Postexercise hypotension in conscious SHR is attenuated by blockade of substance P receptors in NTS

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Chen, Chao-Yin, Paul A. Munch, Anthony W. Quail, and Ann C. Bonham. Postexercise hypotension in conscious SHR is attenuated by blockade of substance P receptors in NTS. Am J Physiol Heart Circ Physiol 283: H1856–H1862, 2002.—In hypertensive subjects, a single bout of dynamic exercise results in an immediate lowering of blood pressure back toward normal. This postexercise hypotension (PEH) also occurs in the spontaneously hypertensive rat (SHR). In both humans and SHRs, PEH features a decrease in sympathetic nerve discharge, suggesting the involvement of central nervous system pathways. Given that substance P is released in the nucleus tractus solitarius (NTS) by activation of baroreceptor and skeletal muscle afferent fibers during muscle contraction, we hypothesized that substance P acting at neurokinin-1 (NK-1) receptors in the NTS might contribute to PEH. We tested the hypothesis by determining, in conscious SHRs, whether NK-1 receptor antagonists of the NK-1 receptor antagonist SR-140333 before exercise attenuated PEH. The antagonist, in a dose (60 pmol) that blocked substance P- and spared D,L-homocysteic acid-induced depressor responses, significantly attenuated the PEH by 37%, whereas it had no effect on blood pressure during exercise. Vehicle microinjection had no effect. The antagonist also had no effect on heart rate responses during both exercise and the PEH period. The data suggest that a substance P (NK-1) receptor mechanism in the NTS contributes to PEH.

NK-1 receptor; exercise; microinjection; blood pressure; hypertension; nucleus tractus solitarius

HYPERTENSION IS A MAJOR ANTECEDENT OF stroke, heart failure, and end-stage renal disease. Hypertension is only adequately controlled in only ~25% of individuals in the United States (44); this downward trend in pharmacological management has led to renewed efforts to reduce the prevalence of hypertension with nonpharmacological approaches (2). In individuals with hypertension, a single bout of mild to moderate dynamic exercise can lead to a postexercise decrease in blood pressure (13, 18, 19, 26, 40). This postexercise hypotension (PEH) can restore blood pressure back toward normal (19, 26) and can persist for up to 12 h (12, 19). Understanding the mechanisms of PEH may be a first step in increasing awareness of this strategy for controlling hypertension and might lead to a greater emphasis on lifestyle modification rather than a sole reliance on pharmacological therapy. The spontaneously hypertensive rat (SHR) appears to be an ideal model for investigating the mechanisms. After single bouts of mild to moderate exercise (treadmill or spontaneous wheel running), SHRs exhibit PEH of the same magnitude and duration as observed in hypertensive humans (5, 7, 28, 37).

Neural, humoral, and vascular changes have been explored to help explain PEH (3, 4, 8, 20, 46, 52). In that regard, one salient characteristic of PEH is a decrease in sympathetic nerve discharge (13, 18, 20, 21, 28), which suggests that central nervous system (CNS) pathways may be in play. There is compelling evidence that mechanisms operating in the CNS may be important. First, endorphins are elevated during exercise in humans (52); gene expression of preproenkephalin has been shown to increase in the NTS, rostral ventral lateral medulla (RVLM), and caudal ventral lateral medulla after treadmill exercise in SHRs (3), and the opioid receptor antagonist naloxone has been shown to attenuate PEH in both humans and SHRs, although to a variable extent (4, 20, 46). Second, a more recent study has implicated vasopressin acting in the CNS (8); injections of a vasopressin V1 receptor antagonist into the lateral cerebral ventricle has been shown to inhibit PEH, although specifically where in the CNS vasopressin might act is still unresolved. Finally, Potts et al. (43) have recently shown that skeletal muscle afferent fibers excited by contractions during exercise release the excitatory neuropeptide substance P in the NTS. This latter finding, coupled with the neurotransmitter or neuromodulator functions of substance P in cardiovascular afferent pathways throughout the NTS (9, 10, 22, 25, 31, 34, 36, 39, 47, 56), suggests that the neuropeptide may play an important role in the effect of exercise on cardiovascular regulation. Of particular relevance to the current study is the finding that exogenous application of substance P (10, 22, 25, 56) or selective neurokinin-1 (NK-1) receptor agonists (34) excites NTS neurons and...
that NTS neurons provide a tonic inhibitory input to sympathetic premotoneurons in the RVLM (9, 31, 47). Taken together, these findings raise the possibility that substance P released during exercise, by increasing the excitability of NTS neurons (36), might contribute to the decreased central sympathetic outflow and hence PEH.

In this study, we tested the hypothesis that a substance P (NK-1) mechanism contributes to PEH by determining, in conscious SHR, that discrete microinjections of a specific substance P neurokinin (NK-1) receptor antagonist in the NTS before exercise prevent the development of PEH.

METHODS

All experimental protocols in this work were reviewed and approved by the Institutional Animal Care and Use Committee in compliance with the Animal Use and Care Administrative Advisory Committee. Rats were anesthetized with ketamine (40 mg/kg im) and xylazine (4 mg/kg im). Each rat was placed on a servocontrolled water blanket, and body temperature was monitored via a rectal temperature probe and maintained within 37 ± 1°C. A catheter was placed in the left jugular vein for common carotid artery for recording arterial blood pressure and body temperature was monitored via a rectal temperature probe and maintained within 37 ± 1°C. The animal was then placed in a stereotaxic frame.

To determine the appropriate concentration of the substance P (NK-1) receptor antagonist SR-140333, we performed some experiments in anesthetized animals in which injections of the antagonist at different concentrations were tested for their ability to block depressor responses to injections of substance P or nα-homocysteic acid (DLH) in the NTS. For these studies, an occipital craniotomy was performed, and the caudal portion of the fourth ventricle was exposed by removing the dura mater and arachnoid membranes. For studies with conscious animals, the skull was exposed by removing the dura mater and arachnoid membranes at the location of the pipette. The animal was then placed in a stereotaxic frame.

General Surgical Preparations

All experiments were performed in male SHRs (322 ± 11 g, range 270–370 g). Surgery was performed under the Guidelines for Survival Surgery in Rodents provided by the Animal Use and Care Administrative Advisory Committee. Rats were anesthetized with ketamine (40 mg/kg im) and xylazine (4 mg/kg im). Each rat was placed on a servocontrolled water blanket, and body temperature was monitored via a rectal temperature probe and maintained within 37 ± 1°C. A catheter was placed in the descending aorta through the left carotid artery for recording arterial blood pressure (ABP). A catheter was placed in the left jugular vein for continuous infusion of anesthesia (5–13 mg/h ketamine and 0.5–1.3 mg/h xylazine) and fluid (0.23–0.56 ml/h). The adequacy of anesthesia was determined every 30 min by pinching the hindlimb paw and monitoring for hindlimb flinch or withdrawal and was maintained by adjusting the anesthesia infusion rate. The animal was then placed in a stereotaxic frame.

To determine the appropriate concentration of the substance P (NK-1) receptor antagonist SR-140333, we performed some experiments in anesthetized animals in which injections of the antagonist at different concentrations were tested for their ability to block depressor responses to injections of substance P or nα-homocysteic acid (DLH) in the NTS. For these studies, an occipital craniotomy was performed, and the caudal portion of the fourth ventricle was exposed by removing the dura mater and arachnoid membranes. To implant the guide cannulae for microinjections in the NTS in the conscious SHRs, the skull was exposed to visualize the landmarks, bregma and lambda. A small hole was drilled in the skull, and a pair of guide cannulae (1.5 mm long, 22-gauge stainless steel, separated by 1–2 mm) was introduced perpendicularly to the skull 4.55 mm caudal to the interaural line, 0.5–1 mm lateral to midline for each cannulae, and 7.9 mm below the surface of the skull. These coordinates placed the tip of the guide cannula ~1 mm above the dorsal surface of the brain stem. Microinjections were made through a 33-gauge needle inserted through and extending 1.5–1.7 mm below the guide cannula and connected by polyethylene (PE)-10 tubing to a 1-µl Hamilton syringe. The placement of the cannulae and the length of the injector required for NTS microinjections were confirmed by a depressor response evoked by DLH microinjections (10 mM, 20–40 nl). After a positive DLH test, the cannula was fixed to the skull with methacrylate and watch screws and then closed with an occluder. The arterial catheter was tunneled beneath the skin and exteriorized at the back of the neck. The venous catheter was closed with a 23-gauge stainless steel occluder. The rats were then allowed to recover (12 ± 5 days, mean ± SD, range 6–22 days), and the arterial catheter was flushed with normal saline and filled with heparin every 2–3 days.

Microinjections in Anesthetized Animals

Microinjection procedures. Unilateral microinjections were made with a three-barrel glass pipette (outside tip diameter 25–50 µm) connected by PE-50 tubing to a 1-µl Hamilton syringe. Each barrel contained DLH (10 mM), substance P (20 μM), or the NK-1 receptor antagonist SR-140333 (0.2 or 1 mM). The pipette was positioned in the NTS under visual guidance with the use of a microscope. On the basis of our previous experience in recording baroreceptor neurons in the NTS (29, 30), the microinjections were made in the intermediate NTS 500 µm rostral to the calamus scriptorius, 500 µm lateral to midline, and 500 µm ventral to the dorsal surface of the medulla.

Experimental protocols. After a 2-min period of recording a stable resting ABP, we unilaterally injected DLH (0.2–0.3 nmol) in the NTS to confirm the location of the pipette. The ABP response to NTS microinjection of substance P (1–2 pmol) was determined 10 min afterward. After the ABP recovered to the control level (10 min after the substance P injection), the NK-1 receptor antagonist (20 or 60 pmol, n = 2 each) was injected to the NTS. The substance P and DLH (to confirm that the antagonist had no nonselective effects that prevented depressor responses evoked by other mechanisms) injections were repeated 5 min after the antagonist injection.

Microinjections in Conscious Animals

Only animals having a positive DLH test on both sides were subjected to the PEH experimental protocol. On the day of the experiment, each rat was placed on the treadmill, and the arterial catheter was connected to a blood pressure transducer. The arterial pressure signals were fed in parallel to an oscilloscope, polygraph, and tape recorder for data analyses. Heart rate was determined from the arterial pulse pressure. The mean ABP (MABP) and heart rate were taken over a 10- to 20-s period every 10 min. After three consecutive and consistent MABP readings (within 6% of each other), the occluders in the guide cannulae were removed, and either the vehicle or the NK-1 receptor antagonist SR-140333 was bilaterally microinjected in a randomized crossover design. The rats then ran on the treadmill at 15 m/min, 10°, for 40 min. The MABP was taken over a 10- to 20-s period every 10 min during exercise and up to 2 h after the exercise period. After exercise, each rat was returned to its individual home cage. The vehicle and antagonist injections were made in a random order with 7 ± 4 days (means ± SD) in between (range 4–13 days).

Histology

At the end of the experiment, the rats were anesthetized with ketamine (50 mg/kg im) and xylazine (8 mg/kg im), and 2% Pontamine sky blue dye in 0.5 M sodium acetate was injected in the NTS to verify the injection site histologically.
postmortem. The rats were killed with an overdose of pentobarbital sodium, and the brain stems were removed and fixed in 4% paraformaldehyde and 10% sucrose. The brain stems were cut in 80-μm coronal sections and counterstained.

**Data Analysis**

All data are expressed as means ± SE unless otherwise indicated. Significance was claimed at *P* < 0.05. For the protocol that established the concentration for the antagonist, the DLH- and substance P-evoked depressor responses [change in (∆) MABP] were compared before and after microinjections of antagonist using a paired *t*-test. For the antagonist effect on baseline MABP, the MABP before and after antagonist injection were compared with a paired *t*-test. For the protocol that determined the contribution of the NK-1 receptor to PEH, the MABP readings every 10 min before, during, and after exercise were compared using a two-way ANOVA with vehicle vs. antagonist as one within factor and time as the other within factor and followed by a Fisher’s least-significant difference (LSD) test. To determine whether the order of antagonist injection (antagonist injection on first trial or second trial) affected the outcome, the MABP readings every 10 min before, during, and after exercise were compared using a two-way ANOVA with order of injection as the between factor and time as the within factor. To further quantify the effect of NK-1 receptor antagonism on PEH, the MABP readings during the 2 h of PEH were averaged for each animal and expressed as the change from control MABP before exercise. The data were compared between vehicle and antagonist with a paired *t*-test. Heart rate readings every 10 min before, during, and after exercise were compared using a two-way ANOVA with vehicle vs. antagonist as one within factor and time as the other within factor and followed by Fisher’s LSD test.

**RESULTS**

**Cardiovascular Effect of Substance P in NTS**

As shown in Fig. 1, microinjection of substance P (2 pmol, 100 nl of 20 μM) or DLH (0.3 nmol, 30 nl of 10 mM) into the NTS of the same SHR decreased ABP. The NK-1 receptor antagonist SR-140333 (20 pmol, 100 nl of 0.2 mM) abolished the substance P-induced depressor response, whereas it had no effect on the DLH-induced depressor response. The group data (*n* = 4; Fig. 2) confirmed that the substance P (NK-1) receptor antagonist (20 pmol (*n* = 2 SHR) or 60 pmol (*n* = 2 SHR)) blocked the substance P (1 or 2 pmol)-induced depressor responses (from ∆-18 ± 4 to ∆4 ± 4 mmHg, *P* = 0.022, paired *t*-test), whereas it had no effect on DLH (0.2–0.3 nmol)-induced depressor responses (from ∆23 ± 2 to ∆23 ± 4 mmHg, *P* = 0.992, paired *t*-test). The NK-1 receptor antagonist microinjected into the NTS had no effect on the baseline MABP (97 ± 5 vs. 95 ± 4 mmHg, before vs. during the antagonist, respectively, *P* = 0.391, paired *t*-test).

**Effect of Substance P (NK-1) Receptor Antagonist on PEH**

The highest dose of the NK-1 receptor antagonist (60 pmol, 60 nl of 1 mM) that blocked the substance P-induced depressor response was used in the conscious animal studies. Microinjection of the NK-1 receptor antagonist before exercise significantly attenuated the peak and duration of PEH after a single bout of dynamic exercise (15 m/min, 10°, 40 min; Fig. 3, two-way ANOVA: *P* = 0.058, vehicle vs. antagonist; *P* < 0.001, time; *P* = 0.008, interaction, *n* = 11). After vehicle injection, a single bout of dynamic exercise evoked PEH; the peak decrease in MABP (measured at the lowest point on the graph) was -36 ± 4 mmHg at 10 min, and MABP remained significantly (*P* < 0.05, Fisher’s LSD test) lower than preexercise MABP at the end of the 2 h of recording (Fig. 3A). When the same animals were injected with the antagonist, the magnitude and duration of PEH were attenuated; the peak

**Fig. 1.** An example of the effects of the substance P (SP) [neurokinin-1 (NK-1)] receptor antagonist SR-140333 on SP- and DL-homocysteic acid (DLH)-evoked responses. SP and DLH microinjected into the nucleus tractus solitarius (NTS) evoked depressor responses (left). The NK-1 receptor antagonist blocked the SP-evoked depressor response, whereas it had no effect on the DLH-evoked depressor response (right). Arrows, drug injections. ABP, arterial blood pressure; MABP, mean ABP.

**Fig. 2.** Group data (*n* = 4) showing the effects of the NK-1 receptor antagonist on SP- and DLH-evoked responses. The NK-1 receptor antagonist (20–60 pmol) blocked the SP (1–2 pmol)-evoked response but not the DLH (0.2–0.3 nmol)-evoked response. *P* = 0.022, control vs. NK-1 antagonist, paired *t*-test.
decrease in MABP was $-25 \pm 3$ mmHg, and MABP returned to within 3% of the preexercise value at the 2-h time point (Fig. 3B). The order of administration of the antagonist did not affect the magnitude of the attenuation of PEH (two-way ANOVA: $P = 0.058$, vehicle vs. antagonist; $P < 0.001$, time; $P = 0.008$, interaction). Hatched bar, exercise period; dotted line, control MABP; arrows, drug or vehicle injection.

Figure 5 shows heart rate before, during, and after a single bout of exercise. Heart rate, which averaged $347 \pm 10$ beats/min before exercise, was significantly lower ($314 \pm 8$ beats/min, average of 2-h PEH period) after a single bout of exercise (vehicle control, $P = 0.002$, paired t-test). There was a significant increase in heart rate during exercise. Microinjection of the NK-1 receptor antagonist before exercise had no effect on the heart rate response during exercise or in the PEH period (two-way ANOVA: $P = 0.621$, vehicle vs. antagonist; $P < 0.001$, time; $P = 0.947$, interaction, $n = 10$).

Histology of Microinjection Sites

Microinjection sites were marked with dye and histologically verified. All animals in the study had at least one injection site located in the intermediate and caudal NTS. Figure 6 shows a photomicrograph of the dye spots marking the microinjection location sites in the NTS in a coronal slice. All injection sites were located from the obex to 1,440 μm caudal to the obex.
DISCUSSION

The principal finding of this study is that microinjection of a substance P (NK-1) receptor antagonist in the NTS before exercise, at a dose that blocked the substance P-induced depressor response, attenuated PEH in SHR by 37%, whereas it had no effect on blood pressure before and during exercise. The data are consistent with the hypothesis that PEH is mediated, at least in part, by a substance P (NK-1) receptor mechanism in the NTS.

An intact baroreflex system (5), hypertension, and exercise are the three key elements of PEH. With regard to the baroreflex system, the NTS, the first central site in the central network, also receives input from muscle afferent fibers (15, 41, 53), making it an ideal site for integrating cardiovascular responses to exercise. Although there is some uncertainty as to the precise site or extent of the contribution of substance P to baroreceptor signal transmission in the NTS, for example, whether the neuropeptide is released by primary baroreceptor afferent fibers or by NTS interneurons (6, 14, 27, 33, 54), it seems clear that the neuropeptide can be released in the NTS when baroreceptor afferent fibers are stimulated electrically (35) or naturally (42), over either monosynaptic or polysynaptic pathways.

The physiological relevance of the substance P release in the NTS may depend on the conditions under which it is released. In the present study, microinjection of the NK-1 receptor antagonist, in doses that blocked the effect of substance P, had no effect on blood pressure in the anesthetized rats, confirming previous findings by others that blockade of NK-1 receptors in the NTS has no effect on blood pressure in anesthetized (45) or conscious (57) animals. Given that NK-1 receptor antagonism in the NTS appears to have an effect on resting blood pressure, it seems unlikely that substance P released at NTS NK-1 receptors contributes importantly to blood pressure under basal conditions. On the other hand, substance P actions in the NTS may be more important in blood pressure regulation under conditions in which the cardiovascular state is altered, such as during and immediately after exercise. Of particular relevance are the findings by Potts et al. (43): that skeletal muscle afferent fibers release substance P in the NTS during muscle contraction. In the context of those data, the present finding that NK-1 receptor antagonism had no effect on the depressor response during exercise suggests that the release of substance P in the NTS sets in motion neuronal mechanisms that, while seemingly unimportant in the pressor response, are required for the full expression of the ensuing hypotension.

Injections of SR-140333, a potent, selective, and long-lasting substance P (NK-1) receptor antagonist (1), made just before exercise attenuated the PEH. In two animals tested, the antagonism of the substance P-evoked depressor responses remained at 33 and 47 min after the injection of the antagonist. Findings in conscious animal models also confirm this duration of antagonism by SR-140333: 1) increases in locomotor activity induced by intracerebroventricular injections of substance P were blocked by SR-140333 injected 40 min before the substance P injections (55); and 2) a time-course evaluation of the antagonism of SR-140333 of substance P-induced contractile responses of guinea pig ileum showed that at 40 min after washout of the antagonist, the substance P-induced contractile response was still inhibited by 70% (11). Although the duration of antagonism of substance P-evoked depressor responses lasted over the approximate time of the exercise period, it seems unlikely that substance P was continually released throughout exercise and into the postexercise period to contribute to the magnitude and duration of the PEH, such that a long-lasting blockade of NK-1 receptors throughout the period of PEH would be required for attenuating the magnitude of the PEH. Network effects of the peptide in other systems, most notably in the lamprey spinal cord, indicate that a single 10-min application of substance P can cause long-lasting (>24 h) modulation of neural network activity (38, 50, 51). The modulation has been mechanistically divided into three phases: an early phase (<2 h) mediated by the activity-dependent effects on glutamatergic synaptic transmission and to the modulation of the excitability of the postsynaptic neurons via substance P receptors; an intermediate maintenance phase of the increased neural activity (2–15 h) that requires de novo protein synthesis; and a final phase (15–24 h) that requires mRNA synthesis (38, 50, 51). These findings are consistent with the idea that substance P released during exercise triggered a series of events that contributed to the development of PEH and that when those events were prevented, PEH was smaller and shorter.

In the current study, in which the animals received both vehicle and antagonist in random order in a crossover design, the NK-1 receptor antagonist attenuated the peak amplitude, overall amplitude, and duration of PEH. Moreover, the antagonist had no effect on the depressor responses evoked by DLH injected in the same site. Thus it seems likely that under these experimental conditions, the substance P-mediated attenuation of PEH was mediated by NK-1 receptors. The extent to which substance P activation of NK-1 receptors in the NTS contributes to PEH is ~37%. The
extent of the contribution of NK-1 receptors has to be considered in light of the inevitable limitations of the microinjection technique, such as uncertainty as to the proportion of NK-1 receptors accessed in the NTS and whether multiple or larger injections would have produced greater effects. Despite these considerations, it seems likely that other mechanisms in the NTS contribute to PEH, possibly substance P acting at NK-2 and NK-3 receptors, also present in the NTS (35), or other neurotransmitters or neuromodulators. There is converging evidence from several laboratories that multiple neurotransmitters and hormones acting throughout the CNS contribute to PEH: the opioids, vasopressin, GABA, and now substance P; however, the question remains as to how these systems interact. Intracerebroventricular injections of a vasopressin V1 receptor antagonist have been shown to have even greater inhibitory effects on the development of PEH than those observed with blockade of NK-1 receptors in the NTS (8). In addition, blockade of opioids with naloxone has also been shown to attenuate PEH in both humans and SHRs (46), although to a variable extent (4, 20, 46) and possibly via κ- in addition to µ-opioid receptor activation (23). Interestingly, activation of κ-opioid receptors has been shown to enhance the release of substance P (49). Whereas substance P excites NTS neurons via NK-1 receptors (34), opioids may excite the neurons through disinhibition of GABAergic mechanisms (32). In the supraoptic nucleus, inhibition of vasopressin neuronal excitability involves pre- and postsynaptic potentiation of GABAergic synaptic activity (48). NTS neuronal output provides a tonic, largely GABA-A-receptor-mediated, inhibition of the activity of RVM cardiovascular sympathetic premotoneurons thought to generate sympathetic vasomotor tone (9, 16, 17, 31, 47). We have recently shown that a tonically increased GABAergic input to the sympathetic cardiovascular RVM neurons also contributes to PEH (24). When the results are viewed together, a picture emerges that opioids, vasopressin, and substance P may interact directly or indirectly through GABAergic systems most likely in hypothalamic and medullary circuitries to contribute to the development of PEH.

In conclusion, the findings of this study provide new information on the physiological relevance of endogenous substance P in the NTS insofar as release of the neuropeptide during exercise contributes to the development of PEH in SHRs.

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