Commissural NTS Lesions and Cardiovascular Responses in Aortic Baroreceptor–Denervated Rats

Monica Akemi Sato, José Vanderlei Menani, Oswaldo Ubirâco Lopes, Eduardo Colombari

Abstract—Both acute (1 day) lesions of the commissural nucleus of the solitary tract (commNTS) and aortic baroreceptor denervation increase pressor responses to bilateral common carotid occlusion (BCO) during a 60-second period in conscious rats. In this study, we investigated the following: (1) the effects of commNTS lesions on basal mean arterial pressure (MAP) and heart rate (HR) of aortic denervated (ADNx) rats; (2) the effects of acute commNTS lesions on pressor responses to BCO in ADNx rats; and (3) the effects of chronic (10 days) commNTS lesions on the pressor response to BCO. ADNx increased basal MAP and HR in sham-lesioned rats. Acute commNTS lesions abolished the MAP and HR increases observed in ADNx rats. Acute commNTS lesions increased the pressor responses to BCO in rats with intact-baroreceptor innervation but produced no additional change in the pressor response to BCO in ADNx rats. Chronic commNTS lesions did not change the pressor responses to BCO in rats with intact-baroreceptor innervation. The data show that acute commNTS lesions abolish the MAP increase produced by aortic baroreceptor denervation. They also suggest that acute commNTS lesions enhance the pressor response to BCO by partial withdrawal of aortic baroreceptor inputs into the NTS. Chronically, reorganization in the remaining aortic baroreceptor or in the baroreflex function as a whole might produce normalization of the cardiovascular responses to BCO. (Hypertension. 1999;34[part 2]:739-743.)

Key Words: blood pressure ■ baroreceptors ■ chemoreceptors ■ solitary nucleus ■ occlusion

Bilateral common carotid occlusion (BCO) for a 60-second period in conscious, freely moving rats produces a pressor response that can be divided into 2 components: the initial peak or first component that arises during the first 20 seconds of occlusion, and the maintained response or second component that arises during the last 30 seconds.1,2 During BCO, carotid baroreceptors are deactivated, signaling for pressor responses, whereas the opposite occurs with aortic baroreceptors, limiting the increase in arterial pressure. Indeed, in aortic baroreceptor–denervated (ADNx) rats, the pressor responses to BCO are much higher than in intact-baroreceptor innervated rats.1-3

Neuroanatomic evidence has shown that arterial baroreceptor and chemoreceptor afferent fibers terminate in the commissural nucleus of the solitary tract (commNTS).4,5 Recent studies from our laboratory have shown that acute lesions of the commNTS enhance the pressor responses to BCO6 and abolish the pressor response to peripheral chemoreflex activation with potassium cyanide, with minor changes in baroreceptor reflex activity.6,7

Acute ADNx produces an increase in arterial pressure.2,8-14 However, the ligation of the artery that irrigates the carotid body attenuates such hypertension that it suggests the involvement of carotid chemoreceptors in its development and maintenance.1,13 If carotid chemoreceptors are important for the hypertensive response observed in ADNx rats, and commNTS lesions abolish the responses induced by peripheral chemoreceptor activation, then commNTS lesions could abolish the hypertension in ADNx rats.

Therefore, in the present study, we investigated the following: (1) the effects of commNTS lesions on basal mean arterial pressure (MAP) and heart rate (HR) of ADNx rats; (2) the effects of acute commNTS lesions on the cardiovascular responses to BCO in animals with ADNx; and (3) the effects of chronic commNTS lesions on the pressor response to BCO.

Methods

Animals

Male Wistar rats weighing 300 to 400 g were used. All experiments were performed in conscious, freely moving rats. The Medical Ethics Committee of the Universidade Federal de São Paulo approved all protocols in this study.

Cerebral Lesion

Rats were anesthetized with 2% halothane mixed with oxygen (100%) and placed in a stereotaxic apparatus (Stoelting Laboratory Standard 51600). Through a partial craniotomy of the occipital bone, the dura mater and arachnoid were incised, and the dorsal surface of the brain stem was exposed. Electrolytic lesions were performed

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with a tungsten electrode (0.010 in; A-M Systems) inserted into the brain stem by means of a micromanipulator with the following coordinates: 0.0 mm lateral, 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 lesion maker (Grass Instruments), and a wire from the anode was attached with a clip to the neck muscles. Lesions were produced with a 3-mA cathodal current delivered for 10 seconds. 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.

**Aortic Baroreceptor Denervation**

After cannulation of the femoral artery, rats were submitted to ADNx. Through an incision in the ventral cervical region, the aortic depressor and superior laryngeal nerves were exposed as described by Krieger.15 ADNx was performed with surgical section of the aortic depressor and superior laryngeal nerves.

**Bilateral Carotid Occlusion**

After cannulation of the femoral artery, the common carotid arteries were exposed. 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 neck. For BCO, the 2 cuffs were connected to a 1-mL syringe filled with isotonic saline by use of Y-shaped PE 50 tubing. Details about the preparation of the cuffs have been described previously.2,3,16,17 During the experiments, the bilateral common carotid arteries were occluded simultaneously for 60 seconds in conscious, freely moving rats. The peak pressor response in the first 20 seconds of BCO was analyzed as the first component of BCO and the peak pressor response in the last 30 seconds of BCO was analyzed as the second component.

**Baroreflex and Chemoreflex Tests**

At the end of BCO tests, in acute and chronic commNTS-lesioned rats, the baroreflex was tested with a pressor dose of phenylephrine (3 μg/kg IV; Sigma Chemical) and depressor doses of sodium nitroprusside (30 μg/kg IV; RBI). The chemoreflex was tested with potassium cyanide (20 μg/0.1 mL per rat IV; Sigma).

**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 ≥48 hours. Serial coronal sections (40 μm) were prepared and stained with Giemsa dye by the Nissl method.7,18,19 Only rats whose lesion sites were located in the commNTS were used for data analysis.

**Statistical Analysis**

Data were expressed as mean±SEM. The pressor responses to BCO were analyzed by 2-way ANOVA followed by Newman-Keuls multiple means comparison analysis with the 2 components of BCO and the different treatments as factors. MAP and HR were analyzed by 1-way ANOVA with repeated measures followed by Newman-Keuls multiple means comparison analysis. Differences were considered significant at the $P<0.05$ level.

**Experimental Protocols**

**Acute commNTS Lesions**

Studies were performed in conscious rats, 24 hours after the surgical procedures (commNTS or sham lesion, cannulation of the femoral artery and vein, ADNx, and implantation of pneumatic cuffs). In the acute experiments, the pressor responses to BCO were studied 1 day after surgery in 4 different groups: intact-baroreceptor commNTS-lesioned rats, intact-baroreceptor sham-lesioned rats, ADNx commNTS-lesioned rats, and ADNx sham-lesioned rats.

**Chronic commNTS Lesion**

The pressor responses to BCO were evaluated 10 days after commNTS or sham lesion. The cannulation of the femoral artery and implantation of pneumatic cuffs were performed 9 days after commNTS or sham lesion.

**Results**

**Histological Analysis**

As shown in Figure 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. Lesions virtually completely 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 vagus that lay adjacent to the lesioned area, sustained only minimal damage.

**Effects of Acute commNTS Lesions on Basal MAP and HR in ADNx Rats**

Figure 2 shows that ADNx increased basal MAP (127±2 mm Hg) and HR (431±19 bpm) of sham-lesioned rats (n=6) compared with intact-baroreceptor sham-lesioned rats (MAP 104±2 mm Hg; HR 366±12 bpm; n=8). Acute (1 day) commNTS lesions abolished the increase in MAP and HR produced by ADNx (ADNx commNTS-lesioned rats: MAP 104±2 mm Hg and HR 380±18 bpm; n=6).

Basal MAP of intact-baroreceptor acute commNTS-lesioned rats (105±3 mm Hg; n=8) was not different from intact-baroreceptor sham-lesioned rats. However, intact-baroreceptor commNTS-lesioned rats presented a reduced HR (332±8 bpm) compared with intact-baroreceptor sham-lesioned rats (Figure 2).

**Pressor Responses to BCO in Rats With Acute commNTS Lesion and ADNx**

ADNx increased both the first and second components of the pressor response to BCO in commNTS-lesioned rats (80±2 and 73±4 mm Hg, respectively) and sham rats (75±3 and 71±4 mm Hg, respectively) compared with intact-baroreceptor commNTS-lesioned rats (54±4 and 44±3 mm Hg, respectively) and intact-baroreceptor sham-lesioned rats (41±2 and 27±2 mm Hg, respectively) (Figures 3 and 4).

commNTS lesions increased the first and second components of BCO in intact-baroreceptor rats but not in ADNx rats.
(Figures 3 and 4). In addition, the differences observed when first and second components of BCO presented by intact-baroreceptor sham- or commNTS-lesioned rats were compared were not observed in ADNx rats (Figures 3 and 4).

Tachycardia in the first and second components during BCO in ADNx commNTS-lesioned rats (5±3 and 32±10 bpm, respectively) was not different from ADNx sham-lesioned rats (22±11 and 38±17 bpm, respectively). Bradycardia in the first and second components during BCO in intact-baroreceptor commNTS-lesioned rats (−12±7 and −1±13 bpm, respectively) was also not different from intact-baroreceptor sham-lesioned rats (−34±8 and −13±12 bpm, respectively). No difference was observed when first

and second components in the same group of rats were compared.

Responses to BCO in Rats With Chronic commNTS Lesion
Basal MAP and HR of chronic (10 days) commNTS-lesioned rats (103±2 mm Hg and 383±15 bpm; n=9) were not different from sham-lesioned rats (101±2 mm Hg and 369±9 bpm; n=9). The pressor responses to BCO in chronic commNTS-lesioned rats (first-component ΔMAP=48±2 mm Hg; second-component ΔMAP=31±3 mm Hg) were not different from the responses of sham-lesioned rats (first-component ΔMAP=44±1 mm Hg; second-component ΔMAP=27±2 mm Hg). A significant difference was observed when the first and second components of the pressor response during BCO were compared in chronic commNTS-lesioned and sham-lesioned rats.

Baroreflex and Chemoreflex in commNTS-Lesioned Rats
In acute intact-baroreceptor commNTS-lesioned rats, the pressor response and bradycardia induced by intravenous phenylephrine (54±4 mm Hg and −63±23 bpm, respec-
tively) were not different from sham-lesioned rats (45±3 mm Hg and −96±18 bpm). The reflex tachycardia produced by intravenous sodium nitroprusside was reduced in acute commNTS-lesioned rats (ΔMAP = 53±5 mm Hg and ΔHR = 65±15 bpm) compared with sham-lesioned rats (ΔMAP = 52±9 mm Hg and ΔHR = 122±11 bpm). Chemoreflex responses produced by potassium cyanide were abolished in acute commNTS-lesioned rats (ΔMAP = 3±6 mm Hg and ΔHR = 2±5 bpm) compared with sham-lesioned rats (ΔMAP = 35±7 mm Hg and ΔHR = 49±20 bpm).

In chronic commNTS-lesioned rats, the pressor response and reflex bradycardia induced by phenylephrine (48±3 mm Hg and −96±9 bpm, respectively) were also not different from sham rats (45±5 mm Hg and −111±20 bpm, respectively). The reflex tachycardia induced by sodium nitroprusside was reduced in chronic commNTS-lesioned rats (ΔMAP = 50±5 mm Hg and ΔHR = 85±14 bpm) compared with sham-lesioned rats (ΔMAP = 44±5 mm Hg and ΔHR = 123±9 bpm). Chemoreflex responses (induced by potassium cyanide) were also attenuated in chronic commNTS-lesioned rats (ΔMAP = 9±5 mm Hg and ΔHR = 41±9 bpm) compared with sham-lesioned rats (ΔMAP = 34±2 mm Hg and ΔHR = 87±15 bpm), confirming previous reports.6,7 Chronic commNTS-lesioned rats also had a decrease in body weight after surgery, with reduction in food intake but no change in water intake, as previously described.19

**Discussion**

Compared with intact-baroreceptor rats, increased pressor responses to BCO were observed in ADNx rats. Acute commNTS lesions increased the pressor response to BCO in rats with intact-baroreceptor innervation but produced no change in the pressor responses to BCO in ADNx rats. These results suggest that aortic baroreceptors seem to be important for the effect of commNTS lesions in increasing the pressor response to BCO. Although commNTS lesions produce only minor changes in the baroreceptor reflex activated by phenylephrine and sodium nitroprusside, it is possible that the increased first and second components of the pressor response to BCO in commNTS-lesioned animals might be due to partial ablation of aortic baroreceptor afferent projections, simulating a partial aortic denervation. These results agree with previously reported neuroanatomic evidence indicating that aortic baroreceptor afferents project to the medial ventrolateral and dorsolateral portions of the solitary complex and also to the commissural subnuclei of the NTS.20

In the present study, sham-lesioned rats submitted to ADNx showed increased arterial pressure similar to that described in other studies,2,8–14 demonstrating that aortic baroreceptor denervation produces sustained neurogenic hypertension in rats. Increased levels of plasma vasopressin after ADNx suggest that vasopressin participates in the mechanisms that induce increased arterial pressure after
aortic denervation. An involvement of carotid chemoreceptors in the development and maintenance of hypertension in aortic denervated rats has been suggested by studies that have shown that the ligation of the artery that irrigates the carotid body attenuates the hypertension produced by aortic denervation. Previous studies have shown that commNTS lesions abolish the chemoreflex induced by potassium cyanide. The present study shows that commNTS lesions abolished the increase in MAP and HR in ADNx rats, which suggests that the integrity of the commNTS is important for the development and maintenance of high blood pressure after ADNx and which supports previous reports that chemoreceptors are important in the hypertension of aortic denervated animals. Taken together, these results indicate that arterial chemoreceptors have a tonic excitatory function on sympathetic activity that is evident only in the absence of aortic baroreceptor afferents. Aortic baroreceptors, in contrast, have a tonic inhibitory influence on sympathetic activity.

Although acute commNTS lesions enhanced the pressor response to BCO, the pressor responses to BCO in chronic commNTS-lesioned animals were not different from sham-lesioned rats. Chronic commNTS lesions produced reduction in body weight associated with decreased food intake, as described previously, and only minor changes in baroreflex responses. As in acute commNTS-lesioned animals, chemoreflex responses were attenuated in chronic commNTS-lesioned animals. These results suggest that in chronically lesioned rats, reorganization of the remaining aortic baroreceptor fibers or of the baroreflex as a whole might occur to normalize the cardiovascular responses to BCO.

Therefore, our results indicate that the integrity of the commNTS is essential for the development and maintenance of the hypertension produced by ADNx. They also suggest that acute commNTS lesions enhance the pressor response to BCO, probably partially removing the aortic baroreceptor inputs into the NTS. Chronically, a possible reorganization of the remaining aortic baroreceptor fibers or of the baroreflex as a whole might result in the recovery of normal cardiovascular responses to BCO.

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