Enhanced pressor response to carotid occlusion in commNTS-lesioned rats: possible efferent mechanisms

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Sato, Monica Akemi, Jose´ Vanderlei Menani, Oswaldo Ubríaco Lopes, and Eduardo Colombari. Enhanced pressor response to carotid occlusion in commNTS-lesioned rats: possible efferent mechanisms. Am J Physiol Regulatory Integrative Comp Physiol 278: R1258–R1266, 2000.—Bilateral common carotid occlusion (BCO) produces the cardiovascular responses produced by BCO in conscious rats and 2) the autonomic and humoral mechanisms activated to produce the pressor response to BCO in sham- and commNTS-lesioned rats. Both the peak and plateau of the pressor response produced by BCO increased in commNTS-lesioned rats despite the impairment of chemoreflex responses induced by intravenous potassium cyanide. In sham rats sympathetic blockade with intravenous prazosin and metoprolol, but not vasopressin receptor blockade with the Manning compound, reduced both components of BCO. In commNTS-lesioned rats the sympathetic blockade or vasopressin receptor blockade reduced both components of BCO. The results showed 1) the sympathetic nervous system, but not vasopressin, is important for the pressor response to BCO during 60 s in conscious sham rats; 2) in commNTS-lesioned rats, despite chemoreflex impairment, BCO produces an increased pressor response dependent on sympathetic activity associated with vasopressin release; and 3) the increment in the pressor response to BCO in commNTS-lesioned rats seems to depend only on vasopressin secretion.

arterial pressure; nucleus of the solitary tract; baroreceptor; chemoreceptor; potassium cyanide; sympathetic; vasopressin

BILATERAL COMMON CAROTID artery occlusion (BCO) produces a pressor response that has been suggested to depend on baro- and chemoreflexes integrated at different levels of the central nervous system (1, 2, 6, 12, 13, 15). BCO lasting 60 s in unanesthetized freely moving rats induces a pressor response that can be divided into two components: 1) an initial peak that arises within the first 20 s and 2) a plateau of lower value that arises within the last 30 s (1, 2, 13, 14).

The peak of the pressor response to BCO is attributed to carotid baro- and chemoreceptor reflexes and is integrated mainly at the medullary level. These same mechanisms with the addition of cerebral ischemia produce a maintained plateau (2, 12, 13). The aortic baroreceptors act to limit the peak and the plateau of the pressor response to BCO, and the result of aortic baroreceptor denervation is an increase in the peak and plateau during BCO (13, 15).

It also has been suggested that sympathetic activation and vasopressin secretion are the efferent mechanisms activated during BCO (9). In dogs, sympathetic nervous system and vasopressin may interact to produce the pressor response (9). Very few studies on mechanisms activated during BCO were performed in unanesthetized animals, and to our knowledge there is no published study showing the efferent mechanisms involved in the pressor response to BCO in conscious rats.

The full development of the pressor response to BCO depends on hypothalamic areas. Lesions of the anteroventral third ventricle region reduce both components of the pressor response to BCO, whereas hypothalamic lesions disconnecting the anterior and middle hypothalamus or lesions of the medial forebrain bundle abolish the plateau of the pressor response (1, 2, 13, 15).

Neuroanatomical studies have shown that arterial baro- and chemoreceptor afferent fibers terminate in the commissural nucleus of the solitary tract (commNTS), (5, 10). According to some electrophysiological studies, it appears that commNTS neurons are sensitive mainly to carotid chemoreceptor stimulation (3, 4). In rats, a recent study showed that electrolytic lesions of the commNTS abolished the pressor response and bradycardia induced by peripheral chemoreceptor activation with potassium cyanide (KCN) without significant changes in baroreflex activity (7). These results suggest that although baro- and chemoreceptor afferent inputs to NTS appear to overlap in the commissural subnucleus, the termination of baroreceptor afferents, in comparison to chemoreceptor afferents, is more widespread, involving regions other than the commNTS. An unexpected result described in the present study is the increased pressor response to BCO in commNTS-lesioned rats.

Therefore, considering the importance of baro- and chemoreceptor reflexes for the pressor response to...
BCO, the effects of commNTS lesions on chemoreflex responses, and the lack of information about the efferent mechanisms responsible for the pressor response to BCO in conscious rats, in the present study we sought to investigate 1) the effects of commNTS lesions on the cardiovascular responses induced by BCO and 2) the involvement of the sympathetic nervous system and vasopressin in the pressor response produced by BCO in unanesthetized sham- and commNTS-lesioned rats.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 300–400 g were used. All experiments were performed on conscious freely moving rats. The Medical Ethics Committee of the Universidade Federal de São Paulo approved the study.

Cerebral lesion. One day before the experiments, rats were anesthetized with 2% halothane mixed with oxygen (100%) and placed in a stereotaxic apparatus (David Kopf Instruments, model 1940). Partial craniotomy was performed through the occipital bone, and the dura mater and arachnoid were incised to expose the dorsal surface of the brain stem. Electrolytic lesions were performed with a tungsten electrode (0.010 inch, A-M Systems) inserted into the brain stem by means of a micromanipulator using the following coordinates: 0.0 mm lateral and 0.1 mm posterior to the calamus scriptorius, and 0.5 mm below the dorsal surface of the brain. The electrode was connected to the cathodal pole of a direct current (DC) lesion maker (Grass Instruments), and a wire from the anode was attached to the neck muscles with a d.i.p. Lesions were produced by a 3-mA cathodal current delivered for 10 s. Sham-operated (control) rats underwent the same procedures, but no current was passed. After placement of electrolytic or sham lesion, the neck muscles were sutured closely.

Bilateral carotid occlusion. Following the cerebral lesion, with the animals still under halothane anesthesia, we exposed the common carotid arteries through an incision in the ventral cervical region. A pneumatic cuff was adjusted around each carotid artery, and the polyethylene tube (PE-50) connected to the cuff was tunneled and fixed to the back of the rat neck. The preparation of the cuffs has been described in detail elsewhere (1, 2, 14, 15). During the experiments, the common carotid arteries were occluded bilaterally simultaneously for 60 s in conscious, freely moving rats. For the occlusion, the two cuffs were connected to a 1-ml syringe filled with isotonic saline using Y-shaped PE-50 tubing.

Arterial pressure recording and intravenous injection. Following the implant of the cuffs around the carotid arteries, we inserted a polyethylene cannula (PE-10 connected to PE-50) into the abdominal aorta through the femoral artery for measurement of pulsatile arterial pressure (PAP), mean arterial pressure (MAP), and heart rate (HR). A second cannula was inserted into the femoral vein for drug administration. Both cannulas were tunneled subcutaneously, exposed, and fixed on the back of the rat neck to allow access when the animal was conscious. PAP and MAP were measured using a strain gauge transducer (Statham P23 Db) connected to a low-level DC preamplifier coupled to a polygraph (Beckman model R-611). HR was derived by a cardiota-ometer (Coupler type 9857B) from arterial pressure waves.

Experimental protocols. All studies were performed in conscious animals 24 h after electrolytic or sham lesion. The pressor responses to BCO were studied in sham- and commNTS-lesioned rats. In each rat baro- and chemoreceptor reflexes were tested after BCO. The baroreflex was activated with pressor doses of phenylephrine (3 µg/kg iv, Sigma) and depressor doses of sodium nitroprusside (30 µg/kg iv, Research Biochemicals). The chemoreflex was tested with KCN (20 and 40 µg·0.1 ml−1·rat−1·iv, Sigma).

To study possible autonomic and humoral components involved in the pressor response induced by BCO, the animals were submitted to two different protocols. In the first protocol, after a control BCO, the animals were treated with the α1-adrenergic antagonist prazosin (1 mg/kg iv, Sigma) and 10 min later a second BCO was performed. Immediately after the second occlusion, and before the effect of prazosin, the animals were treated with the β1-adrenergic antagonist metoprolol (1.7 mg/kg iv, Sigma), and 5 min later a third BCO was performed. Finally, under the effect of prazosin and metoprolol, the animals were treated with vasopressin receptor antagonist (AVPx, 10 µg/kg iv, Mannling compound, Sigma) and 30 min later another BCO was performed.

In the second protocol, in a different group of animals, BCO was similarly performed but the sequential order of drug administration was different (AVP, prazosin, and metopro-

Statistical analysis. All data are expressed as the means ± SE. The peak and the plateau of the pressor response to BCO in both sham- and commNTS-lesioned rats submitted to different treatments were analyzed by two-way analysis of variance (ANOVA), followed by the Newman-Keuls post-test for multiple mean comparisons. Basal MAP and HR of sham- and commNTS-lesioned rats submitted to different treatments were also analyzed by two-way ANOVA followed by the Newman-Keuls post-test for multiple mean comparisons. Chemoreceptor and baroreceptor reflex tests were analyzed by the Student t-test. Differences were considered significant at P < 0.05.

Histology. At the end of the experiments, animals were anesthetized with urethane (1.2 g/kg iv) and perfused intracardially with saline followed by 10% Formalin. The brains were removed and stored in 10% Formalin for at least 48 h. Serial coronal (40 µm) sections were prepared and stained with Giemsa using the Nissl method (11). Only rats with lesions located in the commNTS were used for data analysis.

A photomicrograph showing transverse sections of the brain stem with the lesioned commNTS area is presented in Fig. 1. 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 (15). The lesions virtually destroyed the commNTS but did not destroy the area postrema or lateral regions of the NTS. The extent of the lesion was defined as the area with total destruction of tissue. The hypoglossal nucleus was always intact. Tissues, such as ventromedial portions of the gracile nucleus and medial portions of the dorsal motor nucleus of the vagus, that lay adjacent to the lesioned area sustained only minimal damage.

RESULTS

Responses to BCO. The basal MAP and HR of commNTS-lesioned rats were not different from those of sham-lesioned rats (Tables 1 and 2). An enhanced peak and plateau of the pressor response to BCO (57 ± 1 and 41 ± 2 mmHg, respectively) were observed in acute (1 day) commNTS-lesioned rats compared with sham-lesioned rats (46 ± 2 and 30 ± 2 mmHg, respectively) [F(1, 56) = 34.85; P < 0.0001] (Figs. 2 and 3). In sham- and commNTS-lesioned rats the peak of the pressor response to BCO was different from the plateau [F(1, 56) = 75.36; P < 0.0001] (Figs. 2 and 3). BCO produced no significant changes in HR in sham- or commNTS-
lesioned rats. The pressor response to BCO of rats with lesions outside the commNTS (peak = 39 ± 6 mmHg, plateau = 22 ± 5 mmHg, n = 5) did not differ from the response of sham rats.

Table 1. Effects of sequential pharmacological treatment with prazosin plus metoprolol and AVPx on basal MAP and HR

<table>
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<tr>
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<th>Sham Lesion (n = 8)</th>
<th>commNTS Lesion (n = 8)</th>
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<tr>
<td></td>
<td>MAP, mmHg</td>
<td>HR, beats/min</td>
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<tr>
<td>Control</td>
<td>111 ± 3</td>
<td>350 ± 11</td>
</tr>
<tr>
<td>After PZ</td>
<td>85 ± 6*</td>
<td>421 ± 18*</td>
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<tr>
<td>After Met</td>
<td>103 ± 3</td>
<td>312 ± 16</td>
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<tr>
<td>After AVPx</td>
<td>83 ± 6*</td>
<td>326 ± 15</td>
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Values are means ± SE; n = no. rats studied. Conscious freely moving sham- or commissural NTS (commNTS)-lesioned rats received sequential intravenous injections of prazosin (PZ, 1 mg/kg), metoprolol (Met, 1.7 mg/kg), and vasopressin receptor antagonist (AVPx, 10 µg/kg). MAP, mean arterial pressure; HR, heart rate. *Different from control. †Different from sham submitted to same treatment.

Table 2. Effects of sequential pharmacological treatment with AVPx plus prazosin and metoprolol on basal MAP and HR

<table>
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<tr>
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<th>Sham Lesion (n = 7)</th>
<th>commNTS Lesion (n = 7)</th>
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<tbody>
<tr>
<td></td>
<td>MAP, mmHg</td>
<td>HR, beats/min</td>
</tr>
<tr>
<td>Control</td>
<td>108 ± 2</td>
<td>369 ± 15</td>
</tr>
<tr>
<td>After AVPx</td>
<td>108 ± 3</td>
<td>366 ± 12</td>
</tr>
<tr>
<td>After PZ</td>
<td>73 ± 4*</td>
<td>491 ± 13*</td>
</tr>
<tr>
<td>After Met</td>
<td>97 ± 2</td>
<td>360 ± 14</td>
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Values are means ± SE; n = no. rats studied. Conscious freely moving sham- or commissural NTS-lesioned rats received sequential intravenous injections of AVPx (10 µg/kg), prazosin (1 mg/kg), and metoprolol (1.7 mg/kg). *Different from control. †Different from sham submitted to same treatment.
responses (−76 ± 16 and −130 ± 21 beats/min, respectively) (Fig. 4). CommNTS lesions abolished chemoreceptor reflex responses to the KCN dose of 20 µg·0.1 ml−1·rat−1 (3 ± 3 mmHg and −11 ± 5 beats/min) and reduced the responses to the dose of 40 µg·0.1 ml−1·rat−1 (17 ± 6 mmHg and −71 ± 17 beats/min) (Fig. 4).

Baroreceptor reflex test. The pressor response to phenylephrine (3 µg/kg iv) and the resulting reflex bradycardia in commNTS-lesioned rats (52 ± 3 mmHg and −74 ± 16 beats/min, respectively) did not differ from those of sham-lesioned rats (48 ± 2 mmHg and −118 ± 15 beats/min, respectively; Fig. 5). The depressor responses to sodium nitroprusside (30 µg/kg iv) in animals with commNTS lesions (−49 ± 3 mmHg) did not differ from those of sham-lesioned animals (−47 ± 5 mmHg), but reflex tachycardia was reduced in commNTS-lesioned rats (65 ± 9 beats/min) compared with sham-lesioned rats (125 ± 9 beats/min; Fig. 5).

Effects of sequential treatment with prazosin, metoprolol, and AVPx on pressor responses to BCO. Prazosin (1 mg/kg iv) reduced the peak and plateau of the pressor response to BCO in sham- (26 ± 3 and 18 ± 2 mmHg, respectively, vs. 46 ± 3 and 31 ± 3 mmHg before prazosin) and in commNTS-lesioned rats (33 ± 2 and 31 ± 4 mmHg, respectively, vs. 56 ± 2 and 42 ± 2 mmHg before prazosin; Fig. 6). Before prazosin, the peak and plateau of the pressor response to BCO were enhanced in commNTS-lesioned rats compared with sham rats (Fig. 6). The differences between the peak and the plateau of the pressor response to BCO were absent after prazosin treatment (Fig. 6).

The following treatments with intravenous metoprolol (1.7 mg/kg) or intravenous metoprolol and intravenous AVPx (10 µg/kg) after prazosin in sham- and in commNTS-lesioned rats produced no additional changes in the pressor response to BCO compared with the pressor response after only prazosin (Fig. 6).
ANOVA showed significant differences in the pressor response to BCO between sham- and commNTS-lesioned rats \([F(1, 112) = 35.55; P < 0.0001]\) and in the peak and plateau in the control and after different pharmacological blockades \([F(7, 112) = 29.69; P < 0.0001]\). Changes in the HR during BCO in rats submitted to pharmacological blockades were not analyzed.

Table 1 shows basal MAP and HR in the control situation and after each treatment involving sequential pharmacological blockade. Basal MAP and HR of commNTS-lesioned rats did not differ from sham rats before the treatments. A greater decrease in MAP was produced by the treatment with prazosin in commNTS-lesioned animals. After treatment with metoprolol, MAP was no longer different from control values in sham rats, whereas in commNTS-lesioned rats metoprolol reduced HR below control values. The final additional treatment with AVPx reduced MAP in both groups with a more intense effect in commNTS-lesioned rats and without additional changes in HR.

With pharmacological blockade, ANOVA showed significant differences in MAP \([F(1, 56) = 25.91; P < 0.0001]\) and HR \([F(1, 56) = 6.08; P < 0.05]\) comparing the two groups and in MAP \([F(3, 56) = 37.28; P < 0.0001]\) and HR \([F(3, 56) = 29.82; P < 0.0001]\) comparing the control and the different pharmacological blockades.

Effects of sequential treatment with AVPx, prazosin, and metoprolol on pressor responses to BCO. The treatment with intravenous AVPx (10 µg/kg) reduced both the peak and the plateau of the pressor response to BCO in commNTS-lesioned rats (48 ± 3 and 23 ± 5 mmHg, respectively, vs. 57 ± 1 and 40 ± 4 mmHg before AVPx) but not in sham-lesioned rats (Fig. 7). The subsequent treatment with intravenous prazosin (1 mg/kg) reduced the peak of the pressor response to BCO in sham rats (31 ± 2 mmHg vs. 47 ± 2 mmHg before AVPx) and produced an additional reduction only in the peak of the pressor response to BCO in commNTS-lesioned rats (29 ± 1 mmHg; Fig. 7).

Before AVPx, the peak and plateau of the pressor response to BCO were enhanced in commNTS-lesioned rats compared with sham rats. After AVPx treatment, the responses to BCO in commNTS-lesioned rats were not different from those of sham rats (Fig. 7). The differences between the peak and the plateau of the pressor response to BCO in sham- and commNTS-lesioned rats observed before and after AVPx were absent after prazosin treatment (Fig. 7). In sham-lesioned rats the additional treatment with intravenous metoprolol after AVPx and prazosin produced an additional reduction in the peak and plateau of the pressor response to BCO (18 ± 3 and 13 ± 2 mmHg, respectively; Fig. 7). No additional changes in the pressor response to BCO were observed after metoprolol in commNTS-lesioned rats.
ANOVA showed significant differences in the pressor response to BCO when sham- and commNTS-lesioned rats were compared \( F(1, 94) = 8.05; P < 0.01 \) and in the peak and plateau in the control and after different pharmacological blockades \( F(7, 94) = 39.76; P < 0.0001 \).

Table 2 shows basal MAP and HR before and after each treatment of the sequential pharmacological blockade. AVPx treatment did not change MAP and HR in either group. The subsequent treatment with prazosin reduced MAP to similar values in both groups. Compared with control pretreatment values, HR increased in sham-lesioned but not in commNTS-lesioned rats. With the addition of metoprolol, MAP returned to near control values in sham- and commNTS-lesioned rats. HR returned to control values in sham rats, but was reduced below control levels in commNTS-lesioned rats (Table 2).

With pharmacological blockade, ANOVA showed significant differences in MAP \( F(1, 47) = 7.33; P < 0.01 \) and HR \( F(1, 47) = 12.79; P < 0.001 \) between groups and in MAP \( F(3, 47) = 27.35; P < 0.0001 \) and HR \( F(3, 47) = 14.86; P < 0.0001 \) when comparing the control and the different pharmacological blockades.

### DISCUSSION

The present study demonstrated that commNTS lesions increased both the peak and plateau of the pressor response to BCO despite the strong impairment of peripheral chemoreflex responses to KCN produced by the lesion. The involvement of carotid baro- and chemoreceptors and cerebral ischemia in the pressor response to BCO in rats has been suggested (2, 12, 13). The commNTS receives projections from baro- and chemoreceptor afferent fibers (5, 10). CommNTS lesions almost abolished the chemoreflex responses but produced only small reductions in baroreflex activity, mainly in reflex tachycardia. Considering the effects of commNTS lesions on the baro- and chemoreflex and the importance of these reflexes for the pressor response to BCO, the increase in the pressor response to BCO in commNTS-lesioned rats was an unexpected effect. If carotid chemoreceptor activity were essential for the pressor response to BCO, then the response to BCO in commNTS-lesioned rats should be reduced. However, this is not sufficient evidence to exclude the participation of carotid chemoreceptors in the pressor response to BCO. The lesion, in addition to causing chemoreflex...
impairment, may affect other mechanisms activated during BCO, and changes in these mechanisms may overwhelm any effect of chemoreceptor impairment during BCO.

During BCO carotid baroreceptors are deactivated, signaling for pressor responses, whereas aortic baroreceptors are activated, limiting such pressor response. Aortic denervation enhances the pressor response to carotid occlusion (2, 13, 15). The increase in peak and plateau of the pressor response to BCO caused by commNTS lesions might be due to partial ablation of aortic baroreceptor afferent projections, simulating a partial aortic denervation. Although reflex bradycardia was not affected by a commNTS lesion, the lesion reduced reflex tachycardia, which could be an indication of some impairment of baroreceptor function that might be important for the enhancement of the pressor response to BCO.

The results showed that the peak and plateau of the pressor response to BCO in sham rats were dependent on the sympathetic nervous system, and not on vasopressin, since only sympathetic blockade reduced the response. In commNTS-lesioned rats the sympathetic nervous system and vasopressin were important for the peak and plateau of the pressor response to BCO. The involvement of vasopressin in the pressor response to BCO in commNTS-lesioned rats was very clear when vasopressin receptor blockade was the first step in the sequence of pharmacological blockade. It is interesting to note that with only vasopressin receptor blockade, the peak and plateau of the pressor response to BCO in commNTS-lesioned rats were not different from the responses of sham rats before any blockade. Such observation may suggest that an increment in the pressor response to BCO in commNTS-lesioned rats compared with sham was due to the action of vasopressin during BCO in these rats. After the treatment with prazosin, vasopressin receptor blockade produced no change in the pressor response to BCO. Probably the reduced arterial pressure after sympathetic blockade may induce marked vasopressin secretion, impairing an additional increase in the secretion of this hormone during BCO in commNTS-lesioned rats. The reduction in basal MAP by vasopressin receptor blockade after sympathetic blockade is evidence of vasopressin secretion in rats with sympathetic blockade (Table 1).

The difference between the peak and the plateau of the pressor response to BCO in sham- and commNTS-lesioned rats was abolished by prazosin treatment but not by the AVPx. This suggests that the modulation of sympathetic activity during BCO, perhaps due to aortic baroreceptor activation, is an important mechanism.

![Fig. 7. Peak and plateau of pressor response to BCO during 60 s in sham (A) and commNTS-lesioned (B) rats, before (open bars, control) and after intravenous injection of vasopressin receptor antagonist (AVPx, crosshatched bars, 10 µg/kg) followed by prazosin (hatched bars, 1 mg/kg) and later metoprolol (solid bars, 1.7 mg/kg). ](http://ajpregu.physiology.org/)

* Different from plateau in same group with same treatment.
# Different from after AVPx in same group.
* Different from after prazosin in same group.

**Table 1.**

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<thead>
<tr>
<th></th>
<th>Peak</th>
<th>Plateau</th>
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<tbody>
<tr>
<td>Sham</td>
<td></td>
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<td>CommNTS-lesioned</td>
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Despite chemoreflex impairment, BCO produced an increased pressor response dependent on sympathetic activity associated with vasopressin release; and 3) the increment in the pressor response to BCO in commNTS-lesioned rats seems to depend only on vasopressin secretion.

Perspectives

The data showing the enhanced pressor response to BCO in acute commNTS-lesioned rats open new perspectives for additional studies to investigate whether such response to BCO in these animals might involve partial destruction of aortic baroreceptor afferents in the NTS. Furthermore, the possibility that selective chemoreceptor afferent denervation might alter the BCO response of conscious rats still remains. The evaluation of chronic commNTS lesion effects in the pressor response to BCO also requires further investigation.

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