–Kyoto rats (WKY), and 2) to evaluate the effects of NMU on somatosympathetic, and baroreceptor reflexes in the RVLM.

2. Materials and methods

All procedures in the present study were approved by the Animal Ethics Committee of Macquarie University, Sydney, Australia and conducted in accordance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (http://www.nhmrc.gov.au/guidelines/publications/ea16).

2.1. General procedures

Experiments were performed in 18–20 weeks, age-matched adult male SHR (310–375 g) and WKY (355–385 g). Rats were anaesthetized with an initial dose (1.2–1.4 g kg^{-1}, i.p.) of urethane. Supplemental doses of urethane (20–30 mg, i.v.) were given if BP rose more than 10 mmHg in response to hind paw pinch at every 30 min. Body temperature was monitored and maintained at $37 \pm 0.5\,^{\circ}C$ by placing rats on a feedback-controlled heating blanket for the duration of the experiment (Harvard Apparatus, Holliston, MA, USA).

The left carotid artery and right jugular vein were cannulated for measurement of BP and regular injections of drugs and fluids, respectively. In 5 animals, the left femoral vein was cannulated for administration of phenylephrine hydrochloride (PE). HR was derived from the arterial BP. The trachea was cannulated to permit artificial ventilation and end-tidal CO_2 monitoring. Nerve recordings were obtained from the splanchnic and phrenic nerves. The left greater splanchnic nerve was isolated and dissected through a retroperitoneal approach. The phrenic nerve was accessed from a dorsolateral approach after retraction of the left shoulder blade. The distal end of the nerves were tied with 5/0 silk thread and cut to permit recording of efferent nerve activity. In 5 experiments, the sciatic nerve was isolated, tied and cut. Once the nerves were isolated, they were covered with saline soaked cotton wool for the duration of the remainder of surgical preparation to prevent desiccation.

Animals were secured in a stereotaxic frame and the head tilted downwards at a 45° angle to the horizontal. All animals were paralyzed (pancuronium bromide; 0.8 mg i.v. initially, then 0.4 mg h^{-1}, i.v.), artificially ventilated with oxygen-enriched room air and bilaterally vagotomized to prevent entrainment of the phrenic nerve discharge to the ventilator. End-tidal CO_2 and pH were maintained at 4.0–4.5% and 7.35–7.45, respectively, by adjusting the rate and depth of ventilation after arterial blood gas analysis (pH 7.4 ± 0.05, $PaCO_2$ 40.4 ± 0.6). Animals were infused with 5% glucose in water (1.0–2.0 ml h^{-1}) to ensure hydration. Nerve recordings were made with bipolar silver wire electrodes. The recording electrodes were immersed in a pool of liquid paraffin oil to prevent dehydration and for electrical insulation. After nerves were placed on the recording electrodes, rats were allowed to stabilize for 30–60 min. The neurograms were amplified (10,000×; CWE Inc., Ardmore, PA, USA), band pass filtered (0.1–2 kHz), sampled at 3 kHz (1401 plus, CED Ltd., Cambridge, UK) and recorded on computer using Spike2 software (v7, CED Ltd., Cambridge, UK).

2.2. RVLM microinjections

To enable microinjection into the RVLM, the dorsal surface of the medulla was exposed by partial occipital craniotomy. The dura was cut and reflected laterally. The brainstem was kept moist using phosphate buffered saline (PBS) soaked cotton wool until the experiment protocol began. Brain microinjection, and functional identification of the RVLM, was performed as described previously [32,38]. Briefly, bilateral microinjection of test agents into the RVLM, at a fixed volume of 50 nl, was carried out stereotaxically and sequentially with single barrel glass pipettes over a 10-s period. At the beginning of each experiment, RVLM was identified functionally on either side by an increase of >30 mmHg in MAP following microinjection of L-glutamic acid (5 nmol). The preliminary coordinates used to find the RVLM were 1.8 mm rostral, 1.8 mm lateral, and 3.5 mm ventral to calamus scriptorius. Vehicle solutions, PBS contained 2% rhodamine beads to aid in subsequent histological verification of the injection site. Following euthanasia (3 M KCl, 0.5 ml, i.v.) the brain was removed from the skulls, fixed in 4% paraformaldehyde and sectioned at 100 μm to verify the microinjection sites. Only rats with microinjection sites within the boundaries of the RVLM were used for data analysis.

2.3. I.t. drug administration

I.t. drug injection was performed as described previously [34,37]. Briefly, an i.t. catheter was inserted into the sub-arachnoid space and advanced caudally to the levels of T5–T7 following exposure of the occipital crest and incision of the atlanto-occipital membrane. The patency of the catheter was examined immediately after insertion by withdrawal of cerebrospinal fluid. The slit was left open to prevent increase in i.t. pressure caused by the injection of agents or by flushing. Drug (NMU; 2 mM) and vehicle (10 mM PBS; pH 7.4) were administered i.t. in a total volume of 10 μl using a 25-μl Hamilton syringe. The volume of each catheter was measured before insertion (range 6–8 μl) into i.t. space and this volume was then used to flush the catheter. Injections were made over a 15- to 20-s period. Successful catheterization was confirmed by injecting L-glutamic acid (100 mM, 10 μl). Sharp increases in BP (~20 mmHg), HR (~30 bpm) and sSNA (~30%) indicated a successful catheterization [e.g. 11, 34]. At the end of experiment the location of the injection site was marked by an injection of 10 μl of India ink flushed with PBS. Following euthanasia (3 M KCl, 0.5 ml, i.v.) a laminectomy was performed to verify the location of the cannula tip.

2.4. Sympathetic reflex activation

Baroreflexes were evoked as described previously [6,34]. The sciatic nerve (somatosympathetic reflex) was stimulated intermittently (0.2 ms pulse width; 50 pulses, 1 Hz) at a voltage that was sufficient to generate 2 distinct peaks in the rectified, averaged sSNA trace over the stimulus period (6–18 V) before and after microinjection of NMU. Baroreceptors were activated by intravenous injection of PE (0.1 mg kg^{-1}) before and after microinjection of NMU.

2.5. Experimental protocol

To investigate the effects of NMU on cardiovascular and respiratory responses in the RVLM, NMU (2 mM equivalent to 100 pmol per side, 50 nl) was injected into the RVLM. The dose of NMU was chosen according to dose response curves done in previous experiments [33,34]. PBS was microinjected as a volume and vehicle control 30 min after the completion of glutamate application. This time lag was adopted to ensure complete recovery from the glutamate-induced pressor response before bilateral microinjection into the RVLM of vehicle. Another gap of 30 min was given before injection of NMU. In another set of animals, the somatosympathetic and arterial baroreflexes were evoked before and after