Ablation of NK1 Receptors in Rat Nucleus Tractus Solitarii Blocks Baroreflexes

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Abstract—The neuropeptide substance P (SP) is found in vagal afferent nerves within the nucleus tractus solitarii, where it is released on stimulation of arterial baroreflexes. The neurokinin-1 receptors at which SP may act have been identified in the nucleus tractus solitarii, but there remains uncertainty if the neurons at which SP acts are critical to baroreflex transmission. By using SP conjugated with the toxin saporin, which kills the neurons at which SP may act, we sought to test the hypothesis that neurons expressing the neurokinin-1 receptor are critical to baroreflex transmission in the nucleus tractus solitarii. One and 2 weeks after injection of the toxin into the rat nucleus tractus solitarii, immunoreactivity for the neurokinin-1 receptor was lost. When the toxin had been injected bilaterally, the baroreflex gain was significantly reduced. Therefore, neurons that express SP receptors play a critical role in mediating baroreflexes through the nucleus tractus solitarii of rat. (Hypertension. 2002;40:823-826.)

Key Words: baroreflex ■ blood pressure ■ receptors ■ neuropeptides

Over the past 25 years, considerable effort has been directed toward identifying transmitters that may be involved in transmission of baroreceptor afferent signals on primary central neurons in the nucleus tractus solitarii (NTS). One putative transmitter that has received a great deal of that attention is the peptide substance P (SP). Early studies demonstrated that SP was present in the nodose ganglion and in vagal afferent nerves that terminate in NTS. Others showed that injection of SP into NTS led to depressor/bradycardic responses similar to those seen with baroreflex activation or injection of the excitatory amino acid glutamate into NTS. Attempts to replicate those initial pharmacological studies have led to conflicting results. Despite the uncertain role of SP in baroreflex transmission, it became clear that the neurokinin-1 (NK1) receptors at which SP acts are found in NTS and could mediate cardiovascular responses to SP in NTS. Recognition of those receptors, reports that SP is released in NTS on baroreflex stimulation, and recognition that SP may act as a modulator of the actions of glutamate in NTS renewed our interest in this area.

The present study was designed to test the hypothesis that NTS neurons that expressed the NK1 receptor are integral to the baroreflex. To test that hypothesis we used a novel toxin, SP conjugated with saporin (SP-SAP), which specifically targets cells that express the NK1 receptor and quickly (onset <24 hours) begins to kill those cells.

Methods

All studies were performed in adult male Sprague-Dawley rats and were approved by the institutional animal care and use committees of the University of Iowa and the Iowa City Veterans Administration Medical Center (VAMC). Each rat was anesthetized with halothane (5% induction followed by 1.5 to 2.0% maintenance) delivered in 100% O2 (2 L/min) by a nasal mask. The dorsal surface of the brainstem was exposed, as previously described, and a glass micropipette filled either with SP-SAP (18 ng; supplied by Advanced Targeting Systems) or vehicle (PBS) was stereotaxically placed in the NTS where baroreceptor afferent terminals are most concentrated. After injection of 500 nL over 15 minutes, the pipette was left in place for 15 minutes to limit efflux of injectate from the pipette track. In some animals (n = 8), injections were made unilaterally, allowing us to compare histological changes in response to SP-SAP with normal histology on the contralateral side in the same animal. In others, injections of toxin (n = 12) or vehicle (n = 12) were made bilaterally. After withdrawal of the pipette, wounds were closed with silk suture, buprenorphine (0.1 mg/kg) was administered for analgesia, and halothane was discontinued. When fully recovered from anesthesia, the animal was returned to the animal care facility, where it was carefully observed at least twice a day (morning and night) for 1 to 2 weeks.

After 1 or 2 weeks, the animal was again anesthetized with halothane and instrumented with femoral arterial and venous cannulae for recording arterial pressure and delivering drugs intravenously. Halothane was discontinued, and anesthesia was maintained with chloralose (40 mg/kg IV for induction and 20 mg/kg per hour for maintenance) for the duration of the study. Depth of anesthesia and need for supplemental chloralose was assessed as we have previously reported. Fifteen minutes after conversion from halothane to chloralose anesthesia, we tested arterial baroreflexes by administering varying doses of phenylephrine (1, 5, 10, 15, and 20 μg/kg; 1 to 3 μL) or nitroprusside (1, 10, 20, 30, and 50 μg/kg; 1 to 3 μL) intravenously and assessing reflex changes in heart rate with respect to changes in arterial pressure. Doses and drugs were administered in random order. Upon completion of baroreflex testing, we administered Nembutal (50 mg/kg IP) and euthanized the animal by
transcardiac perfusion with 4% paraformaldehyde in PBS. The brain was then removed and processed for immunohistochemical analysis of NK1 as described below. Data from baroreflex testing were subjected to \( t \) test and regression analysis.

Thirty-micrometer transverse sections of brainstem through the NTS were made by using a cryostat. The sections were treated with 0.3% \( \text{H}_2\text{O}_2 \), blocked with 10% goat normal serum in PBS, and then incubated with rabbit anti-NK1 antibody (1:100, Novus Biologicals, raised against a 15-residue synthetic peptide at the C-terminus of rat NK1) overnight. Sections were then incubated with biotinylated goat anti-rabbit antibody (Histostain-SP kit, Zymed Labs) for 2 hours, followed by incubation with streptavidin conjugated horseradish peroxidase (Histostain-SP kit, Zymed Labs) for 60 minutes. The final visualization of NK1 immunoreactivity (NK1-IR) was achieved by incubating sections with 0.005% 3'-3' diaminobenzidine tetrahydrochloride in the presence of 0.6% nickel ammonium sulfate and 0.006% \( \text{H}_2\text{O}_2 \) for 2 to 4 minutes. Sections were then transferred to slides, air dried, mounted with Permount, and examined microscopically. Tissue processed without addition of primary antibody served as a negative control. In this control preparation, no NK1-IR was present.

Results

After injections had been made into the NTS and animals had recovered from anesthesia, the treated rats, like the controls, appeared healthy and to be eating and drinking normally. However, 4 animals that had received bilateral injections died suddenly, with no apparent premonitory warning signs, before baroreflex testing. One animal died 8 days after treatment, two died 9 days after treatment, and one died 12 days after treatment. Each animal had been examined twice daily before its death, and each had appeared healthy and in no distress at the final examination. The cause of death could not be determined because the animals, having died unobserved, could not be properly fixed for postmortem analysis of tissues. No animal that received unilateral injections died as a result of the injection.

The remaining animals studied 1 and 2 weeks after bilateral administration of SP-SAP demonstrated significant \((P<0.0001)\) reduction of the baroreflex gain when compared with that of animals with sham lesions, ie, injections of vehicle. Because there was no difference in the gain of the reflex at 1 and 2 weeks, we combined data from those 2 times (Figure 1). A reduction of the gain was apparent for reflex responses both to pressor and depressor effects of phenylephrine and nitroprusside, respectively.

We analyzed NK1-IR in intact control NTS, in NTS 1 and 2 weeks after unilateral SP-SAP, in NTS 1 and 2 weeks after bilateral SP-SAP injection, and in NTS after bilateral injection of vehicle. In all cases, NK1-IR was strikingly reduced after injection of SP-SAP but not after injection of vehicle (Figure 2). At 1 and 2 weeks, the loss of NK1-IR was found in regions of NTS that are most associated with termination of baroreceptor afferents. Those regions included the dorsolateral and medial subnuclear regions of the nucleus, as well as the interstitial subnucleus. There was no difference in the reduction of NK1-IR 1 and 2 weeks after injection of SP-SAP; however, at 1 week some infiltration of glia into the NTS was noted. Most neurons had no apparent damage within the same regions of NTS where loss of NK1-IR was most prominent. Any reduction in the concentration of neurons seen on hematoxylin stained sections was minimal compared with loss of NK1-IR (Figure 2).

Discussion

The present study confirms that SP receptors are present in the cardiovascular region of the NTS. Furthermore, it shows that administration of SP-SAP into the dorsal vagal complex in the medulla oblongata produces neurotoxic effects, as has been reported, when it was administered to the spinal cord.\(^{15}\) Those neurotoxic effects are manifest as loss of NK1 immunoreactivity centered in the region of NTS where baroreceptor afferents are known to be concentrated,\(^{18}\) although the affected region contains terminals of other cardiovascular and noncardiovascular afferents as well.\(^{20}\) The significantly reduced gain of the baroreflex after loss of NK1-IR in NTS suggests that neurons expressing NK1 receptors are integral to baroreflex transmission in NTS. We recognize that the affected neurons and fibers likely express other receptors and other putative transmitters.\(^{21}\) Therefore, although SP may act at those neurons and fibers, the source of endogenous SP may not be baroreceptor afferents.

SP-SAP has been shown\(^{15,22,16}\) to kill selectively central neurons, including spinal cord neurons that express NK1 receptors, and to interfere with nociception as a result. The neurotoxic effects of SP-SAP can be seen within 24 hours of its administration and can seem first to consist of lack of transport of the receptor unit to the cell membrane.\(^{15}\) Toxicity rapidly progresses, and cell loss can be documented within 4 days. Within 7 days, 95% of neurons in affected regions have died. We extrapolated the dose used in the present study from doses used in earlier studies\(^{15}\) and found relative preservation of NTS neuronal architecture though neurons expressing

![Figure 1. Baroreflex sensitivity was significantly (P<0.0001) decreased within 2 weeks of bilateral injection of SP-SAP into the NTS. The baroreflex was tested in 8 rats anesthetized with chloralose. Reflex bradycardia, in response to pressor effects of varying doses of phenylephrine, and tachycardia, in response to depressor effects of nitroprusside, were assessed and analyzed by regression analysis. Animals were anesthetized with chloralose during baroreflex testing. Because there was no significant difference in baroreflex responses at 1 week (n=5) and 2 weeks (n=3) after administering SP-SAP, data were combined in this analysis.](image)
NK1 receptors were virtually undetectable. Therefore, our findings are consistent with a relatively selective toxic action of SP-SAP on neurons and fibers that express the NK1 receptor. Our finding of such well-preserved neuronal architecture in NTS, despite the marked loss of NK1-IR, is fully consistent with work from others, who, as did we, showed that the bulk of NK1 staining in NTS lies within fibers rather than within neurons. Because NK1 immunoreactivity in the NTS has been shown to be associated only with neurons and not with glia, it is unlikely that effects of the toxin were secondary to effects on glia.

Before baroreflex testing in animals with bilateral lesions, 4 animals died suddenly and unexpectedly. Currently, we cannot explain their death. Arterial pressure was not recorded chronically in these animals. Therefore, neurogenic hypertension resulting from the lesions cannot be excluded. However, death from NTS lesions typically occurs with acute pulmonary edema. The latter was clearly not present immediately before death.

The physiological portion of the present study was performed in anesthetized animals. It is well recognized that anesthesia may blunt or abolish lability of arterial pressure that follows chronic NTS lesions. Therefore, this study does not establish the long-term effects of lesions created in NTS by SP-SAP; but because of the profound effects that the SP-SAP lesions have on the baroreflex, we conjecture that further study will reveal chronically labile arterial pressure in treated animals.

Perspectives

The present study supports the hypothesis that neurons expressing the NK1 receptor, and thus neurons that could respond to SP, are critical to processing baroreflex transmission in NTS. The data would be consistent with a primary transmitter role for SP in the baroreflex, a modulatory role in the reflex, or potentially a role for the peptide in integrating nonbaroreflex signals with the baroreflex at the level of NTS. Animals treated with SP-SAP in NTS provide another model of chronic compromise of central baroreflex pathways and could likewise be a model of sudden cardiac death.

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References


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