IL-6 microinjected in the nucleus tractus solitarii attenuates cardiac baroreceptor reflex function in rats

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Takagishi M, Waki H, Bhuiyan ME, Gouraud SS, Kohsaka A, Cui H, Yamazaki T, Paton JF, Maeda M. IL-6 microinjected in the nucleus tractus solitarii attenuates cardiac baroreceptor reflex function in rats. Am J Physiol Regul Integr Comp Physiol 298: R183–R190, 2010. First published November 11, 2009; doi:10.1152/ajpregu.00176.2009.—Recent gene array and molecular studies have suggested that an abnormal gene expression profile of interleukin-6 (IL-6) in the nucleus tractus solitarii (NTS), a pivotal region for regulating arterial pressure, may be related to the development of neurogenic hypertension. However, the precise functional role of IL-6 in the NTS remains unknown. In the present study, we have tested whether IL-6 affects cardiovascular control at the level of the NTS. IL-6 (1, 10, and 100 fmol) was microinjected in the NTS of Wistar rats (280–350 g) under urethane anesthesia. Although the baseline levels of arterial pressure and heart rate did not change following IL-6 injections, the cardiac baroreflex in response to increased arterial pressure was dose-dependently attenuated. In addition, IL-6 (100 fmol) microinjections also attenuated L-glutamate-induced bradycardia at the level of the NTS. Immunohistochemical detection of IL-6 in naïve rats demonstrated that it was predominantly observed in neurons within the brain stem, including the NTS. These findings suggest that IL-6 within the NTS may play an important role for regulating cardiovascular control via modulation of input signals from baroreceptor afferents. Whether the abnormal gene expression of IL-6 in the NTS is associated in a causal way with hypertension remains to be resolved.

interleukin-6; blood pressure

IT IS WELL KNOWN that destruction of the nucleus tractus solitarii (NTS) leads to fulminating hypertension (8). This is highly suggestive that the NTS is a central brain stem structure that plays a vital role in maintaining the set point of arterial pressure. Equally, because baroreceptor afferents terminate in this nucleus (4), it is also one of the most effective central sites for modulating baroreceptor reflex function, a process that is critically important for blood pressure homeostasis (9, 18, 21, 26). Based on this evidence, we hypothesized that abnormal function of the NTS contributes to the development of neurogenic hypertension through effects on both set-point and baroreceptors reflex function (33).

Recently, we identified that the NTS of an animal model of essential hypertension [the spontaneously hypertensive rat (SHR)] exhibits a specific inflammatory condition compared with normotensive Wistar-Kyoto rat (WKY) (32). This includes gene expression profile of interleukin-6 (IL-6), a major cytokine that is known to be expressed in the brain stem (34). IL-6 mRNA expression in the NTS of SHR was ~60% downregulated compared with the NTS of WKY (34). Although the functional roles of brain IL-6 have yet to be fully established, it is not necessarily limited to either a proinflammatory or an anti-inflammatory action (23, 29). For instance, it is known that IL-6 mRNA levels in the brain increase during postnatal development (10), indicating that IL-6 expression may be important for the normal development of brain function. Indeed, IL-6 has crucial roles to promote neuronal survival and astrocyte differentiation (20). In addition, IL-6 can modulate synaptic transmission (6, 14). All told, abnormal IL-6 expression in the brain regions that control the baroreceptor reflex, such as the NTS, may be associated with cardiovascular malfunctions. However, the cardiovascular effects of IL-6 in the NTS have not yet been studied.

There is other evidence suggesting that IL-6 in the NTS may have a functional role to regulate the cardiovascular system. There are several reports demonstrating a functional association between systemic IL-6 and blood pressure (3, 5, 17). Lee et al. (17) demonstrated that ANG II-induced hypertension was attenuated in animals deficient of IL-6. The mechanisms by which IL-6 contributes to this type of hypertension are not fully understood (17). However, because circulating ANG II is known to attenuate baroreceptor reflex via activation of ANG II type 1 receptors expressed in the NTS (22, 28), IL-6 in the nucleus may be involved in the etiology of the hypertensive state. In this regard, it is suggested that IL-6 in the NTS may attenuate baroreflex function.

In the present study, we initiated our studies to characterize functionally the role of IL-6 at the level of the NTS by examining changes in cardiovascular variables and baroreceptor cardiac reflex responses in normotensive rats. In addition to the functional examinations, cellular localization of IL-6 in the NTS was also studied. Here, we show that the IL-6 within the NTS neurons may play an important role for modulating input signals from baroreceptor afferents. Our data support a novel mechanism underlying the maintenance of cardiovascular homeostasis.

METHODS

Animals and animal care. Male Wistar rats (280–350 g) obtained from Kiwa Laboratory Animal (Wakayama, Japan) were used for all experiments in this study. WKY rats (280–300 g) were obtained from Japan SLC (Shizuoka, Japan) and used in some experiments (see Experimental protocol). The animals were housed in a temperature-controlled room with a fixed 12:12-h light-dark cycle (8:00 A.M. to 8:00 P.M. and 8:00 P.M. to 8:00 A.M.). Food and tap water were
given ad libitum. All experiments were approved by the Ethics Committee for Animal Experiments at Wakayama Medical University and complied with the guidelines of the Physiological Society of Japan.

**General procedures.** The animals were anesthetized with urethane (1.45 g/kg) given intraperitoneally. The level of anesthesia was monitored regularly by assessing the limb withdrawal response to a noxious pinch, and, if necessary, an additional dose of urethane (0.145 g/kg ip) was administered. Rectal temperature was monitored and maintained at 37°C using a heating pad (BWT-100; Bio Research Center). The trachea was cannulated to facilitate artificial breathing using a rodent respirator (SN-480-7; Shimano Respirator). A polyethylene catheter (PE-50 tubing filled with heparinized saline) was inserted in the right femoral artery to record pulsatile arterial pressure (AP-601G and P23ID; Nihon Kohden) and the pulsatile pressure signal using a cardiotachometer (AT-601G; Nihon Kohden). A polygraph system (RM-7000; Nihon Kohden) was used for continuous recording of mean arterial pressure (MAP) and heart rate (HR), which was derived from the pulsatile pressure signal using a cardiotachometer (AT-601G; Nihon Kohden) and the MacLab system (PowerLab/8s; ADInstruments). The femoral veins were also cannulated (PE-50) for continuous infusion of physiological saline (0.8 ml·h⁻¹ containing pancuronium bromide (0.08 mg·kg⁻¹·h⁻¹) and vasoactive drugs for baroreceptor reflex tests as shown below.

**NTS microinjections.** The experimental procedures followed an established protocol (1, 19). The rats were placed in a stereotaxic head holder (SR-5; Narishige), and the caudal dorsal medulla was exposed through a midline incision in the dorsal neck. Microinjections of equal volumes of either drug or vehicle were made in the NTS using either a single or multibarrel glass micropipettes (GC200F-10, outside diameter = 20–30 μm; Harvard Apparatus). For unilateral microinjection in the NTS, the tip of the micropipette was positioned 0.5 mm rostral to the calamus scriptorius, 0.5 mm lateral from midline, and 0.5 mm deep from the dorsal surface of the brain stem. This site was chosen since it is considered to be an area where barosensitive neurons are located predominantly (1). To assess site specificity of the cardiovascular effects evoked by IL-6, we also studied a more caudal NTS area where chemosensitive neurons are located predominantly (i.e., 0.3–0.4 mm caudal to the calamus scriptorius, 0.0–0.2 mm lateral from midline, and 0.4–0.5 mm deep from the dorsal surface of the brain stem (1, 31)). A Hamilton microsyringe connected hydraulically to a syringe pump (CFV-3200; Nihon Kohden) was used for microinjections with the volume injected set by the pump. At the end of the experiment, the position of the micropipette was marked by injection of 100 nl of India ink. The animals were killed by an overdose of urethane (3 g/kg iv), and the brain stem was removed and postfixed with 4% paraformaldehyde for at least 24 h and transferred transcardially perfused with 4% paraformaldehyde. The brain stem was chosen since it is considered to be an area where barosensitive neurons are located predominantly (1). To assess site specificity of the cardiovascular effects evoked by IL-6, we also studied a more caudal NTS area where chemosensitive neurons are located predominantly (i.e., 0.3–0.4 mm caudal to the calamus scriptorius, 0.0–0.2 mm lateral from midline, and 0.4–0.5 mm deep from the dorsal surface of the brain stem (1, 31)). A Hamilton microsyringe connected hydraulically to a syringe pump (CFV-3200; Nihon Kohden) was used for microinjections with the volume injected set by the pump. At the end of the experiment, the position of the micropipette was marked by injection of 100 nl of India ink. The animals were killed by an overdose of urethane (3 g/kg iv), and the brain stem was removed and postfixed with 4% paraformaldehyde for at least 24 h and transferred to PBS containing 30% sucrose before sectioning (30 μm) using a cryostatic microtome. Finally, sections were mounted for viewing. Only those rats with microinjection sites precisely within the NTS were used in the analysis.

**Experimental protocol.** In the first set of experiments, L-glutamate (3.4 nmol/100 nl, L-glutamic acid monosodium salt dissolved in saline, Sigma, St. Louis MO, USA) was unilaterally microinjected in the NTS to characterize the microinjection site. Following the recovery of MAP and HR to baseline levels, baroreceptor reflex tests were performed as described below. After complete recovery from the reflex test, a single dose of rat IL-6 (dissolved in saline; PeproTech EC) was microinjected unilaterally in the NTS. Initial pilot studies indicated instability in arterial pressure and HR when bilateral injections were made, but unilateral injections did not affect baseline arterial pressure and HR. The doses of IL-6 were 1, 10, and 100 fmol, and they were delivered in a volume of 100 nl (n = 6 for each dose). The dose of IL-6 used in this study was based on the dose-dependent response of the baroreflex-induced bradycardia in anesthetized rats as presented in this manuscript. Immediately after IL-6 microinjection in the NTS, the baroreceptor reflex gain was determined pharmacologically as described below. The same experiments using NTS microinjections of saline as a vehicle control were also performed (n = 4). In addition to Wistar rats, WKY (n = 5) were also used to assess whether there is a species difference in regard to the effect of NTS microinjection of IL-6 on the baroreceptor reflex gain in normotensive animals. For these experiments, we used 100 fmol of IL-6.

In a second set of experiments, we tested whether IL-6-mediated changes in baroreceptor reflex function were because of modulation of NTS neurons. Thus the effect of IL-6 microinjection in the NTS on L-glutamate-induced cardiovascular responses was tested. Two different concentrations of L-glutamate (i.e., 0.5 or 3.4 nmol/100 nl) were tested. First, L-glutamate was unilaterally microinjected in the NTS by a multibarrel pipette. Second, following recovery of measured variables, a single dose of IL-6 was unilaterally microinjected in the NTS by the same pipette. Finally, immediately after IL-6 microinjection, the same dose of L-glutamate was again microinjected unilaterally, and the changes in MAP and HR in response to L-glutamate injection before and after IL-6 administration were compared. To identify whether the cardiovascular effects of IL-6 were specific to L-glutamate, acetylcholine chloride (500 pmol; Sigma), another neurotransmitter that exhibits a similar cardiovascular response to that of L-glutamate when microinjected in the NTS (7), was also tested.

In the third set of experiments, the specificity of IL-6-induced cardiovascular responses was tested by using a IL-6 blocking antibody (Anti Rat IL-6 Antibody; R & D Systems) (25). IL-6 was incubated with the blocking antibody (1/10 of IL-6) for 120 min at room temperature. The mixed solution was then microinjected in the NTS (100 nl, dose of IL-6: 100 fmol), and the baroreceptor reflex test was performed before and after the microinjection.

**Evaluation of baroreceptor reflex.** In all experiments, the baroreflex function was evaluated by measuring the bradycardiac reflex gain as follows. Phenylephrine (10–20 μg/kg; Sigma-Aldrich, Steinheim, Germany) was administered by a bolus injection through the venous catheters with a 1-ml syringe (SS-01T; Terumo, Tokyo, Japan) mounted on a syringe pump (CFV-3200; Nihon Kohden) to raise MAP between 40 to 60 mmHg. Pressor challenges before and after IL-6 injections were performed at least two times. To assess whether the cardiovascular effects of IL-6 were specific to L-glutamate, the peak changes in MAP (ΔMAP) and the corresponding peak reflex changes in HR (ΔHR) were measured, and the ΔHR-to-ΔMAP ratios (ΔHR/ΔMAP) were averaged and used as an index of baroreceptor bradycardiac reflex gain. When 100 fmol of IL-6 were tested, the reflex function was also evaluated by determining logistic sigmoidal curves of best fit for the MAP-HR relationships to examine the effect on the entire reflex function. Increases and decreases in MAP were produced by intravenous infusions of phenylephrine (31.25 μg/ml) and sodium nitroprusside (25 μg/ml; Calbiochem). They were administered through the venous catheters in successive ramped infusions at an initial rate of 0.8 ml/h for phenylephrine or 1.25 ml/h for sodium nitroprusside, increasing every 30 s by a further 0.8 ml/h (phenylephrine) or 1.25 ml/h (sodium nitroprusside). The approximate rate of the linear changes in MAP was ~1.0 mmHg/s. The sigmoidal curves are described by the following equation: HR = A1/[1 + exp[A2(MAP – A3)]], + A4, where A1 is the HR range, A2 is the gain coefficient, A3 is the MAP at the midpoint of the HR range, and A4 is the value of HR at the bottom plateau (28). The maximum gain of the baroreceptor reflex is defined as the maximum slope of the sigmoid curve (i.e., −ΔA1/ΔA2). The threshold and saturation pressure were calculated with the following equations: threshold pressure = A3 − 2.944/A2 and saturation pressure = A3 + 2.944/A2 (28).

**Immunohistochemistry for IL-6.** Naïve animals (n = 5) were transcardially perfused with 4% paraformaldehyde. The brain stem was then removed, incubated with 4% paraformaldehyde for at least 24 h, and transferred to PBS containing 30% sucrose. Serial sections (30 μm) through the NTS were obtained using a cryostatic microtome. The sections were rinsed in PBS, placed in 10% serum with 0.3%...
Trigon X-100 for 15 min at room temperature, rinsed again, and then incubated with an IL-6 antibody (sc-1265, dilution 1:100–200 in PBS with 1% serum and 0.3% Trigon X-100; Santa Cruz Biotechnology, Santa Cruz, CA). After overnight incubation at 4°C, the sections were rinsed in PBS and incubated with biotinylated horse anti-IgG (dilution 1:500; Vector Laboratories) for 1 h. The sections were rinsed and then incubated in streptavidin-conjugated Alexa-Fluor 488 (dilution 1:500; Molecular Probes) for 1 h. Finally, sections were washed in PBS before mounting in Vectashield (Vector Laboratories). Sections were photographed using a scanning laser confocal microscope (LSM 5 Pascal; Carl Zeiss).

To determine the cell types expressing IL-6, we used double-labeling fluorescence immunohistochemistry with either a glial cell marker (anti-GFAP; Invitrogen) or neuron marker (anti-NeuN; Millipore).

Gene expression profiles of IL-6 receptor and gp-130 in the NTS. As far as we know, no standard antibodies for IL-6 receptor (IL-6R), which can be used for the rat brain, are commercially available. We therefore assessed gene expression of IL-6R by performing RT-PCR to test whether IL-6 potentially exert a functional role at the level of the NTS. Because IL-6R acts with the common signal transduction component, gp-130 (also called CD130), gene expression of gp-130 was also assessed. First, the rats (300–325 g, n = 5) were humanely killed by cervical dislocation. The NTS between 1.0 mm rostral and 0.5 mm caudal to the calamus scriptorius from each animal was rapidly dissected out of the brain stem by fine forceps under a dissecting microscope and homogenized in 400 μl TRIzol reagent (Invitrogen, Carlsbad, CA). To avoid contamination with genomic DNA, the RNA samples were treated with RNase-free DNase I (Bio-Rad Laboratories, Hercules, CA), and the QuantiFast SYBR Green RT-PCR kit (Qiagen) was used according to the manufacturer’s protocol as described previously (32). Expression of target genes was confirmed by melting curve analysis and gel electrophoresis.

Statistical analysis. All values are expressed as means ± SE for each group. To evaluate dose-dependent effects, comparisons of maximum elicited responses in MAP and HR with different dosages were made using one-way ANOVA or one-way repeated-measures ANOVA followed by Scheffé’s test. Comparisons between two groups (e.g., data obtained before and after antagonist treatment) were evaluated using the Student’s paired t-test. The criterion for statistical significance was set at P < 0.05.

RESULTS

All data were obtained from Wistar rats unless indicated as the WKY strain.

IL-6 expression within the NTS. The expression of IL-6 protein in the brain stem was immunohistochemically identified. We found that IL-6 was widely distributed in the brain stem area, including the dorsal vagal nucleus, hypoglossal nucleus, caudal/rostral ventrolateral medulla, and NTS. However, it was extensively colocalized with NeuN, a neuronal marker, but not with GFAP, a glial cell marker (data not shown), indicating that IL-6 is expressed in neurons predominantly (Fig. 1). RT-PCR also revealed that mRNA of both IL-6R and gp-130 was expressed in the NTS, demonstrating that the NTS is a central site where endogenous IL-6 can exert a functional role (Fig. 1).

Effects of IL-6 microinjection in the NTS on baroreceptor reflex. The baseline levels of MAP and HR in urethane-anesthetized rats were 84.7 ± 1.5 mmHg and 396 ± 11 beats/min, respectively (n = 22). Unilateral administration of IL-6 (1, 10, and 100 fmol) in the NTS, where barosensitive neurons are located predominantly, did not affect the baseline levels of MAP (e.g., 100 fmol, before: 84.8 ± 2.7 mmHg, after: 87.4 ± 4.7 mmHg, n = 6) and HR (e.g., 100 fmol, before: 390 ± 22 beats/min, after: 390 ± 22 beats/min).
386 ± 22 beats/min, n = 6; Figs. 2 and 3). The baseline level of
the baroreceptor bradycardiac reflex gain ($\Delta$HR/$\Delta$MAP) was
$-1.67 \pm 0.16$ beats·min$^{-1}$·mmHg$^{-1}$ ($n = 22$). This was signifi-
cantly inhibited by IL-6 injections in the same area of NTS (e.g.,
100 fmol, before: $-1.44 \pm 0.23$ beats·min$^{-1}$·mmHg$^{-1}$, after:
$-0.90 \pm 0.13$ beats·min$^{-1}$·mmHg$^{-1}$, $P < 0.05$, $n = 6$; Figs. 2
and 3). This inhibitory effect on reflex gain was found to be
dose dependent (1 fmol, 11.0 ± 3.3%; 10 fmol, 20.4 ± 3.7%; 100 fmol, 36.7 ± 3.5%; Fig. 4) and reversible (Figs. 2
and 3). The reflex gain measured by logistic sigmoidal
curves between HR and MAP changes was also found to be
decreased significantly by IL-6 injections (100 fmol, before:
$-1.98 \pm 0.13$ beats·min$^{-1}$·mmHg$^{-1}$, after: $-0.89 \pm 0.35$
beats·min$^{-1}$·mmHg$^{-1}$, $P < 0.05$, $n = 5$; Table 1 and Fig. 5).
The HR range was also significantly reduced by IL-6 injections
(before: 101.9 ± 2.3 beats/min, after: 62.4 ± 8.0 beats/min,
$P < 0.05$, $n = 5$; Table 1 and Fig. 5) while the midpoint of
MAP was not altered (Table 1). We also confirmed that
microinjection of vehicle had no significant effect on the
baseline level of MAP, HR, and baroreceptor bradycardiac
reflex gain (data not shown). Moreover, IL-6 microinjections
(100 fmol) in the area where chemosensitive neurons are
dominantly located did not affect any cardiovascular parame-
ters, including the baroreceptor bradycardiac gain [before:

\[
\begin{align*}
\text{Baseline MAP} & = 90 
\text{Baseline HR} & = 400 
\text{Baroreceptor reflex gain} & = -1.0
\end{align*}
\]

$*P < 0.05$ between before and after IL-6 administration.

![Fig. 2. Representative recordings illustrating the inhibition of the baroreceptor reflex by unilateral IL-6 (100 fmol) microinjection in the NTS. Baroreceptor
cardiac reflex was induced by iv injection of phenylephrine (15 μg/kg). AP, arterial pressure; MAP, mean AP; HR, heart rate.]

![Fig. 3. Group data showing the effects of unilateral IL-6 microinjection (100 fmol) in the NTS on MAP, HR, and cardiac baroreceptor reflex gain ($\Delta$HR/$\Delta$MAP). The baseline levels of MAP and HR were not changed, whereas baroreceptor reflex gain was inhibited significantly by IL-6 injection. \( *P < 0.05 \) between before and after IL-6 administration.]

![Fig. 4. Group data showing the dose-dependent inhibitory effects of IL-6 (1, 10, and 100 fmol) microinjected in the NTS on baroreceptor reflex gain. The inhibitory effect was more pronounced when a higher concentration of IL-6 was microinjected in the NTS. However, this effect was inhibited by NTS microinjection of both IL-6 (100 fmol) with IL-6 blocking antibody. Saline was used as a control. \( *P < 0.05 \) and \( **P < 0.01 \) vs. saline; \( $$$P < 0.01 \) vs. 1 fmol; \( #P < 0.05 \) vs. 10 fmol; and \( ++P < 0.01 \) vs. 100 fmol.]

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IL-6 in NTS Attenuates Baroreflex

Table 1. Parameters describing modulation of cardiac baroreceptor reflex function by IL-6 in NTS

<table>
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<tr>
<th></th>
<th>n</th>
<th>Midpoint, mmHg</th>
<th>Lower Plateau, beats/min</th>
<th>Upper Plateau, beats/min</th>
<th>Maximum Gain, beats·min⁻¹·mmHg⁻¹</th>
<th>HR Range, beats/min</th>
</tr>
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<tbody>
<tr>
<td>Before IL-6 microinjection</td>
<td>5</td>
<td>99.4±1.0</td>
<td>353±17</td>
<td>455±17</td>
<td>−1.98±0.13</td>
<td>101.9±2.3</td>
</tr>
<tr>
<td>After IL-6 microinjection</td>
<td>5</td>
<td>98.6±1.1</td>
<td>377±15</td>
<td>427±26</td>
<td>−0.89±0.35*</td>
<td>62.4±8.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. IL-6, interleukin-6; NTS, nucleus tractus solitarii; HR, heart rate. Parameters were derived from sigmoidal curve of best fit for the mean arterial pressure-HR relationships (see text). Maximum gain is gain of the sigmoidal curve. IL-6 (100 fmol) microinjected in the NTS attenuated cardiac baroreceptor reflex function. *P < 0.05 vs. before IL-6 microinjection.

−1.99 ± 0.20 beats·min⁻¹·mmHg⁻¹, after: −1.90 ± 0.18 beats·min⁻¹·mmHg⁻¹, not significant (NS; n = 4).

With regard to WKY, the baseline level of the bradycardiac reflex gain (i.e., ΔHR/ΔMAP) in WKY was found to be lower than Wistar rats (−0.84 ± 0.08 beats·min⁻¹·mmHg⁻¹, n = 5 vs. −1.44 ± 0.23, n = 6, P < 0.05), showing a species-related difference in the basal level of reflex function. However, we found that IL-6 microinjected in the NTS of WKY also inhibits the baroreceptor bradycardiac gain (100 fmol, −0.66 ± 0.07 beats·min⁻¹·mmHg⁻¹, n = 5, P < 0.01), suggesting that the IL-6 effects on the reflex are common physiological functions, at least in normotensive rats.

Effects of IL-6 blocking antibody on IL-6-induced attenuation of baroreceptor reflex. The baroreceptor bradycardiac reflex gain was −35% decreased by microinjection of IL-6 (100 fmol) as shown above (see Fig. 4). On the other hand, NTS microinjection of IL-6 (100 fmol) with IL-6 blocking antibody did not affect the level of bradycardiac reflex gain (ΔHR/ΔMAP, before: −1.92 ± 0.45 beats·min⁻¹·mmHg⁻¹; after: −1.75 ± 0.42 beats·min⁻¹·mmHg⁻¹, P = 0.12, n = 5, see Fig. 4). We also confirmed that IL-6 blocking antibody itself did not affect the level of reflex gain (data not shown).

Effects of IL-6 microinjection in the NTS on L-glutamate-induced cardiovascular responses. To test whether IL-6-mediated changes in baroreceptor reflex function are because of modulation of NTS neurons, the effect of IL-6 microinjection in the NTS on L-glutamate-induced cardiovascular responses was tested. Unilateral administration of L-glutamate (0.5 or 3.4 nmol) in the NTS significantly decreased both MAP and HR (Figs. 6 and 7). After IL-6 microinjection in the NTS, L-glutamate-induced bradycardia was significantly attenuated (ΔHR, 0.5 nmol: −108.3 ± 9.2 vs. −90.5 ± 6.6 beats/min, P < 0.01, n = 5; 3.4 nmol: −66.8 ± 8.8 vs. −45.2 ± 10 beats/min, P < 0.01, n = 6; Figs. 6 and 7), whereas L-glutamate-induced MAP changes were not altered by IL-6 injection in the NTS (ΔMAP, 0.5 nmol: −32.5 ± 2.9 vs. −39.1 ± 2.8 mmHg, NS, n = 5; 3.4 nmol: −30.9 ± 3.1 vs. −26.9 ± 3.4 mmHg, NS, n = 6; Figs. 6 and 7). It should be noted that similar cardiovascular responses were also found when ACh (500 pmol) was tested. ACh-induced bradycardia was attenuated significantly (ΔHR, before: −75.4 ± 9.4 beats/min; after: −56.3 ± 6.6 beats/min, P < 0.01, n = 5), whereas ACh-induced MAP changes were not altered by IL-6 injection in the NTS (ΔMAP, before: −34.1 ± 2.8 mmHg; after: −31.9 ± 2.8 mmHg, NS, n = 5).

Histological verification. Figure 8 shows a photomicrograph of a coronal section of the brain stem of a rat representative of the group that received unilateral microinjection of drugs in the NTS. We confirmed that microinjection sites were precisely within the restricted area of the NTS in all rats used for data analysis.

Discussion

We have demonstrated for the first time that IL-6 modulates cardiovascular control at the level of the NTS of normotensive rats. The cardiac baroreflex function evaluated by measuring full functional curves between MAP and HR changes was inhibited by IL-6 microinjected in the NTS. Moreover, we confirmed that the cardiac baroreflex in response to increased arterial pressure was dose-dependently attenuated by IL-6 microinjected in the NTS. However, it should be noted that IL-6 microinjections did not modulate the set point of arterial pressure. Because IL-6 attenuated L-glutamate-induced bradycardia at the level of the NTS, we propose that IL-6 may be acting postsynaptically to inhibit NTS neurons controlling HR. Moreover, we identified gene expression of both the IL-6R and signal-transducing component gp-130 at the level of the NTS. Finally, immunohistochemical detection of IL-6 demonstrated that NTS neurons may be a potential source for IL-6 production and release.

There are several reports demonstrating a functional association between systemic IL-6 and blood pressure. For example, Chae et al. (3) demonstrated that plasma levels of IL-6 were strongly associated with high blood pressure. Furthermore, stress or ANG II-induced hypertension was attenuated in animals deficient of IL-6 (5, 17). These findings suggest that systemic IL-6 is important functionally for the hypertensive state. In this study, we identified that IL-6 can also act in a brain region that controls the baroreceptor reflex and that a functional role of it is to attenuate the cardiac baroreceptor reflex. Indeed, an attenuated cardiac baroreceptor reflex gain is...
found in both SHR and humans with essential hypertension (11, 12, 15, 24). We surmise that altered IL-6 levels in the NTS may be a potential novel mechanism underlying the development and maintenance of hypertension. However, we previously found that IL-6 gene was less expressed in the NTS of SHR compared with WKY (34). We suggest that the lower gene expression profile of IL-6 in the NTS of the SHR may not be causative for the development of hypertension but secondary (i.e., responsive) to the hypertensive condition, perhaps acting as a compensatory mechanism for the attenuated cardiac baroreceptor reflex gain. This hypothesis is also supported by our previous finding that the level of IL-6 gene expression in the NTS remains normal in young prehypertensive SHR compared with their age-matched WKY (34), suggesting it is secondary to the hypertension itself.

As with many cytokines, astrocytes are known to be a major source of IL-6 within the central nervous system (30). How-

![Fig. 6. Representative recordings illustrating the inhibition of L-glutamate (3.4 nmol)-induced bradycardia by unilateral IL-6 (100 fmol) microinjection in the NTS. It should be noted that the L-glutamate-induced hypotensive effect was not affected by IL-6 administration.](image)

![Fig. 7. Group data showing the effects of unilateral IL-6 microinjection (100 fmol) in the NTS on L-glutamate (3.4 nmol)-induced cardiovascular responses. L-glutamate-induced bradycardia was inhibited significantly while the hypotension was not affected by a unilateral IL-6 (100 fmol) microinjection in the NTS. **P < 0.01 compared with before IL-6 administration. $$$P < 0.01 compared with after recovery.](image)

![Fig. 8. Histological identification of microinjection sites. Coronal section (30 μm, CS) of the medulla ~0.5 mm rostral to the CS. Left: arrow indicates the site of microinjection in the NTS (0.5 mm lateral and 0.5 mm deep from the dorsal surface of the medulla), marked by Indian ink (100 nl). Right: major nuclei in that region are shown. ap, Area postrema; cc, central canal; dmnX, dorsal motor nucleus of vagus; gr, gracile nucleus; nts, nucleus tractus solitarius; nXII, hypoglossal nucleus; py, pyramids. The microinjection sites were within the NTS in all rats used for data analysis.](image)
ever, in this study, we demonstrated that neurons may be a source of IL-6 in the NTS of Wistar rats. It should be noted that this finding is not limited to the NTS. IL-6 protein expression has also been found in neurons in other parts of brain areas, including the hippocampus and hypothalamus (13). D’Arcangelo et al. (6) found that IL-6 inhibits glutamate release in the cerebral cortex, suggesting that IL-6 may act as a neuronal modulator of excitatory neurons. Because the inhibitory effect was rapid and transient, it was suggested that IL-6R-activated tyrosine phosphorylation processes modulate synaptic transmission independently from activation of transcription (6). This may be one mechanism to explain the attenuated baroreceptor reflex by IL-6 microinjections in the NTS, since glutamate release from the baroreceptor afferent terminals excites barosensitive second-order neurons in the NTS to produce the bradycardic effect (27). In addition, because we found that IL-6 in the NTS inhibited the bradycardia induced by L-glutamate microinjection, IL-6 may also affect NTS neurons postsynaptically that mediate the baroreceptor reflex cardiac component. However, because the ACh-induced bradycardia from NTS was also attenuated by IL-6, it is likely that this cytokine is not restricted to a single transmission system (glutamate) or its receptors (NMDA, AMPA, or kainate). Whether IL-6-positive neurons found in the NTS directly innervate afferent terminals or the neurons mediating the baroreceptor reflex remains unknown. Moreover, IL-6 could be mediated by actions on gamma-aminobutyric acid (GABA) containing NTS neurons (6). Consistent with our findings with IL-6, activation of GABA receptors in the NTS is known to reduce the gain of baroreflex bradycardia without alteration in the baseline of arterial pressure and HR (2, 22). Because our findings demonstrate that there appears to be no IL-6-mediated effect on the set point of arterial pressure and basal levels of HR despite alterations in the cardiac baroreflex gain, mechanisms mediated by GABAergic interneurons may be involved in IL-6-induced attenuation of baroreceptor cardiac reflex. Finally, the issue of how IL-6 modulates HR but not arterial pressure also requires comment. Our observation is consistent with the ability to modulate these baroreflex components (arterial pressure and HR) independently as recently discussed (24). The ability of IL-6 to selectively attenuate the cardiac component induced by L-glutamate within the NTS suggests that some neurons are already committed to cardiac parasympathetic vs. sympathetic outflows. We would postulate that IL-6Rs are expressed on neurons that ultimately control cardiac vagal motoneurons but not on circuitry regulating sympathetic outflow. To confirm this, future work will need to assess the effects of IL-6 in NTS on the sympathetic component of baroreflex.

In the present study, we focused on studying the acute cardiovascular regulation of IL-6 at the level of the NTS. However, chronic effects of IL-6 in the NTS on the cardiovascular system should also be considered, since IL-6 mRNA level in the brain is known to increase during postnatal development (10), indicating that IL-6 expression may be important for the normal development of brain function. Indeed, IL-6 has crucial roles to promote neuronal survival and astrocyte differentiation (20). The NTS may not be excluded from the brain regions where IL-6 plays these functional roles. Although Lee et al. (16, 17) found that the resting levels of arterial pressure and HR were not altered in IL-6-deficient animals, chronic manipulation of IL-6 gene expression in the NTS will be required to characterize the long-term effects of IL-6 in the NTS on the cardiovascular system. More importantly, however, the acute hypertensive effect of mental stress appeared to be attenuated in animals deficient of IL-6. These altered pressure responses may be partially explained by the inhibitory effect of IL-6 on baroreceptor reflex function at the level of NTS as described herein. Moreover, we speculate that a similar scenario may explain the blunted hypertensive effects of ANG II in animals deficient of IL-6. Clearly, further experiments including assessment of baroreflex function in IL-6 knockout animals will be required.

In summary, our findings indicate that IL-6 within the NTS may play an important role in the acute regulation of the cardiovascular system via modulation of input signals from baroreceptor afferents. Because psychosocial stress or ANG II-induced hypertension was attenuated in animals deficient of IL-6 (5, 16, 17), this cytokine acting within the NTS may play an important role under these conditions. Moreover, the lower gene expression profile of IL-6 in the NTS of the SHR found in our previous study (34) may be secondary to the hypertensive condition, acting as a compensatory mechanism for the attenuated cardiac baroreceptor reflex gain. Mechanisms underlying IL-6 release and subsequently attenuation of baroreceptor reflex at the level of the NTS have yet to be elucidated. Finally, to allow translation of our work, these novel insights need to be assessed in conscious animals.

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**DISCLOSURES**

No conflicts of interest are declared by the authors.

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