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TARGETED DELETION OF NEUROKININ-1 RECEPTOR EXPRESSING NUCLEUS TRACTUS SOLITARII NEURONS PRECLUDES SOMATOSENSORY DEPRESSION OF ARTERIAL BARORECEPTOR–HEART RATE REFLEX

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Abstract—Neurokinin-1 receptor (NK1-R) expressing neurons are densely distributed throughout the nucleus tractus solitarii (NTS). However, their fundamental role in arterial baroreflex function remains debated. Previously, our group has shown that activation of contraction-sensitive somatic afferents evoke substance P (SP) release in the NTS and resets the arterial baroreflex via activation of a GABAergic NTS circuit. Based on these findings, we hypothesized that modulation of arterial baroreflex function by somatic afferents is mediated by NK1-R dependent inhibition of barosensitive NTS circuits. In the present study, SP-conjugated saporin toxin (SP-SAP) was used to ablate NK1-R expressing NTS neurons. Contraction-sensitive somatic afferents were activated by electrically-evoked muscle contraction and the arterial baroreceptor–heart rate reflex was assessed by constructing reflex curves using a decerebrate, arterially-perfused preparation. Baseline baroreflex sensitivity was significantly attenuated in SP-SAP-treated rats compared with control rats receiving either unconjugated SAP or vehicle. Muscle contraction significantly attenuated baroslope in SAP and vehicle-treated animals and shifted the baroreflex curves to higher systemic pressure. In contrast, somatic afferent stimulation failed to alter baroslope or shift the baroreflex curves in SP-SAP-treated animals. Moreover, when reflex sensitivity was partially restored in SP-SAP animals, somatic stimulation failed to attenuate baroreflex bradycardia. In contrast, SP-SAP and somatic stimulation failed to blunt the reflex bradycardia evoked by the peripheral chemoreflex. Immunohistochemistry revealed that pretreatment with SP-SAP significantly reduced the number of NK1-R expressing neurons in the caudal NTS, while sparing NK1-R expressing neurons rostral to the injection site. This was accompanied by a significant reduction in the number of glutamic acid decarboxylase (GAD67) expressing neurons at equivalent levels of the NTS. These findings indicate that immunolesioning of NK1-R expressing NTS neurons selectively abolishes the depressive effect of somatosensory input on arterial baroreceptor–heart rate reflex function.

Key words: nucleus tractus solitarii, neurokinin receptors, substance P, GABA, arterial baroreflex, exercise.

The nucleus tractus solitarii (NTS) receives afferents from visceral, somatic and cardiopulmonary structures. Recently, considerable attention has been placed on the functional role of intrinsic excitatory and inhibitory NTS interneurons in the processing of primary baroreceptor afferent inputs (Andresen et al., 2004; Chen and Bonham, 2005; Kawai and Senba, 1996; Paton, 1999). However, the precise role for NTS neurons in modulating arterial baroreflex function during physiological perturbations, such as exercise, remains unresolved.

Over a decade ago, we showed that the operational properties of the carotid baroreflex were reset during volitionally exercise in humans, while retaining overall reflex sensitivity (Potts et al., 1993). Subsequently, these findings have been confirmed in humans and animals by many other laboratories (Boscan et al., 2002; Burger et al., 1998; Fadel et al., 2001; McIlveen et al., 2001; Norton et al., 1999; Papelier et al., 1994; Raven et al., 1997). In addition, neural feedback from skeletal muscle has been shown to reset the arterial baroreflex in a manner similar to the changes that occur during exercise (Carrington et al., 2003; Gallagher et al., 2001; Ichinose et al., 2002; Iellamo et al., 1997; McIlveen et al., 2001; McWilliam et al., 1991; Potts, 2002; Potts et al., 1998; Potts and Li, 1998; Potts and Mitchell, 1998). Collectively, these findings suggest that an alteration in the central neurotransmission of arterial baroreceptor afferent signals may be involved in baroreflex resetting during exercise. Although the precise mechanism(s) mediating this process remains unknown, there are several lines of evidence suggesting that GABAergic and tachykininergic mechanisms may be involved. First, it has been shown that substance P (SP) influences the activity of cardiovascular and respiratory circuits within the medulla (Helke and Seagard, 2004; McKay et al., 2005; Nattie and Li, 2002; Riley et al., 2002; Wang et al., 2001). SP exerts its effect by acting on a diverse class of neurokinin receptors (NK1, NK2 and NK3)

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Abbreviations: BIC, bicuculline; CED, Cambridge Electronic Design; GAD67, glutamic acid decarboxylase; HR, heart rate; NA, nucleus ambiguus; NaCN, sodium cyanide; NK, neurokinin receptor; NTS, nucleus tractus solitarii; PBS, phosphate-buffered saline; PND, phrenic nerve discharge; PP, perfusion pressure; SAP, saporin; SP, substance P; X, dorsal motor nucleus of the vagus; XII, hypoglossal motor nucleus.

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that share 65% amino acid homology (Mantyh, 2002; Regoli et al., 1994). Medullary regions, including the NTS, nucleus ambiguus (NA), hypoglossal motor nucleus (XII) and the ventral respiratory group (VRG), express high levels of NK1-R (Dixon et al., 1998; Guyenet et al., 2002; Lawrence and Jarrott, 1996; Wang et al., 2003).

Previous studies have reported that stimulation of somatic and arterial baroreceptor afferents release SP in the NTS (Potts and Fuchs, 2001; Potts et al., 1999; Williams and Fowler, 1997; Williams et al., 2002) and that neural feedback from skeletal muscle depresses arterial baroreceptor signaling, in part, through activation of a local GABAergic NTS circuit (Boscan et al., 2002; Potts et al., 2003). Since depression of the arterial baroreflex can be prevented by pharmacological blockade of NK1-R in the NTS (Boscan et al., 2002), we propose that somatosensory input blunts baroreflex function by targeting a population of NK1-R expressing GABAergic neurons in the NTS.

The purpose of the present study is to determine whether somatosensory evoked depression of the arterial baroreceptor–heart rate (HR) reflex could be prevented by selective loss of NK1-R expressing NTS neurons. Recent development of the ribosomal inactivating protein saporin (SAP) has provided an effective and efficient means to selectively eliminate NK1-R expressing neurons (Wiley and Lappi, 1997). This approach has previously been used to successfully ablate NK1-R expressing neurons in the NTS (Riley et al., 2002) and ventrolateral medulla (Nattie and Li, 2002; Wang et al., 2002). Using SP-conjugated SAP toxin (SP-SAP), we hypothesized that elimination of NK1-R expressing NTS neurons would abolish the depressive effect of somatic afferents on arterial baroreflex function.

EXPERIMENTAL PROCEDURES

Experiments were performed on male Sprague–Dawley rats (70–100 g) in accordance with the regulations stipulated in the US Department of Health and Human Services and were approved by the Animal Investigation Committee at the University of Missouri-Columbia and Wayne State University School of Medicine. Every attempt was made to minimize the number of animals used in these studies and to limit their distress and potential suffering. Rats ($n=26$) were anesthetized with ketamine (60–80 mg/kg, i.p.) and xylazine (6–7 mg/kg, i.p.). Adequacy of anesthesia was periodically determined by lack of a withdrawal response to firm hindlimb compression and absence of corneal reflex. When a response was evoked supplemental anesthesia (ketamine, 35 mg/kg, i.m.; xylazine 3.5 mg/kg, i.m.) was administered. The skull was shaved, the skin sterilized with Betadine and alcohol and the animal was positioned in a stereotaxic head frame (Kopf, Tujunga, CA, USA). A limited craniotomy was performed and the cerebellum retracted to expose the dorsal surface of the brainstem.

Immunolesioning of NK1-R expressing neurons

A single-barrel glass micropipette (tip diameter <10 μm, o.d.) was stereotaxically positioned in the caudal NTS (250 μm lateral of midline, 350 μm ventral of dorsal surface) at the level of calamus scriptorius. Rats received bilateral microinjection (1.5 ng/100 nl per side; Advanced Targeting Systems, San Diego, CA, USA) of either SP-SAP (experimental group, $n=5$), free SAP (toxin control; $n=5$) or phosphate buffered saline (PBS, vehicle control, $n=5$) via a pneumatic picopump (model PV 820; WPI, Sarasota, FL, USA). The dose of SP-SAP was based on preliminary experiments using several SP-SAP doses (0.5–10 ng in 100 nl). We found that 1–3 ng of SP-SAP produced lesions that were confined to the medial and commissural subdivisions of the NTS caudal to calamus scriptorius. Smaller doses produced inconsistent or partial lesions, while larger doses (5–10 ng) lesioned regions outside the NTS, including the dorsal motor nucleus of the vagus (X), dorsal column nuclei and the XII. All injection volumes were measured directly using a surgical microscope (Zeiss Stemi SV11; Zeiss Surgical, Thornhill, NY, USA) equipped with a calibrated eyepiece reticule and were performed over 3–5 min to limit potential damage to NTS circuits. Following the first microinjection, the pipette remained in place for 10 min prior to repositioning it into the contralateral region of the NTS. Following completion of the second injection, the wound was closed with sterile suture and buprenorphine (0.5 mg/kg, i.m.) was administered for analgesia. Animals were permitted to recover for 5–7 days. This was based on preliminary time-course experiments performed in our laboratory, as well as findings reported from a previous study (Riley et al., 2002).

Assessment of NK1-R ablation on arterial baroreflex and chemoreflex function

Experiments were performed using the *in situ* arterially-perfused, decerebrate working heart–brainstem preparation (WHBP) (Paton, 1996). Briefly, rats were deeply anesthetized via inhalation with halothane and bisected sub-diaphragmatically. The rostral portion (head, forelimbs and thorax) was immediately submerged in ice-cold Ringer solution bubbled with 95% O_2–5% CO_2 gas mixture. A precollicular decerebration was performed and a portion of the posterior thorax was removed to expose the heart and lungs. The thoracic aorta was isolated by blunt dissection and the left phrenic nerve was identified visually and sectioned at the level of the diaphragm. Finally, the cerebellum was removed to expose the dorsal surface of the brainstem. The preparation was transferred to an acrylic chamber where the thoracic aorta was cannulated with a double lumen catheter (16 ga/18 ga; Braintree Scientific, Braintree, MA, USA) and perfused with Ringer solution containing an oncotic agent (Ficoll, 70 kDa, 1.25%; Sigma, St. Louis, MO, USA) by a roller pump (model 505S pump; model 314DW2 pump head; Watson-Marlow, Chicago, IL, USA). The perfusate was gassed with 95% O_2–5% CO_2, warmed to 32–34 °C and passed through two in-line bubble traps and a filter (polypropylene mesh, pore size: 40 μm). The time to the beginning of perfusion following induction of anesthesia was <10 min. Pump flow rate was monitored via an in-line volumetric flow probe (model 2N; Transonic Systems, Ithaca, NY, USA) and perfusion pressure (PP) was measured using a strain-gauge pressure transducer (model P23; Statham, Oxnard, CA, USA). At flow rates used in the present study (25–35 ml/min), systemic PP averaged 85 ± 8 mm Hg. Phrenic nerve discharge (PND) was recorded with a glass suction electrode (tip diameter: 0.2–0.3 mm). The raw phrenic neurogram was amplified 10,000–20,000 (Neurolog NL104; Digitimer, Letchworth Garden City, UK), integrated using a time constant of 100 ms (Neurolog NL703), filtered (Neurolog NL126; 500 Hz–5 kHz) and displayed on an analog oscilloscope (model 7603, Tektronic). The electrocardiogram (EKG) was amplified (Grass P511-Telefactor, West Warwick, RI, USA), filtered (100 Hz–3 kHz) and displayed on an oscilloscope. A discriminator circuit (Neurolog NL 201) generated a TTL pulse to threshold crossing of the upstroke of R-wave and instantaneous HR was derived from the measured inter-beat interval. Neuromuscular paralysis was produced by addition of vecuronium bromide (50 μg) directly to the perfusate.

Arterial baroreceptor afferents were activated using rapid pressure ramps (25–30 mm Hg/s, 1–2 s) generated by a digital