Antihypertensive effects of central ablations in spontaneously hypertensive rats

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Moreira TS, Takakura AC, Colombari E, Menani JV. Antihypertensive effects of central ablations in spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol 296: R1797–R1806, 2009. First published April 1, 2009; doi:10.1152/ajpregu.90730.2008.—Commisural nucleus of the solitary tract (commNTS) lesions transitorily (first 5 days) reduce mean arterial pressure (MAP) in spontaneously hypertensive rats (SHR), and lesions of the tissue surrounding the anteroventral third ventricle (AV3V region) chronically reduce MAP in other models of hypertension. In the present study, we investigated the effects of combined AV3V+commNTS electrolytic lesions on MAP and heart rate (HR) in conscious SHR. Baseline MAP and HR were recorded in male SHR before and for the next 40 days after sham or AV3V lesions combined with sham or commNTS lesions. The AV3V lesions produced no change in MAP in SHR, while commNTS lesions reduced MAP acutely (121 ± 2 to 127 ± 3 mmHg) in the 1st and 5th days, respectively, vs. prelesion: 192 ± 4 mmHg) but not chronically (from 10 to 40 days). However, combined AV3V+commNTS lesions reduced MAP of SHR chronically (119 ± 2 to 161 ± 4 mmHg, in the 1st and 40th day, respectively, vs. prelesion levels: 186 ± 4 mmHg) or sham-lesioned SHR (187 ± 4 to 191 ± 6 mmHg). Sympathetic and angiotensinergic blockade produced less reduction in MAP in SHR with AV3V+commNTS-lesions, and there was no relationship between changes on water and food intake, body weight, or urinary excretion produced by AV3V+commNTS lesions with the changes in MAP. The present findings suggest that in the absence of the commNTS, the AV3V region contributes to the hypertension observed in SHR by mechanisms that appear to involve enhanced angiotensinergic and sympathetic activity.

IN SPONTANEOUSLY HYPERTENSIVE rats (SHR), arterial pressure starts rising a few weeks after birth and reaches hypertensive levels 12 to 14 wk later, mainly due to increases in sympathetic and angiotensinergic activities and the involvement of central mechanisms (7, 20, 24, 29). Central mechanisms for the development and maintenance of hypertension in SHR are present in the brain stem, particularly in the commissural nucleus of the solitary tract (commNTS) and area postrema (AP) (25, 36, 37, 38). Inhibition of the commNTS neurons with injections of γ-aminobutyric acid (GABA) reduces sympathetic nerve discharges (SND) and mean arterial pressure (MAP) in SHR (37). Moreover, electrolytic lesions of the commNTS reduce MAP to normotensive levels in SHR in the first 5 days after lesions with MAP returning to hypertensive prelesion levels around 10 days after lesions (36). However, in contrast to the effects in SHR, in normotensive rats, electrolytic lesions or GABA injection in the commNTS produce no change in baseline MAP, which suggests that commNTS pressor mechanisms are particularly active in SHR increasing SNA and MAP (37). Besides the antihypertensive effects in SHR, commNTS lesions also abolish the pressor responses to peripheral chemoreceptor activation without changing baroreflex control of heart rate (HR) in normotensive rats and SHR (13, 36, 38). A relationship between increases in SNA and increases in chemoreflex activation in SHR has been reported (16, 19, 33). Therefore, a suggestion is that the antihypertensive effects of commNTS lesions in SHR might be related to the blockade of chemoreflex by these lesions (36, 37). However, one important question is why is MAP only transitorily reduced by commNTS lesions in SHR, while chemoreflex is permanently impaired? Probably commNTS pressor mechanisms are replaced by another pressor mechanism allowing MAP to return to hypertensive levels 10 days after commNTS lesions in SHR. Electrolytic lesions of the AP located close to the commNTS in young SHR also impair development of hypertension chronically by mechanisms not yet completely understood (25).

In the forebrain, one area strongly involved in development and maintenance of different models of experimental hypertension is the tissue surrounding the anteroventral third ventricle (AV3V region) (7, 8, 9, 22, 23, 26). The AV3V region includes periventricular preoptic nuclei surrounding the anteroventral part of the third ventricle and the organum vasculosum of the lamina terminalis, an area lacking blood-brain barrier and rich in ANG II receptors (9, 10, 11, 22). In rats, electrolytic lesions of the AV3V region abolish or reduce pressor responses dependent on sympathetic activation and/or vasopressin secretion like pressor responses to central ANG II or cholinergic activation, without changing baroreflex and peripheral chemoreflex (7, 8, 26, 27, 43, 44). AV3V lesions impair the development of neurogenic hypertension produced by sinoaortic denervation or by ablation of the medial NTS and the development of renal ANG II-dependent hypertension (7, 9, 22, 23). However, AV3V lesions produce no effect on hypertension in SHR (9, 17).

Based on previous studies, it seems that the main mechanisms involved in the development and maintenance of hypertension in SHR are located in the brain stem, while other models of hypertension are more dependent on forebrain mechanisms (7, 8, 9, 22, 23, 26, 36–38). However, recent studies showed that AV3V lesions or blockade of cholinergic or angiotensinergic mechanisms in the forebrain strongly reduces pressor responses produced by the activation of the NTS and the rostroventrolateral medulla (RVLM), which suggests that the activation of brain stem hypertensive mechanisms are
strongly dependent on forebrain cholinergic and angiotensinergic mechanisms (43, 44, 45).

Important pressor mechanisms are present in different forebrain areas, like septal area, subfornical organ (SFO), preoptic area, medial hypothalamus, and paraventricular nucleus of hypothalamus (PVN) that are connected with brain stem areas related to cardiovascular regulation directly or through connections with the AV3V region (9, 12, 22, 23, 42). Studies have also shown specific neural connections between the AV3V region and the NTS (34, 35). Therefore, considering that: 1) previous studies showed that commNTS and AV3V region are strongly involved in the development and/or maintenance of one or more models of hypertension, 2) the existence of anatomical connections between the two structures, and 3) the importance of forebrain mechanisms for pressor responses that arise with the activation of brain stem pressor mechanisms, in the present study we investigated the effects of AV3V and commNTS lesions alone or combined on MAP, HR, and cardiovascular reflexes in SHR. Because the AV3V region is also strongly involved in the control of fluid-electrolyte balance (9, 27), we also evaluated the possible influence of changes in water intake and renal excretion for the effects of AV3V associated with commNTS lesions on MAP in SHR. In addition, we also tested whether AV3V and commNTS lesions might affect the activation of sympathetic and angiotensinergic mechanisms involved in the maintenance of hypertension in SHR. The results show that AV3V and commNTS lesions combined in the same animal chronically reduce hypertension in SHR by impairing activation of angiotensinergic and sympathetic mechanisms, while changes in fluid-electrolyte balance seem to have no role for the antihypertensive effects of these lesions.

MATERIALS AND METHODS

Animals

Experiments were performed in 80 adult male SHR and in 12 control normotensive male Holtzman rats weighing 320–350 g (14 to 17 wk old). The animals were housed individually in stainless steel cages in a room with controlled temperature (23 ± 2 °C) and humidity (55 ± 10%). Lights were on from 7:00 AM to 7:00 PM. Standard Guabi chow (Paulinia, SP, Brazil) and tap water were available ad libitum. All the experiments were performed in conscious, freely moving rats. The experimental protocols were approved by the Animal Experimentation Ethics Committee of the School of Dentistry, São Paulo State University (UNESP).

Electrolytic Lesions

Commissural NTS lesions. Rats were anesthetized with intraperitoneal injection of ketamine (80 mg/kg of body wt) combined with xylazine (7 mg/kg of body wt) and placed in a stereotactic frame (model 900; David Kopf Instruments). A partial craniotomy of the occipital bone was performed, and the dorsal surface of the brain stem was exposed. A tungsten electrode (0.1 mm in diameter) bared at the tip (0.5 mm) was inserted into the brain 0.1 and 0.5 mm caudal to the calamus scriptorius, in the midline, and 0.4 mm below the dorsal surface of the brain stem. Electrolytic lesions were performed using a cathodal current of 1 mA during 10 s in each one of the two stereotoxic coordinates cited above as previously described (13, 36, 38). A clip attached to the tail was used as the indifferent electrode. Sham-lesioned rats were submitted to the same surgical procedures and had the electrode placed along the same coordinates, except that no current was passed.

AV3V lesions. The AV3V lesions were also performed in rats anesthetized with intraperitoneal injection of ketamine (80 mg/kg of body wt) combined with xylazine (7 mg/kg of body wt) using a tungsten wire electrode (0.4 mm in diameter) bared at the tip (0.5 mm) that was inserted into the brain using the following coordinates: 0.0 mm from bregma, in the midline, and 7.0 mm below the dura mater. Electrolytic lesions were performed using a cathodal current (2 mA during 10 s) as previously described (12, 27, 41–44). A clip attached to the tail was used as the indifferent electrode. Sham-lesioned rats had the electrode placed along the same coordinates, except that no current was passed. For combined lesions, rats had lesions in the commNTS followed immediately by AV3V lesions. Rats received a prophylactic dose of penicillin (30,000 IU) given intramuscularly and a subcutaneous injection of the analgesic Ketoflex (ketoprofen 1%, 0.03 ml/rat) postsurgically.

Arterial Pressure and HR Recordings

Pulsatile arterial pressure (PAP), MAP, and HR were recorded in unanesthetized freely moving rats. Under intraperitoneal injection of ketamine (80 mg/kg of body wt) combined with xylazine (7 mg/kg of body wt) anesthesia, a polyethylene tubing (PE-10 connected to a PE-50) was inserted into the abdominal aorta through the femoral artery. Next, a second polyethylene tubing was inserted into the femoral vein for drug administration. Both cannulas were tunneled subcutaneously to the back of the rats and connected to stainless steel “elbows” made of 23-gauge hypodermic tubing. One end of this elbow was kept under the skin, and the other end was exteriorized through the skin. The cannulas were filled with sterile saline containing heparin (50 U/ml) and penicillin G (1.3 mg/ml, the equivalent to 2,000 U/ml). The external end of the elbow was closed with a plastic cap, as previously described (39). This method of arterial cannulation allows daily arterial pressure recordings chronically (for more than a month) (39). To record PAP, MAP, and HR, a polyethylene tubing filled with sterile saline connected the arterial line of the external end of the elbow to a strain gauge transducer (Statham P23Db) coupled to a preamplifier (model ETH-200; Bridge Bio Amplifier) that was connected to a Powerlab computer data acquisition system (model Powerlab 16SP; ADInstruments). Arterial pressure and HR were recorded from 2 days before until 40 days after brain surgery.

On 1, 2, 3, 5, 10, 15, 20, 30, and 40 days after brain surgery, MAP and HR were recorded once a day from 8:00 AM to 12:00 AM. In each rat, the recording period took ~1 h. Rats had the arterial line connected to the transducer, and after 15 min, the recording of MAP and HR started. MAP and HR were recorded for the next 30–40 min, and the mean of the values of arterial pressure and HR recorded before starting the intravenous injections were used as the result. One day before and 1, 5, 10, 20, 30, and 40 days after cerebral lesions, baro- and chemoreflex were tested with intravenous injections of phenylephrine (PHE), sodium nitroprusside (SNP), and potassium cyanide (KCN) at the end of the recording sessions. The interval between intravenous injections was 5 min.

Daily Food and Water Intake, Body Weight, Urinary Volume, and Na+ and K+ Excretion

To test whether changes on MAP were a consequence of changes in fluid-electrolyte balance or food intake, the effects of AV3V and commNTS lesions alone or combined on daily water and food intake, body weight, urinary volume, and Na+ and K+ excretion, in SHR were investigated. Rats were housed in metabolic cages, and daily recordings were started 2 days before surgery (control) and continued until 40 days after lesions. Four groups of SHR (sham, AV3V lesion, commNTS lesion, and combined lesions) not implanted with arterial or venous catheters were tested. Plasma osmolality, protein, Na+, K+, and hematocrit were also analyzed at the end of 40 days.

For the same purpose, MAP and HR were also recorded for 12 days in a control group of intact SHR and in a group of intact SHR that had
available the same amount of water and food that AV3V+commNTS-lesioned SHR ingested. MAP and HR were recorded for 2 days in control conditions before starting the reduction in water and food intake and continued until the 10th day of restriction.

**Histology**

At the end of the experiments, rats were deeply anesthetized with sodium thiopental (70 mg/kg of body wt ip). Saline followed by 10% buffered formalin was perfused through the heart. The brains were frozen, cut coronally into 50-μm sections, stained with Giemsa stain, and analyzed by light microscopy to confirm the AV3V and commNTS lesions.

**Statistical Analysis**

Statistical analysis was done with Sigma Stat version 3.0 (Jandel, Point Richmond, CA). All data are reported as means ± SE. One- or two-way parametric ANOVA followed by the Newman-Keuls multiple comparisons test were used, as appropriate. Significance was set at P < 0.05.

**Experimental protocols**

**Effects of AV3V and commNTS lesions alone or combined on baseline MAP and HR in SHR.** To evaluate the effect of specific brain lesions on baseline MAP and HR in conscious SHR, resting PAP, MAP, and HR were recorded for the next 3 days after cannulation of the femoral artery and vein. At the end of the third recording period, animals were submitted to sham or commNTS lesions combined with sham or AV3V lesions. On 1, 2, 3, 5, 10, 15, 20, 30, and 40 days after central lesions, resting MAP and HR were recorded. Four groups of SHR were tested: sham AV3V lesion+sham commNTS lesion, sham AV3V lesion+commNTS lesion, AV3V lesion+sham commNTS lesion, and AV3V lesion+commNTS lesion.

**Effects of AV3V and commNTS lesions on chemoreflexes and baroreflexes in SHR.** One day before and 1, 5, 10, 20, 30, and 40 days after cerebral lesions, at the end of the session of MAP and HR recording, rats had baroreflexes and chemoreflexes tested to investigate whether the reflexes were influenced by brain lesions. Chemoreflex was tested with an intravenous bolus injection of KCN (40 μg·0.1 ml⁻¹·rat⁻¹). Baroreflex was tested injecting intravenously a pressor dose of phenylephrine (PHE; 5 μg/kg of body wt) and a depressor dose of SNP (30 μg/kg of body wt).

Daily water and food intake, renal excretion, and body weight in AV3V- and commNTS-lesioned SHR. Four groups of SHR (sham, AV3V lesion, commNTS lesion, and combined lesions) different from those used to record MAP and HR, were used to test specifically daily water and food intake, renal Na⁺, K⁺, and volume excretion, and body weight. Daily recordings started 2 days before surgery (control) and continued until 40 days after lesions.

Arterial pressure and HR in intact SHR with reduced amount of food and water available. To show that the reduction in baseline MAP after combined AV3V+commNTS lesions is not related to the reduction in water and/or food ingestion produced by these lesions, MAP and HR were recorded for 13 days in a control group of intact SHR and in a group of intact SHR that had available the same amount of water and food that AV3V+commNTS-lesioned SHR ingested. MAP and HR were recorded for 3 days in control conditions before starting the reduction in water and food intake and continued until the 10th day of restriction.

**Sympathetic and angiotensinergic activities in AV3V- and commNTS-lesioned SHR.** Previous studies have shown that sympathetic activity and ANG II are increased in SHR (7, 15, 24, 30). To test whether the reduction in MAP produced by AV3V+commNTS lesions would be dependent on changes in sympathetic activity and/or ANG II mechanisms, at the 40th day after lesions, unanesthetized freely moving SHR received intravenous injections of losartan (ANG II AT₁ receptor antagonist, 10 mg/kg of body wt). On the next day, in the same groups of rats, autonomic blockade was performed with intravenous injections of hexamethonium (10 mg/kg of body wt). Doses of losartan and hexamethonium were based on previous studies that showed the efficacy of these doses for blocking AT₁ receptors and sympathetic activity (2, 6, 14, 18, 28, 32, 46, 47).

**Effects of combined AV3V and commNTS lesions on baseline MAP, HR, and cardiovascular reflexes in normotensive rats.** To confirm results from previous studies (36–38) showing that commNTS lesions do not affect MAP in normotensive rats and to demonstrate that combined AV3V+commNTS lesions specifically reduce MAP in SHR and not in normotensive rats, the effects of these lesions on MAP and HR were tested also in normotensive rats.

In normotensive rats, recording of resting MAP and HR started 2 days after cannulation of femoral artery and vein. On the third day after starting recordings, rats were submitted to sham or combined commNTS and AV3V lesions, and the recordings were continued for the next 5 days. One day before and 1, 2, 3, and 4 days after cerebral lesions, at the end of the session of MAP and HR recording, the rats had baroreflexes tested with intravenous injections of PHE and SNP and chemoreflexes tested with intravenous injection of KCN.

**RESULTS**

**Histological Analysis**

Lesions of the commNTS were located in the midline above the central canal and extended from the level of the obex to ~1 mm caudal to the obex (Fig. 1A). Lesions completely destroyed the commNTS but did not destroy the area postrema or lateral regions of the NTS. The extension of the lesions was similar to that performed in previous studies (13, 36, 38). The AV3V lesions (Fig. 1B) were located between the anterior commissural nucleus of the solitary tract (commNTS) and anteroven-tral third ventricle (AV3V) lesions. Photomicrographs showing the typical lesions (arrowheads) of commNTS (A) and AV3V region (B). The commNTS lesions extended for ~800 μm rostrocaudally. According the atlas of Paxinos and Watson (31), the sections extend from bregma −13.8 to bregma −14.6 mm. The AV3V lesions extended for ~540 μm rostrocaudally. According the atlas of Paxinos and Watson (31), the sections extend from bregma −0.26 to bregma −0.8 mm. AP, area postrema; cc, central canal; XII, hypoglossal nucleus; AC, anterior commissure; oc, optic chiasm. Scale bar = 0.5 mm.
sure and the floor of the third ventricle with bilateral damage of the periventricular tissues from the lamina terminalis through the preoptic and anterior hypothalamus, never extending caudally to the arcuate nucleus or medial hypothalamus (8, 10, 12, 26, 27, 41, 43, 44). The brain structures that were consistently destroyed by AV3V lesions were the preoptic periventricular nuclei, the ventral part of median preoptic nucleus, and the anterior wall of the third ventricle with the associated organum vasculosum of the lamina terminalis. Partial destruction of the medial preoptic nuclei and the medial region of the anterior hypothalamic nuclei were also observed in some animals.

From a total of 61 rats submitted to commNTS and/or AV3V lesions, 19 had complete lesions in the commNTS, 20 had complete lesions of the AV3V region, and 22 had complete lesions in both structures. The criteria for exclusion of animals was based upon histological analysis. Only data from rats with complete lesions of the AV3V region and/or commNTS confirmed by histological analysis were included in the results.

Effects of AV3V and commNTS Lesions Alone or Combined on Baseline MAP and HR in SHR

From a prelesion level of 192 ± 4 mmHg, MAP was reduced to a level between 121 ± 2 and 127 ± 3 mmHg from 1 to 5 days after commNTS lesions alone in SHR. After 5 days of lesion, MAP started increasing and returned to prelesion levels 15 days after commNTS lesions (Fig. 2A). CommNTS lesion alone did not affect HR (Fig. 2B).

Lesions of the AV3V region alone did not affect baseline MAP in SHR (Fig. 2A). However, by the combining of AV3V+commNTS lesions in SHR, MAP was reduced during the whole period of recording (40 days) (Fig. 2, A and C). From a prelesion level of 186 ± 4 mmHg, MAP was reduced to a level between 119 ± 2 and 150 ± 4 mmHg in the first 10 days after combining AV3V+commNTS lesions and reached 161 ± 4 mmHg 40 days after lesions [F (3, 29) = 128.12, P < 0.01] (Fig. 2, A and C). AV3V lesions alone or combined with commNTS lesions increased HR [F (3, 29) = 378.31, P < 0.01] (Fig. 2, B and C).

Effects of AV3V and commNTS Lesions on Chemoreflexes and Baroreflexes in SHR

AV3V lesions alone did not affect the pressor and bradycardic responses produced by peripheral chemoreceptor activation with intravenous injection of KCN in SHR (Fig. 3A). However, commNTS lesions alone or combined with AV3V lesions almost abolished the pressor responses to intravenous KCN for the whole period of study [F(3, 29) = 63.4, P < 0.01] (Fig. 3A). The bradycardia induced by intravenous KCN in SHR was attenuated from 1 to 10 days after commNTS lesions alone or combined with AV3V lesions [F(3, 29) = 102.6, P < 0.01] (Fig. 3A).

AV3V and commNTS lesions (alone or combined) did not modify the pressor and bradycardic responses to intravenous PHE in SHR (Fig. 3B). However, commNTS lesions (1 to 10 days),

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**Fig. 2.** Baseline mean arterial pressure (MAP) and heart rate (HR) in AV3V- and/or commNTS-lesioned spontaneously hypertensive rats (SHR). A: baseline MAP and B: baseline HR on control days (prelesion) and 1, 2, 3, 5, 10, 15, 20, 30, and 40 days after sham, AV3V, commNTS, or AV3V+commNTS lesions in SHR. C: representative recordings showing changes in pulsatile arterial pressure (PAP), MAP, and HR in 1 SHR submitted to sham lesions (C1) and 1 SHR submitted to combined AV3V+commNTS lesions on the control day (prelesion) and 1, 5, 10, 20, 30, and 40 days after lesions (C2). Results in A and B are represented as means ± SE. *P < 0.01 compared with sham-lesioned group by 2-way repeated-measures ANOVA followed by Newman-Keuls multiple comparison test. Dashed line shows prelesion levels of MAP and HR.
alone or combined with AV3V lesions, attenuated the reflex tachycardia to intravenous injection of SNP [F(3, 29) = 111.23; \( P < 0.05 \)] without changing the hypotension in SHR (Fig. 3C).

**Fluid-Electrolyte Balance and Body Weight in AV3V- and commNTS-Lesioned SHR**

All four groups of rats reduced water and food intake in the next 2 days after brain surgery (Fig. 4, A and B). In the first 10 days after AV3V lesion alone or combined with commNTS lesion, daily water intake was reduced [F(3, 29) = 322.74; \( P < 0.05 \)] (Fig. 4A). Strong reduction of water intake (80%) occurred in the first 4 days after lesions. No significant difference in daily water intake was observed between sham- and commNTS-lesioned rats (Fig. 4A).

In sham- or AV3V-lesioned rats, food intake and body weight were reduced only in the first 2 days after lesions (Fig. 4, A and B). However, food intake was reduced in the first 10 days and body weight in the first 15 days after commNTS lesion alone or combined with AV3V lesion (Fig. 4, A and C). Strong reduction of food intake (80%) occurred in the first 4 days after commNTS lesion alone or combined with AV3V lesion. The maximum reduction of body weight occurred between 5 and 10 days after commNTS lesion alone or combined with AV3V lesion. After 10 days of lesion, rats started to gain weight, and their body weight paralleled with sham- or AV3V-lesioned rats after 15 days of lesion (Fig. 4, B and C). The changes in water intake and food intake in rats with AV3V and commNTS lesions seem not to be due to impairment of motor activity. Although motor activity was not quantified, visual observation in their home cages and during handling revealed no apparent differences in reactivity or locomotion between sham and lesioned SHR.

Sodium excretion was reduced in the first 7 days in all groups of SHR, probably as a result of the reduction in water intake and/or food intake (Fig. 4E). After 7 days, Na\(^+\) excretion returned to normal level for all groups and was maintained in this level until the 40th day. There were no significant changes in K\(^+\) excretion and urinary volume in all group of SHR over the 40 days after AV3V and commNTS lesion (Fig. 4, D and F).

Considering only daily water intake and urinary volume, daily water balance was reduced in the first 3 days after lesions in all groups tested. In the first 5 days after lesions, reduction of water balance was more intense in AV3V- or AV3V+commNTS-lesioned SHR (−11 to 7 ml/day) than in sham- or commNTS-lesioned SHR (4 to 29 ml/day) [F(3, 29) = 153.34; \( P < 0.05 \)]. There were also no significant differences in plasma osmolality, protein, Na\(^+\) and K\(^+\), and hematocrit between sham- and AV3V+commNTS-lesioned SHR at the end of 40 days (Table 1).

**Arterial Pressure and HR in Intact SHR with Reduced Amount of Food and Water Available**

As described above, water and food intake were significantly reduced after AV3V and commNTS lesions during ~10 days. Daily water intake in AV3V+commNTS-lesioned rats ranged from 8 ± 4 ml in the first day after lesions to 12 ± 5 ml in the 10th day, and food intake ranged between 3 ± 5 and 5 ± 6 g in the same period, while controls ingested an average of 32 ± 5 ml of water and 23 ± 4 g of food daily along the same period.

Before food and water restriction were started, MAP values were 183 ± 4 and 185 ± 3 mmHg, respectively, in the control group and in the group to be submitted to food and water restriction. Food and water restriction did not change MAP,
and values ranged between 185 ± 2 and 188 ± 4 mmHg from the first to the 10th day after the reduction of water and food intake \(F(1, 21) = 1.12, P > 0.05\) (Fig. 5A). Food and water restriction also did not affect baseline HR in SHR \(F(1, 21) = 1.35, P > 0.5\) (Fig. 5B).

**Sympathetic and Angiotensinergic Activities in AV3V- and commNTS-Lesioned SHR**

Compared with sham-lesioned SHR, intravenous losartan produced a smaller reduction of MAP in AV3V-lesioned rats and in AV3V+commNTS-lesioned SHR (Fig. 6). Ganglionic blockade with hexamethonium produced a smaller reduction in MAP only in AV3V+commNTS-lesioned SHR (Fig. 6). Compared with sham-lesioned SHR, losartan and hexamethonium did not produce significant changes in HR in all groups of SHR (data not showed). The efficacy of the blockade of AT1 receptors by losartan was also confirmed with intravenous injection of ANG II (50 ng/kg) (4 ± 3 mmHg vs. ANG II: 29 ± 6 mmHg, \(P < 0.05\)).

**Effects of Combined AV3V and commNTS Lesions on Baseline MAP, HR, and Cardiovascular Reflexes in Normotensive Rats**

Combined AV3V+commNTS lesions in normotensive rats abolished the pressor responses and reduced the bradycardia produced by intravenous injection of KCN (40 μg·0.1 ml⁻¹·rat⁻¹), during the whole period of study (5 days) \(F(1, 44) = 103.18; P < 0.05\), without changing the pressor and bradycardic responses to intravenous PHE (5 μg/kg of body wt) (See Suppl. Fig. 1 in online version of this article). However, similar to SHR, combined AV3V+commNTS lesions in normotensive rats attenuated the reflex tachycardia to intravenous injection of SNP (30 μg/kg of body wt) \(F(1, 44) = 78.64; P < 0.05\), without changing hypotension (see Suppl. Fig. 2, online).

### Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>(Na^+), meq/l</th>
<th>(K^+), meq/l</th>
<th>Protein, g/dl</th>
<th>Hematocrit, %</th>
<th>Osmolality, mOsm</th>
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<tr>
<td>Sham</td>
<td>141 ± 0.7</td>
<td>5.6 ± 0.1</td>
<td>6.2 ± 0.04</td>
<td>42 ± 0.6</td>
<td>1586 ± 38</td>
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<tr>
<td>Lesion</td>
<td>142 ± 0.4</td>
<td>5.7 ± 0.4</td>
<td>6.1 ± 0.14</td>
<td>39 ± 0.2</td>
<td>1648 ± 30</td>
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Results are means ± SE, \(n = 6–7\) rat. AV3V, anteroventral third ventricle; commNTS, commissural nucleus of the solitary tract; SHR, spontaneously hypertensive rats.
DISCUSSION

Present results provide the first direct evidence that AV3V/commNTS electrolytic lesions produce a long-lasting reduction in arterial pressure (for at least 40 days) in adult SHR, which suggests that forebrain and brain stem mechanisms, particularly those related to AV3V region and commNTS, act to maintain hypertension in SHR. The reduction in MAP produced by AT1 receptor or ganglionic blockade in AV3V/commNTS-lesioned SHR was smaller than the changes in MAP produced by the same treatments in sham-lesioned SHR, which suggests that the antihypertensive effects of these lesions are probably related to reduction in ANG II and sympathetic-mediated pressor mechanisms. In normotensive rats, combined commNTS/AV3V lesions produced only a small and transitory reduction of MAP (≤ 20 mmHg only in the first day after lesions), which is evidence that these lesions affect only mechanisms activated to produce hypertension, not those that maintain normotensive levels of arterial pressure.

Caution must be exercised when interpreting the effects of electrolytic lesions, because these lesions also destroy fibers of passage in addition to cell bodies. In spite of this, electrolytic lesions have been used as a first approach to investigate the importance of central areas in different physiological or pharmacological responses (12, 13, 36–38, 41, 43, 44). Specifically, considering the present results, it is important to remember that similar to electrolytic lesions of the commNTS, the blockade of neuron activity with muscimol injected into the commNTS also reduces MAP (37). Analysis of the effects of AV3V lesions is a little more complex because this lesion, besides fibers of passage, also encompasses more than one nucleus (9, 10, 23), and only specific lesions in each one of these structures included in the AV3V region can show which one plays a role in the maintenance of hypertension in SHR. However, if the effects of AV3V and commNTS lesions were due to destruction of cell bodies or fibers of passage, the interesting point is that hypertension in SHR is maintained by activation of forebrain and brain stem pressor mechanisms that deserve more detailed investigation in future studies.

AV3V lesions alone or combined with commNTS lesions reduced water intake, renal excretion, and water balance, while commNTS lesions alone or combined with AV3V lesions reduced food intake. However, the antihypertensive effects of AV3V+commNTS lesions in SHR cannot be attributed to changes in fluid-electrolyte balance or food intake because: 1) the changes in fluid-electrolyte balance produced by combined AV3V+commNTS lesions were similar to the changes produced by AV3V lesions alone that have no effect on MAP in SHR and 2) MAP did not change in intact SHR that had restriction of food and water to the same level ingested by AV3V+commNTS-lesioned SHR. In addition, the antihypertensive effects of commNTS lesions alone or combined with AV3V lesions cannot be attributed to changes in baroreflexes, because reflex responses to intravenous PHE or SNP were not significantly modified by these lesions in normotensive rats or SHR (13, 36, 38, 43, 44, and present results).

The AV3V region is rich in ANG II receptors and is reciprocally connected with different central areas, including those that are part of the medullary circuitry related to cardiovascular regulation (7, 8, 22, 23, 34, 35). The AV3V region receives important direct descending afferent connections from the SFO, another circumventricular organ in the forebrain lacking blood-brain barrier and rich in ANG II receptors, and also direct ascending afferent projections from the parabrachial...
nucleus and from different medullary regions containing catecholamine cell bodies. It was also described direct efferent projections from the AV3V region to the SFO, PVN, supraoptic nucleus, ventral medullary area, parabrachial nucleus, and mesencephalic central gray matter (8, 9, 23, 35). Therefore, AV3V lesions may affect the activity of areas like supraoptic nucleus and PVN involved in vasopressin secretion. The PVN is also an important area involved in the control of sympathetic activity through direct connections with the intermediolateral cell column, or RVLM, and AV3V lesions may also impair this function. The NTS that receives important cardiovascualr afferent signals sends projections to hindbrain areas like caudal ventrolateral medulla (CVLM), RVLM, parabrachial nucleus, and medullary regions containing catecholamine cell bodies and to forebrain areas like specific hypothalamic nuclei (34, 35). Therefore, the commNTS lesion may impair important signals that control the activity of CVLM and RVLM, two main areas of the medullary circuitry involved in cardiovascular regulation and, in addition, also signals that might reach directly or indirectly the AV3V region. Besides the inhibitory signals that reach the RVLM through CVLM, the NTS and PVN are also important sources of excitatory inputs to RVLM and the sympathetic system. Lesion of the commNTS disrupts mainly excitatory inputs from the NTS to RVLM without changing the inhibitory inputs, and AV3V lesion may impair excitatory inputs from the PVN. According to the present results, it seems that the blockade of both mechanisms is necessary to reduce MAP in SHR.

The fall in MAP produced by the blockade of ANG II receptors with intravenous losartan was reduced by 60 to 70% in AV3V, or commNTS+AV3V-lesioned SHR, while commNTS lesions did not affect the responses to intravenous losartan, which suggests that angiotensinergic pressor mechanisms are impaired in rats with AV3V lesions. The fall in MAP produced by intravenous hexamethonium was reduced by 50% only in SHR with combined AV3V and commNTS lesions, not in SHR with lesions in only one structure, which suggests that sympathetic activity is reduced in SHR with combined lesions. Therefore, reduction in sympathetic and angiotensinergic activities in SHR with AV3V+commNTS lesions seems to be the cause of the antihypertensive effects of these combined lesions. The AV3V lesions impair the pressor responses to central angiotensinergic and cholinergic activation, to glutamate injected into the NTS and RVLM, to the pressor response to carotid occlusion, and prevent the development of neurogenic hypertension produced by sinoaortic denervation or by ablation of the medial NTS and the development of many other models of experimental hypertension, including renal ANG II-dependent hypertension, without changing baroreflex and peripheral chemoreflex in normotensive rats (7, 8, 9, 12, 42, 43, 44). All of these effects suggest that AV3V lesions reduce sympathetic- and angiotensinergic-mediated pressor responses in normotensive rats. However, as shown by the present results, the fall in MAP produced by intravenous hexamethonium was not modified by AV3V lesions, suggesting that AV3V lesions alone do not affect the high sympathetic activity in SHR. Considering that AV3V lesions strongly reduce the action of angiotensinergic pressor mechanisms, the maintenance of hypertension in AV3V-lesioned SHR seems to depend mainly on the high sympathetic activity not affected by AV3V lesions. Chronic commNTS lesions do not affect the fall in MAP produced by intravenous losartan or hexamethonium, which suggests that angiotensinergic and sympathetic pressor mechanisms are acting normally in chronic commNTS-lesioned SHR. However, reduced MAP and sympathetic activity occur in commNTS+AV3V-lesioned SHR, which in addition also have reduced angiotensinergic activity. Therefore, the maintenance of high sympathetic activity is an important mechanism for hypertension in SHR and only the combined lesions that reduce sympathetic and angiotensinergic activities also reduce resting MAP chronically in SHR. Probably when one area is destroyed the other replace the function, i.e., in the absence of AV3V region, commNTS maintains the high sympathetic activity and hypertension and vice versa. Reduction in the action of angiotensinergic mechanisms may also contribute for the antihypertensive effects of AV3V+commNTS lesions in SHR; however, as evidenced in AV3V-lesioned rats, only the reduction of angiotensinergic mechanisms is not enough to reduce MAP chronically in SHR. In spite of the reduction in baseline MAP by AV3V+commNTS lesions in SHR, it is important to note that MAP was still a little above normotensive level, which suggests that other mechanisms, not dependent on these two areas, are also involved in the maintenance of hypertension in SHR.

It has been suggested that sympathetic activity consists of rhythmic discharges produced by supraspinal structures, particularly multiple oscillatory networks in the brain stem (3–5). Acute inhibition of commNTS neurons similar to PVN inhibition decreases sympathetic activity in SHR but not in normotensive control Wistar-Kyoto rats (1, 37). These results are usually interpreted as evidence that RVLM neurons are more active in SHR, either because they receive more excitatory inputs or less GABAergic inhibition (21). There is a suggestion that under resting conditions, arterial chemoreceptors have a high activity in SHR, which causes sympathetic hyperactivity and high arterial pressure in this strain (16, 19, 33, 40). Acute or chronic lesions of the commNTS abolish the pressor responses to peripheral chemoreflex activation (13, 28), which might be a mechanism to reduce sympathetic activation and arterial pressure in SHR (36, 37, 38). However, in commNTS-lesioned SHR, MAP recovers prelesion hypertensive levels chronically, in spite of the chronic impairment of chemoreflex responses, which suggests that other mechanisms are activated to recover high arterial pressure in chronic commNTS-lesioned SHR. An impairment of respiration increasing blood Pco2 and/or reducing Po2 in commNTS-lesioned SHR might also increase arterial pressure, allowing the recovery of precommNTS lesion hypertensive levels. In this case, the blockade of the pressor responses to changes in blood gases might be the effect of AV3V lesions. However, unpublished results from our laboratory have shown that electrolytic lesions or muscimol injections into the commNTS did not affect baseline respiration, although they reduced the increase in breathing produced by peripheral chemoreceptor activation in normotensive rats. Therefore, probably an AV3V-dependent mechanism, not related to respiratory changes, recovers sympathetic activity chronically after commNTS lesions.

Electrolytic lesions of the area postrema also impair the development of hypertension in SHR (25). However, as commented by the authors, lesions of the area postrema may also partially affect NTS, particularly commNTS. Indeed, the present and previous studies have suggested that commNTS...
lesions, which do not affect the integrity of the area postrema, reduce hypertension (36, 38). Therefore, it is still not clear whether the reduction of arterial pressure in SHR by lesions of the area postrema is due to impairment of the area postrema alone or commNTS mechanisms. Perhaps commNTS and area postrema are part of the same mechanism that can be disrupted by area postrema or commNTS lesions. It is also important to note that lesions of the area postrema were performed in 4-wk-old SHR or before they became hypertensive, and we found no study showing effects of lesions of the area postrema in adult hypertensive SHR.

In summary, the present results suggest that hypertension in SHR is strongly dependent on central complex mechanisms involving multiple central areas and probably different mechanisms. One mechanism may compensate for the absence of the other, which increases the probability of hypertension in these animals. The antihypertensive effects of commNTS+AV3V lesions in SHR seem to depend on reduction of angiotensinergic and sympathetic pressor mechanism activation. Changes in fluid-electrolyte balance or food intake are probably not important.

**Perspectives and Significances**

Future studies using different approaches are necessary to confirm the mechanisms dependent on the AV3V region and commNTS involved in the maintenance of hypertension in SHR. The existence of more than one mechanism to maintain hypertension in SHR is an important point to be considered in future studies, because the absence of effects blocking one particular mechanism does not mean that it is not involved in the development or maintenance of hypertension in SHR. Studies observing an absence of the effect of altering one brain region may overlook the importance of that region unless multiple regions are considered. Clearly the maintenance of hypertension in SHR involves complex, redundant, central mechanisms that should be examined simultaneously.

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