Recovery of High Blood Pressure After Chronic Lesions of the Commissural NTS in SHR

Monica Akemi Sato, Gerhardus Hermanus Maria Schoorlemmer, José Vanderlei Menani, Oswaldo Ubríaco Lopes, Eduardo Colombari

Abstract—Acute electrolytic lesions of the commissural nucleus of the solitary tract (commNTS) reduce blood pressure (BP) in SHR but not in normotensive Wistar-Kyoto and Wistar rats and abolish the pressor response to intravenous injection of potassium cyanide. We investigated the chronic effect of commNTS lesions on mean arterial pressure (MAP), and on baroreceptor and chemoreceptor reflex responses in SHR. The contribution of the sympathetic nervous system and the hormones vasopressin and angiotensin II to maintenance of BP in lesioned SHR was also investigated. MAP fell to normotensive levels the day after lesioning the commNTS but returned to the hypertensive level 9 days later. The reflex tachycardia evoked by sodium nitroprusside remained attenuated for 10 days after commNTS lesions but became enhanced 30 days after commNTS lesions. The pressor component of the chemoreflex elicited by potassium cyanide remained blocked for 30 days after lesions. Vasopressin antagonist or ACE blocker did not change MAP in sham or commNTS-lesioned SHR. Ganglionic blockade with hexamethonium elicited similar reductions in MAP in sham and commNTS-lesioned SHR. Results demonstrated that commNTS lesions in SHR produce a transient fall in BP and a long-lasting inhibition of the pressor response of the chemoreflex. Therefore, the blockade of the pressor response to peripheral chemoreflex activation is not sufficient to chronically reduce MAP in SHR. In the chronic absence of the commNTS, other subnuclei of the NTS or other brain stem nuclei may reorganize to replace the function of commNTS neurons, restoring sympathetic activity and high BP in SHR. (Hypertension. 2003;42[part 2]:713-718.)

Key Words: baroreceptors ■ chemoreceptors ■ blood pressure ■ rats, spontaneously hypertensive

Blood pressure in spontaneously hypertensive rats (SHR) starts rising a few weeks after birth and reaches a hypertensive level 12 to 14 weeks later.1 The hypertension in this strain appears to be related to increased sympathetic activity.2,3

Previous studies have shown a link between increased chemoreceptor reflex sensitivity and high blood pressure in SHR.4 Anatomic studies showed that the carotid body is larger in SHR than in normotensive Wistar-Kyoto rats (WKY).5 Extracellular recordings from carotid sinus afferent nerves have also demonstrated that the sensitivity of arterial chemoreceptors to hypoxia is increased in adult SHR compared with normotensive WKY and Wistar rats.4 These observations suggest the involvement of arterial chemoreceptors in hypertensive mechanisms.4,6,7

The nucleus of the solitary tract (NTS) is the primary site of termination of afferent fibers arising from arterial baroreceptors and chemoreceptors.8,9 Our previous studies10 have shown that acute (from 1 to 4 days) electrolytic lesions of the commissural (comm) NTS reduce arterial pressure in SHR to normotensive levels but do not affect arterial pressure in normotensive WKY control rats. These lesions also abolished the pressor response of the chemoreflex evoked by potassium cyanide and attenuated the reflex tachycardia elicited by sodium nitroprusside either in SHR or in normotensive control rats.10 Acute inhibition of commNTS neurons by GABA microinjection reduced splanchnic sympathetic nerve activity, arterial pressure, and heart rate in anesthetized SHR but not in normotensive WKY and Sprague-Dawley rats.11 Therefore, it appears that the commNTS is important for the maintenance of high sympathetic nerve activity and arterial pressure in SHR.

Since previous studies only analyzed relatively acute effects (4 days) of commNTS lesions on cardiovascular function, the current study was designed to investigate the effect of chronic lesions of commNTS on blood pressure, heart rate, and the cardiovascular reflex responses in SHR. The neural and humoral mechanisms that maintain arterial pressure in chronic commNTS lesioned SHR were also analyzed.

Methods

Animals
Experiments were performed in adult male SHR (250 to 300 g, 14 to 16 weeks old) supplied by the Animal Care of the University of Sao Paulo, Brazil.
Paulo, which obtained this strain from B.R.L. Biological Laborato-
ries (Shizuoka, Japan). Rats were housed individually in a
temperature-controlled room with a 12-hour light/12-hour dark
cycle. Standard chow pellets and tap water were available ad libitum.
The medical ethics committee of the Universidade Federal de Sao
Paulo approved all the experiments before they were carried out. All
the experiments were performed in conscious, freely moving rats.

Cerebral Lesions
Rats were anesthetized with ketamine (50 mg/kg IP) and xylazine
(50 mg/kg IM) and placed in a stereotaxic apparatus (Stoelting
Laboratory Standard 51600). A partial craniotomy of the occipital
bone was performed, and the dorsal surface of the brain stem was
exposed. A tungsten electrode was positioned in the commNTS
(0.1 mm caudal to the calamus scriptorius, 0.4 mm below the dorsal
surface of the brain stem, on the midline) and a 3-mA current was
passed for 10 seconds, as we have previously described.12,13 Sham-
lesioned rats were submitted to the same procedures, but no electric
current was passed.

Arterial Pressure Recording and Intravenous Injection
Polyethylene tubing (PE-10, drawn above steam to a smaller diam-
eter, 4 cm), welded to PE-50, was inserted into the abdominal aorta
through the femoral artery for measurement of pulsatile arterial
pressure (PAP), mean arterial pressure (MAP), and heart rate (HR).
A silastic cannula (Dow Corning) was inserted into the femoral vein
for drug administration. Cannulas were tunneled subcutaneously to
the area between the shoulder blades and connected to stainless steel
“elbows” made of 23-gauge hypodermic tubing. One end of this
elbow protruded through the skin. The cannulas were filled with
sterile saline containing heparin (50 U/mL) and penicillin G (2000
U/mL=1.3 mg/mL), and the external end of the elbow was closed
with a plastic cap, as previously described.14 On the day of
experiments, polyethylene tubing filled with sterile saline was
connected to the external end of the elbow. PAP and MAP were
measured by connecting the arterial cannula to a strain gauge
transducer (Staham P23Db) connected to a data acquisition system
(Power Laboratory/4SP, AD Instruments). HR was measured from
the period between peaks in the arterial pressure waves.

Figure 1. A through C, Photomicrographs showing NTS of a single rat that received a lesion of the commNTS (arrow). D through F,
Drawing depicting the extent of commNTS lesion sites in the rostral-caudal axis at ~13.7, 14.1, and 14.6 mm caudal from bregma. AP
indicates area postrema; X, dorsal motor nucleus of the vagus; XII, hypoglossal nucleus; cc, central canal; cu, fasciculus cuneatus; Cu,
nucleus cuneatus; C1/A1, C1 and A1 catecholaminergic groups; gr, nucleus gracilis; LRT, nucleus reticularis lateralis; NA, nucleus
ambiguous; py, pyramid; pyX, pyramidal decussation; Sp5C, nucleus trigeminus spinalis caudalis; Sol, nucleus of the solitary tract; RVL,
rostral ventrolateral medulla; CVL, caudal ventrolateral medulla.
Histology
At the end of the experiments, rats were deeply anesthetized with an overdose of urethane (1.5 g/kg IV), and an intracardiac perfusion with saline followed by 10% formalin was performed. The brain stem was removed and stored in 10% formalin. Serial coronal sections (40 μm) were prepared and stained with neutral red. Only rats whose lesion sites were located in the commNTS were considered for data analysis. Lesions of the commNTS were located on the midline above the central canal and extended from the level of the obex to ~1 mm caudal to the obex (Figure 1). Lesions virtually completely destroyed the commNTS but did not destroy the area postrema or lateral regions of the NTS. The extent of the lesions was similar to that in our earlier studies.10,12,13,15

Statistical Analysis
All data are expressed as mean±SEM. The results were analyzed by 2-way ANOVA followed by the Tukey posttest for multiple mean comparisons. Significance level was set at P<0.05.

Experimental Protocol
Five days after cannulation of the femoral artery and vein, resting MAP and HR were recorded, and baroreceptor and chemoreceptor reflexes were tested. The chemoreflex was evoked by an intravenous bolus injection of potassium cyanide (40 μg/0.1 mL per rat), and the baroreflex was induced by pressor doses of phenylephrine (3 μg/kg body wt IV) and depressor doses of sodium nitroprusside (30 μg/kg body wt IV). Two days later, animals were submitted to sham or commNTS lesions. One, 10, 20, and 30 days after commNTS lesions, basal MAP and HR were recorded again, and chemoreceptor and baroreceptor reflexes were tested as before. Ten days after lesioning, blood pressure responses induced by an intravenous injection of the V1 receptor antagonist (Manning Compound, 10 μg/kg body wt IV) were measured, and 15 minutes later the blood pressure responses were measured to intravenous injection of an ACE inhibitor (captopril, 5 mg/kg body wt). Blood pressure responses to intravenous injection of the ganglionic blocker hexamethonium (10 mg/kg body wt) were measured 11, 20, and 30 days after lesioning.

Results
Effect of Chronic commNTS Lesions (10, 20, and 30 Days) on Basal MAP and HR
MAP and HR were low the day after lesioning the commNTS (Figure 2, n=10), as we have previously shown.19 However, in the chronic phase of commNTS lesions, MAP increased and returned to prelesion hypertensive levels. At 10, 20, and 30 days after commNTS lesions, MAP was not different from prelesion level or from sham-lesioned SHR (Figure 2). Sham lesions (n=9) produced no significant changes on MAP and HR compared with the control day (before sham lesions) (Figure 2).

Chemoreceptor Reflex Test
In commNTS-lesioned SHR (n=9), chemoreflex activation with potassium cyanide (KCN 40 μg/0.1 mL per rat) produced hypotension 1 day, 10 days, and 20 days after lesions or reduced pressor responses 30 days after lesions compared with prelesion response or with sham-lesioned SHR (n=8) (Figure 3). The bradycardia evoked by intravenous KCN was attenuated 1 day after commNTS lesions compared with sham-lesioned SHR (Figure 3).

Baroreceptor Reflex Test
CommNTS lesions did not alter the pressor response (49±4, 48±2, 41±4, 40±3 mm Hg at days 1, 10, 20, and 30) and the reflex bradycardia (−46±13, −47±4, −49±5, and −53±12 bpm at days 1, 10, 20, and 30) induced by intravenous injection of phenylephrine from the prelesion values (46±2 mm Hg and −73±16 bpm), and differences between sham and commNTS-lesioned SHR were not significant.

However, the lesions did change responses to intravenous injection of sodium nitroprusside. They attenuated the reflex tachycardia (1 and 10 days after lesions, Figure 4). Twenty days after lesions, reflex tachycardia had returned to normal, and 30 days after lesions it was enhanced, even though the fall in blood pressure was slightly smaller (Figure 4).

Effect of Vasopressin Receptor Blockade and Inhibition of ACE on Basal MAP and HR in SHR 10 Days After commNTS Lesions
Injection of the vasopressin receptor antagonist Manning compound (AVPx, 10 μg/kg body wt IV) did not significantly reduce MAP (161±9 mm Hg versus 163±10 mm Hg control baseline) or HR (292±26 bpm versus 286±16 bpm control baseline) in rats with a lesion in the commNTS and similarly had no effect in sham-lesioned control rats.

Figure 2. Basal MAP (mm Hg) and HR (bpm) in sham or commNTS-lesioned SHR on the control day (prelesion) and 1, 10, 20, and 30 days after lesions. *Different from prelesion; + different from sham.
Injection of the ACE inhibitor captopril (5 mg/kg body wt IV, injected 15 minutes after AVPx) had no effect on MAP and HR in sham or commNTS-lesioned SHR (155±10 mm Hg and 311±21 bpm versus 159±9 mm Hg and 296±25 bpm, control baseline).

**Effect of Ganglionic Blockade in SHR 11, 20, and 30 Days After commNTS Lesions**

Ganglionic blockade with intravenous injection of hexamethonium (10 mg/kg body wt) at 11, 20, and 30 days after commNTS lesions produced similar reductions in MAP in sham and commNTS-lesioned SHR (Figure 5). Hexamethonium-induced changes in HR were small except for an increased tachycardia 30 days after commNTS lesions.

**Discussion**

The present study shows that commNTS lesions produce a dramatic acute fall in arterial pressure in SHR, confirming our previous findings. This previous study shows that blood pressure remains reduced for 4 days after commNTS lesion but did not investigate longer-term effects. The present results show that 10 days after lesioning, blood pressure had returned to the normal hypertensive level, and it remained there for the duration of the study. Although the hypotension induced by the lesions was transient, the lesions caused a permanent impairment in the pressor component of the chemoreflex evoked by potassium cyanide. The restoration of arterial pressure in SHR after lesions of the commNTS suggests that the role of the commNTS in the maintenance of hypertension in SHR was replaced by other central nuclei. The idea of reorganization is supported by the restoration, by 20 days, of the reflex tachycardia evoked by sodium nitroprusside. In fact, this reflex tachycardia appeared to be hyperactive 30 days after the lesion. In addition, 30 days after lesioning, it appeared that the pressor response of the chemoreflex started to recover. Since both the commNTS and the intermediate NTS receive inputs of the primary afferents arising from baroreceptors and chemoreceptors, it is possible that the function of commNTS neurons was replaced by neurons of the intermediate NTS.
Previous studies have indicated that the activity of arterial chemoreceptors in SHR is increased under resting conditions. This could lead to sympathetic hyperactivity, which would contribute to the high blood pressure in SHR. Our previous studies have shown that acute electrolytic lesions of the commNTS in SHR abolished the pressor component of the chemoreflex induced by potassium cyanide. The present study shows that blood pressure recovered to hypertensive levels within 10 days after lesions, although the pressor component of the chemoreceptor reflex was absent. However, the bradycardic component of the chemoreflex was not abolished after chronic commNTS lesions. These results suggest that signals arising from these receptors could reach the brain and be processed in nuclei adjacent to the commNTS such as the intermediate NTS. This portion of the NTS projects to the nucleus ambiguus and to the dorsal motor nucleus of the vagus to excite the parasympathetic neurons to the heart that evoke the bradycardic response of the chemoreflex. These findings also indicate that the arterial chemoreceptors send signals through afferent fibers to the NTS despite the lesion in the commNTS. The sympathetic and parasympathetic components of the chemoreflex response appear to undergo a dissociation in different subnuclei of the NTS in SHR. These data also suggest that arterial chemoreceptors are not essential to recover and maintain high blood pressure after commNTS lesions in SHR.

Because the fall in blood pressure induced by commNTS lesions was transient, we tested if humoral factors contributed to the restoration of blood pressure. Our results suggest that neither vasopressin nor angiotensin II was contributing to arterial pressure. On the other hand, ganglionic blockade with hexamethonium decreased arterial pressure in commNTS-lesioned SHR, but this response was not different from sham-lesioned SHR. That finding suggests that sympathetic activity probably is similar in sham-lesioned or chronic commNTS–lesioned SHR. Previous studies have shown that sympathetic nerve activity is increased in SHR. The components of this network are not completely known, but it is possible that the commNTS is part of this network. The acute inhibition of commNTS neurons decreases the splanchnic sympathetic nerve activity in SHR but not in normotensive control Wistar-Kyoto or Sprague-Dawley rats. Therefore it is possible that this subnucleus of the NTS participates in the central network that controls sympathetic activity in SHR. Since the inhibition of commNTS neurons decreases the sympathetic nerve activity acutely but no difference was observed in sympathetic activity after chronic lesions of the commNTS, it is likely the central network underwent reorganization to reestablish the high sympathetic activity in SHR.

In normotensive Wistar rats, lesions of the commNTS attenuate the reflex tachycardia to sodium nitroprusside and the pressor response to chemoreflex activation without changing arterial pressure. The differences between the effect of commNTS lesions in SHR and in normotensive rats suggest that the commNTS plays an important role in the maintenance of increased sympathetic activity and high blood
pressure in SHR, whereas in normotensive rats, these neurons are not involved in the maintenance of blood pressure.

Lesions of the commNTS failed to chronically reduce arterial pressure in SHR. Previous studies showed that chronic lesions of the rostral ventrolateral medulla with N-methyl-D-aspartic acid (NMDA) acutely reduced blood pressure in SHR, but the hypertension returned 15 days after lesions.25 Other studies showed that bilateral lateral tegmental lesions interrupt the dorsal noradrenergic bundle and deplete forebrain norepinephrine. However, these lesions do not prevent the development of hypertension in SHR.26 Taken together, this evidence suggests that the central nervous system or different mechanisms are activated chronically to reestablish the high blood pressure in SHR.

Perspectives
Although the reduction of blood pressure by commNTS lesions is transient, the reduction suggests that the commNTS plays an important role in the maintenance of high sympathetic activity and blood pressure in SHR. The recovery of high blood pressure in SHR with chronic commNTS lesions in the absence of a pressor response to chemoreflex activation suggests that arterial chemoreflex activity is not essential to recover and maintain hypertension after commNTS lesions in SHR. In the absence of the commNTS, other subnuclei of the NTS or other brain stem nuclei appear to reorganize to replace the function of the commNTS, increasing sympathetic activity and recovering the high blood pressure in SHR. Further studies are necessary to evaluate the mechanisms activated by commNTS to increase sympathetic activity and blood pressure and identify the brain areas related to these effects. Such studies may provide more information on brain mechanisms involved in the maintenance of hypertension in SHR.

Acknowledgments
This study was supported by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq-PRONEX).

References
17. Chitravanshi VC, Sapru HN. A midline area in the commissural sub-nucleus of NTS mediates the phrenic nerve responses to carotid chemoreceptor stimulation. Brain Res. 1994;662:127–133.
Recovery of High Blood Pressure After Chronic Lesions of the Commissural NTS in SHR
Monica Akemi Sato, Gerhardus Hermanus Maria Schoorlemmer, José Vanderlei Menani, Oswaldo Ubriaco Lopes and Eduardo Colombari

Hypertension. 2003;42:713-718; originally published online August 4, 2003;
doi: 10.1161/01.HYP.0000086523.51029.EC

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/42/4/713

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office.
Once the online version of the published article for which permission is being requested is located, click Request
Permissions in the middle column of the Web page under Services. Further information about this process is
available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/