Ouabain- and central sodium-induced hypertension depend on the ventral anteroventral third ventricle region

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Veerasingham, Shereeni J., and Frans H. H. Leenen. Ouabain- and central sodium-induced hypertension depend on the ventral anteroventral third ventricle region. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H63–H70, 1999.—To examine the role of the ventral anteroventral third ventricle (vAV3V) in the hypertension induced by chronic subcutaneous ouabain and intracerebroventricular hypertonic saline, neurons in this area were destroyed by microinjection of an excitotoxin, ibotenic acid. Sham-operated or lesioned Wistar rats were administered ouabain (50 µg/day) or placebo for 3 wk from subcutaneously implanted controlled release pellets or artificial cerebrospinal fluid (CSF) or CSF containing 0.8 mol/l NaCl (6 µl/h) infused intracerebroventricularly for 2 wk. At the end of the experiment, mean arterial pressure (MAP) and heart rate at rest and in response to ganglionic blockade by intravenous hexamethonium (30 mg/kg) were assessed. In rats infused with hypertonic saline, responses to air jet stress were also assessed. Baseline MAP in sham-operated rats receiving intracerebroventricular hypertonic saline or subcutaneous ouabain was significantly higher than in control rats (115 ± 1 vs. 97 ± 3 and 121 ± 3 vs. 103 ± 3 mmHg, respectively). vAV3V lesions abolished the increase in MAP elicited by chronic infusion of hypertonic saline or administration of ouabain. Sham-operated rats treated with hypertonic saline or ouabain exhibited significantly increased depressor responses in MAP to hexamethonium, but lesioned rats did not. Rats infused with hypertonic saline demonstrated enhanced responses to air jet stress that were similar in sham-operated and lesioned rats. These results demonstrate that neurons in the vAV3V are essential for the hypertension induced by intracerebroventricular hypertonic saline and subcutaneous ouabain, possibly by increasing sympathetic tone. Cardiovascular responses to air jet stress appear not to be mediated by the vAV3V.

hypertonic saline; excitotoxic lesion; air jet stress; ganglionic blockade

THE PRESENCE OF endogenous ligand inhibitors of Na⁺-K⁺-ATPase activity in humans and other mammalians has been well established. An inhibitor characterized as ouabain or a stereosomer of ouabain (7, 24) has been described in mammalian plasma and may be secreted by the adrenal cortex (22). Endogenous Na⁺-K⁺-ATPase inhibitors have also been isolated from mammalian brain (9) and may be produced in the hypothalamus (1, 6, 27). Tymiak et al. (35) have identified one of these inhibitors as an isomer of ouabain.

Chronic administration of exogenous ouabain for 10 days or more, either peripherally or centrally, induces hypertension in normotensive rats (11, 23, 39). The mechanisms responsible for the ouabain-induced hypertension are still unresolved. A central mechanism is supported by the fact that intracerebroventricular administration of Fab fragments of antibodies that bind ouabain and related steroids with high affinity (2) prevent the hypertension induced by subcutaneous ouabain and that ganglionic blockade reverses the hypertension (11).

In normotensive rats, intracerebroventricular administration of hypothalamic extracts containing endogenous brain ouabain-like compounds, ouabain, or hypertonic saline cause similar sympathoexcitatory and pressor responses (10). These responses are abolished by intracerebroventricular pretreatment with anti-ouabain Fab fragments (10). This suggests that these responses to acute intracerebroventricular administration of hypertonic saline are mediated by a brain ouabain-like compound. Chronic intracerebroventricular infusion of hypertonic saline increases brain ouabain-like compound concentrations accompanied by a sustained increase in blood pressure, sympathoexcitation, and enhanced pressor responses to air jet stress in normotensive rats (13, 25). Concurrent infusion intracerebroventricularly of anti-ouabain Fab fragments prevents the hypertension induced by chronic intracerebroventricular infusion of hypertonic saline, indicating that a brain ouabain-like compound mediates this chronic pressor effect (13).

One central region that may be involved in mediating the pressor effects of chronic hypertonic saline and ouabain is the tissue surrounding the most anteroventral part of the third ventricle (AV3V). This region integrates fluid and electrolyte homeostasis and autonomic regulation (14–16). Ventricular obstruction studies demonstrate that hypertonic saline administered intracerebroventricularly needs to contact the anterior third ventricle wall to cause pressor effects (4). In addition, electrolytic ablation of the AV3V abolishes pressor responses to intracerebroventricular hypertonic saline (4) and attenuates pressor responses to intracerebroventricular ouabain (33). We demonstrated that neurons in the ventral AV3V (vAV3V) partly mediate pressor and tachycardic responses to acute intracerebroventricular administration of hypertonic saline and ouabain in rats with systemic vasopressin blockade (36). The organum vasculosum laminae terminalis (OVLT), the most ventral component of the AV3V, contains nerve terminals densely immunoreactive to ouabain (38). Endogenous ouabain-like compounds in the vAV3V may therefore be involved in mediating the pressor effects of an increase in cerebrospinal fluid (CSF) sodium concentration.

In this study, we utilized microinjections of the excitotoxin ibotenic acid to destroy neurons in the vAV3V to determine whether the hypertension induced by chronic intracerebroventricular administration of hypertonic saline and chronic subcutaneous administration...
tion of ouabain is dependent on neurons in this area. Ouabain was administered subcutaneously, because long-term intracerebroventricular administration may cause neurotoxic effects. To assess the involvement of autonomic mechanisms, we evaluated the effects of ganglionic blockade. Cardiovascular responses to air jet stress were assessed in rats treated with intracerebroventricular hypertonic saline to ascertain involvement of the vAV3V in the enhancement of these responses seen in this model (13).

**METHODS**

Male Wistar rats (Charles River, Montreal, Canada) weighing 150–200 g were housed in a climatized room at 24°C on a 12:12-h light-dark cycle and were fed regular rat chow and tap water ad libitum for at least 3 days before entering the study. Body weight was monitored weekly. All experimental procedures were approved and carried out in accordance with the guidelines of the University of Ottawa Animal Care Committee for the care and use of laboratory animals. All chemicals were purchased from BDH (Toronto, Ontario, Canada) except where otherwise noted.

**Experimental Protocols**

**Experiment 1.** Controlled time-release ouabain pellets (50 μg/day) or placebo pellets were implanted subcutaneously 1 day after either vAV3V or sham lesion. Lesioned rats were provided access to graded sucrose solutions postsurgically (10, 5, and 2% sucrose in water for 2 days each) followed by regular water to ensure fluid intake comparable to that in sham-lesioned rats (36). Rats were fed a high-sodium diet (1,370 mmol sodium/g; Harlan Sprague Dawley, Madison, WI) for the last 10 days of the experimental period to accelerate increases in blood pressure (11). At 20–21 days, blood pressure and heart rate (HR) at rest and in response to an intravenous injection of the ganglionic blocker hexamethonium (30 mg/kg) were assessed.

**Experiment 2.** Sham- or vAV3V-lesioned rats received chronic intracerebroventricular infusions of either artificial CSF (aCSF) or aCSF containing 0.8 mol/l NaCl via osmotic minipumps. Lesioned rats were provided sucrose solutions as in the previous experiment. After 14 days of infusion, blood pressure and HR were assessed at rest followed by responses to a standardized air jet stress of a 1.5-psi air stream directed on the face of the rat for 20 s. After a 10-min recovery period in which mean arterial pressure (MAP) and HR returns to a standardized air jet stress of a 1.5-psi air stream directed on the face of the rat for 20 s. After a 10-min recovery period in which mean arterial pressure (MAP) and HR returned to the skull with small screws and acrylic cement (HCG Hydrogen; St. Catharines, Ontario, Canada). The intracerebroventricular cannula was connected by polyethylene tubing (PE-50 and PE-60 combination) to an osmotic minipump (model 2ML2; Alza, Palo Alto, CA) that was implanted subcutaneously on the back of the rat. Osmotic minipumps were filled with 2.3 ml of either aCSF or aCSF containing 0.8 mol/l NaCl and infusate was delivered at a mean pumping rate of 5.0 μl/h. Penicillin G (30,000 IU; Longsil, Victoriaville, PQ, Canada) was injected subcutaneously after surgery for prophylaxis.

**Pellet implantation.** The day after excitotoxic/sham lesioning, rats were reanesthetized by halothane/oxygen inhalation. A small incision was made on the back of the rat, and two controlled time-release ouabain/placebo pellets (Innovative Research, Sarasota, FL) were implanted subcutaneously. Each pellet (0.5 mg) releases a constant amount of ouabain (25 μg/day) or vehicle over a 21-day period.

**Arterial and venous cannulation.** On the morning of the experiment, rats were reanesthetized by halothane/oxygen inhalation and instrumented with right femoral artery and vein catheters (polyethylene catheter, PE-10 and PE-50 combination) filled with heparinized saline (1,000 IU/ml heparin). The catheters were tunneled subcutaneously and exteriorized at the nape. Blood pressure measurements were taken after a 4-h recovery period.

**Blood Pressure and HR Measurements**

The arterial catheter was connected to a pressure transducer for recording MAP and HR. The bridge output signal of the transducer was amplified (Transbridge TM84; World Precision Instruments, Sarasota, FL) and fed to an IBM-compatible computer programmed by a data acquisition program (Dataquest LabPro; Data Science International, St. Paul, MN) that allowed on-line analysis of the pulsatile blood pressure signal (sampling rate 500 Hz) and storage of data. MAP and HR measurements represent averages of 10-s periods, except for air stress data in which momentary changes in MAP and HR were used. Rats were allowed an accommodation period of 30 min before resting MAP and HR were recorded.

**Intracerebroventricular Cannula Placement and Lesion Verification**

At the end of the experiment, the rats were deeply anesthetized with pentobarbital sodium (65 mg/kg ip) and injected intracerebroventricularly with 2 ml of 20% India ink in saline to verify guide cannula placement. Patency and connection of the catheter to the intracerebroventricular cannula and osmotic minipump were also checked. The rats were then
perfused transcardially with 100 ml of 10 mmol/l phosphate-buffered saline (PBS) followed by 150 ml of 4% paraformaldehyde in 100 mmol/l PBS containing 0.4% picric acid. The brain was removed, postfixed for 90 min, and cryoprotected in 10% sucrose in PBS for 1 wk. Serial 16-µm sections were cut on a cryostat, and lesions were verified by visualizing an absence of immunoreactivity to A60, an antibody that recognizes a neuron-specific nuclear antigen, NeuN (26). All antibodies were diluted in 10 mmol/l PBS (pH 7.4) containing 0.3% Triton X-100, and incubations were carried out in a humidified chamber at 35°C. Sections were washed between incubations in 10 mmol/l PBS three times. An 80-min primary incubation with A60 (1:200) was followed by PBS washes and incubation in a sheep anti-mouse biotinylated Ig (1:100; Amersham, Oakville, Ontario, Canada) for 60 min. After further PBS washes, sections were incubated with streptavidin-horseradish peroxidase complex (1:200; Amersham, Oakville, Ontario, Canada) for 30 min. Immunoreactive cells were visualized using diaminobenzidine as a chromogen. The extent of the lesions was visualized as an absence or marked

Fig. 1. Photomicrographs of neuron-specific nuclear antigen (NeuN) immunoreactivity of a typical sham-operated (A, C, and E) and lesioned (B, D, and F) rat through the anteroventral part of the third ventricle (AV3V) region (coordinates with reference to bregma: A and B, −0.10 mm; C and D, −0.25 mm; and E and F, −0.35 mm). Arrowheads point to lesioned area. Scale bar indicates 500 µm. 3V, third ventricle; ac, anterior commissure.
decrease of immunoreactivity to NeuN and mapped onto projection drawings of the rat brain from individual animals using a stereotaxic atlas as a guide (28). In a separate group of sham and lesioned rats \( (n = 4/\text{group}) \), the degree and extent of the lesion was assessed by counting immunoreactive neurons in specific AV3V nuclei using an image analysis system equipped with MetaMorph software (Universal Imaging, West Chester, PA) calibrated for the objective used \( (\times 10) \). After image acquisition and contrast enhancement, thresholding was performed to exclude weakly stained nuclei, and criteria were set to exclude nuclear fragments <8 μm. Immunoreactive neurons were counted within a 100-μm² area in each nucleus in two representative sections per rat, and averages from the two counts were used in statistical analysis. Slides were coded so that experimental groups were not known at the time of quantification.

**Statistical Analysis**

A total of seven rats from the four lesioned groups were excluded prospectively from the analysis due to unilateral vAV3V ablation. In the second experiment, a further seven rats were excluded due to incorrect intracerebroventricular cannula placement \( (n = 2) \) or blocked/disconnected catheters from minipumps \( (n = 5) \). Final numbers of rats per group were as follows: sham-operated groups receiving placebo or aCSF \( (n = 7/\text{group}) \), sham-operated rats receiving ouabain or 0.8 M NaCl \( (n = 7 \text{ and } 8, \text{ respectively}) \), lesioned rats receiving placebo or aCSF \( (n = 7 \text{ and } 6, \text{ respectively}) \), and lesioned rats receiving ouabain or 0.8 M NaCl \( (n = 8/\text{group}) \). Values are presented as means ± SE. Changes in MAP or HR in response to either hexamethonium or air jet stress from resting levels within groups were determined by paired t-tests. Comparisons of NeuN positive neurons between sham and lesioned groups were determined by unpaired t-tests, and all other group comparisons were determined by two-way ANOVA followed by the Student-Newman-Keuls test. The level of significance was set at \( P < 0.05 \).

**RESULTS**

**Extent of Lesions**

Histological verification of lesions confirmed consistent lesioning of tissue limited to the vAV3V region. Specifically, there was a marked decrease in NeuN immunoreactivity in lesioned versus sham-operated rats within the OVLT \( (9 \pm 1 \text{ vs. } 32 \pm 2 \text{ neurons/100 μm}^2, P < 0.05; \text{Fig. 1B}) \) and the most ventral part \( (\sim 100 \text{ μm}) \) of the subcommissural median preoptic nucleus (MnPO, \( 6 \pm 1 \text{ vs. } 14 \pm 2 \text{ neurons/100 μm}^2, P < 0.05; \text{Fig. 1D}) \). NeuN immunoreactivity was not significantly different between sham and lesioned rats in the remaining MnPO \( (42 \pm 3 \text{ vs. } 40 \pm 2 \text{ neurons/100 μm}^2) \), the periventricular hypothalamic nucleus (8 ± 1 vs. 10 ± 1

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**Fig. 2.** Baseline mean arterial pressure (MAP) and heart rate (HR) in sham- and vAV3V-lesioned rats treated for 3 wk with ouabain (50 μg/day sc) or placebo. bpm, Beats/min. Values are presented as means ± SE; \( n = 7-8 \text{ rats}. \) *\( P < 0.05 \) vs. other groups.

**Fig. 3.** Peak decreases in MAP and HR in response to hexamethonium \( (30 \text{ mg/kg iv}) \) in sham- and vAV3V-lesioned rats treated for 3 wk with ouabain \( (50 \text{ μg/day sc}) \) or placebo. Values are presented as means ± SE; \( n = 7-8 \text{ rats}. \) *\( P < 0.05 \) as indicated.

**Fig. 4.** Baseline MAP and HR in sham- and vAV3V-lesioned rats treated with artificial cerebrospinal fluid (aCSF) or aCSF containing 0.8 mol/l NaCl \( (5 \mu \text{l/h icv}) \) for 2 wk. Values are presented as means ± SE; \( n = 6-8 \text{ rats}. \) *\( P < 0.05 \) vs. other groups.

**Fig. 5.** Peak decreases in MAP and HR in response to hexamethonium \( (30 \text{ mg/kg iv}) \) in sham- and vAV3V-lesioned rats treated with aCSF or aCSF containing 0.8 mol/l NaCl \( (5 \mu \text{l/h icv}) \) for 2 wk. Values are presented as means ± SE; \( n = 6-8 \text{ rats}. \) *\( P < 0.05 \) as indicated.
neurons/100 µm²), anterior medial preoptic nucleus (14 ± 2 vs. 17 ± 2 neurons/100 µm²), and anteroventral preoptic nucleus (18 ± 2 vs. 16 ± 1 neurons/100 µm²). Figure 1 shows NeuN immunoreactivity in sections taken through the AV3V region of typical lesioned and sham-operated rats. Final body weights were not significantly different between sham- and vAV3V-lesioned rats in either experiment (data not shown).

Effect of vAV3V Lesion on Responses to Chronic Subcutaneous Ouabain Administration

Resting MAP in sham-operated rats receiving chronic ouabain was significantly higher compared with those on placebo treatment. vAV3V lesions abolished the increase in MAP elicited by chronic ouabain treatment (Fig. 2). Resting HR did not differ significantly between groups.

The extent of decrease in MAP caused by intravenous hexamethonium was significantly enhanced in sham-operated rats treated with ouabain but not in lesioned rats treated with ouabain (Fig. 3), resulting in similar MAP in all groups after hexamethonium (sham + placebo: 60 ± 5; sham + ouabain: 60 ± 3; lesion + placebo: 54 ± 3; and lesion + ouabain: 59 ± 4 mmHg; not significant). Decreases in HR were not significantly different between groups.

Effect of vAV3V Lesion on Responses to Chronic Intracerebroventricular Hypertonic Saline Administration

Resting MAP in sham-operated rats receiving chronic intracerebroventricular hypertonic saline infusion was significantly higher than those receiving intracerebroventricular aCSF. Lesions of the vAV3V prevented the increase in MAP elicited by chronic intracerebroventricular hypertonic saline infusion (Fig. 4). Resting HR was not significantly different between groups.

The extent of decrease in MAP caused by intravenous hexamethonium was significantly enhanced in sham-operated rats receiving intracerebroventricular hypertonic saline but was not enhanced in lesioned rats treated similarly (Fig. 5). After hexamethonium treatment, MAP was not significantly different between groups (sham + aCSF: 56 ± 4; sham + 0.8 M NaCl: 59 ± 3; lesion + aCSF: 61 ± 2; and lesion + 0.8 M NaCl: 59 ± 3 mmHg). Decreases in HR were not significantly different between groups.

Sham-operated rats infused with hypertonic saline exhibited significantly enhanced MAP and HR responses to air jet stress compared with either sham or lesioned rats treated with aCSF infusion (Table 1). Lesioned rats receiving intracerebroventricular hypertonic saline exhibited similarly enhanced responses to air jet stress.

DISCUSSION

The present study demonstrates that the vAV3V region is essential for the hypertension induced by chronic administration of hypertonic saline intracerebroventricularly or of ouabain subcutaneously. Because excitotoxic lesions spare axons passing through and nerve terminals within the area of lesion (30), it is likely that neuronal somata in the vAV3V mediate this effect. In addition, vAV3V lesions prevent enhanced depressor responses to ganglionic blockade but not enhanced cardiovascular responses to air jet stress.

Consistent with other recent studies, chronic treatment with ouabain caused moderate hypertension in conscious rats (11, 23, 39). Takahashi et al. (33) reported an attenuation of the pressor effect of acute intracerebroventricular administration of ouabain after electrolytic AV3V lesions involving the whole subcommissural MnPO and the anterior hypothalamic periventricular area. Lesions limited to the vAV3V also attenuate the pressor and tachycardic responses to acute intracerebroventricular administration of ouabain in rats with systemic vasopressin blockade (36). In the present study, vAV3V lesions fully prevented hypertension induced by chronic subcutaneous administration of ouabain likely because a comparatively smaller dose was administered, which presumably would act only on nuclei most sensitive to ouabain. A larger (acute) dose would be able to act on less sensitive nuclei as well as the vAV3V. The finding that the enhanced depressor responses to ganglionic blockade elicited by chronic administration of ouabain were prevented by vAV3V lesions suggests that this region mediates sympathetic activation in ouabain-induced hypertension. After ganglionic blockade, blood pressure was similar in all groups, consistent with a role for sympathetic activation in ouabain-induced hypertension. Administration of a vasopressin receptor antagonist after ganglionic blockade only caused a further 5-mmHg decrease in MAP in rats treated chronically with ouabain (11), suggesting that release of vasopressin contributes only in a minor way to the pressor effect of chronic treatment with ouabain. Ouabain was administered subcutaneously and therefore could also act peripherally to cause hypertension, e.g., by inhibiting the Na⁺-K⁺-ATPase in vascular smooth muscle, resulting in vasoconstriction (31). However, it is not likely that peripheral mechanisms play a significant role in the hypertension induced by subcutaneous ouabain, because central blockade of the effects of ouabain prevents hypertension in this model (11).
We previously reported that treatment with ouabain for 2 wk at a dose similar to that used in the present study caused hypertension accompanied by tachycardia (11). In the present study, rats were treated with ouabain for 3 wk and did not exhibit tachycardia. This is consistent with the findings of Manunta et al. (23), who did not find an accompanying tachycardia after 5 wk of treatment with ouabain. It is likely that, in the more chronic phase of treatment, control systems adapt, causing the tachycardia to diminish.

Consistent with an earlier study (5) intracerebroventricular infusion of 0.8 mol/l NaCl caused mild hypertension in conscious rats. Because sympathoexcitatory and pressor responses are elicited by intracerebroventricular hypotonic saline but not by other hypotonic solutions or ammonium chloride, it is likely that the responses to hypotonic saline are attributable to sodium ions rather than hyperosmolality or chloride ions (5). Infusion of 0.8 mol/l NaCl at 5.0 µl/h for 14 days increases CSF sodium ion concentration by ~6 mmol/l (13).

Our results complement earlier studies that demonstrated a complete blockade of pressor responses to an acute intracerebroventricular administration of hypertonic saline by electrolytic AV3V ablation (3, 4). vAV3V lesions attenuated the pressor and tachycardic effect of acute intracerebroventricular infusions of 0.3 mol/l NaCl by ~30% in rats with systemic blockade of vasopressin mechanisms (36). In the present study, vAV3V lesions fully prevented hypertension induced by chronic intracerebroventricular infusion of hypotonic saline. This difference in extent of blockade may be due to the fact that the concentration of sodium obtained chronically would likely be less than the concentration achieved acutely as an ~10-fold lower rate was administered chronically. Presumably smaller concentrations of sodium acted only on the nuclei most sensitive to sodium. Higher concentrations may act on less sensitive nuclei in addition to the vAV3V. Rohmeiss et al. (29) demonstrated that an angiotensinergic mechanism within the subfornical organ (SFO) mediates the pressor response to an acute intracerebroventricular injection of hypertonic saline and suggested that this response may be mediated via a pathway from the SFO to the paraventricular nucleus. This pathway traverses or synapses in the subcommissural MnPO, explaining why electrolytic AV3V lesions are able to abolish pressor responses to acute intracerebroventricular hypertonic saline. As vAV3V lesions leave the subcommissural MnPO unaffected to a large extent, it appears that the OVLT plays a greater role in mediating pressor effects of chronic intracerebroventricular infusion of hypertonic saline. In dogs, an additional site, the area postrema, has been implicated in mediating pressor responses to intracerebroventricular hypertonic saline (17).

Deficits in vasopressin release mechanisms have been demonstrated with electrolytic AV3V lesions (20, 37). It is not known if excitotoxic lesions of the vAV3V also cause a deficit in vasopressin release. Because blockade of vasopressin mechanisms was not used in the present study, it is possible that release of vasopressin may have contributed to the hypertension induced by hypotonic saline. Vasopressin contributes to the pressor effect of hypotonic saline in the early phase (day 1) of chronic intracerebroventricular infusion. In the chronic phase (day 7), elevated plasma catecholamine levels and augmented depressor responses to ganglionic blockade suggest an increase in sympathetic activation (18). In the present study, vAV3V lesions prevented the enhanced depressor response to ganglionic blockade elicited by chronic intracerebroventricular administration of hypertonic saline. In addition, despite mild hypertension in sham-operated rats receiving hypertonic saline, resting MAP was similar in all groups after ganglionic blockade. It is therefore likely that sympathetic activation mediated by the vAV3V persisted in sham-operated rats and accounts for the greater part of the pressor effect of chronic intracerebroventricular administration of hypertonic saline.

In rats receiving chronic intracerebroventricular infusion of hypotonic saline, vAV3V lesions prevent enhanced depressor responses to ganglionic blockade but not enhanced cardiovascular responses to air jet stress. Therefore, although the vAV3V appears to mediate the increase in baseline sympathetic tone due to intracerebroventricular administration of hypertonic saline, it does not mediate the enhanced cardiovascular responses to air jet stress exhibited by this model. This is consistent with studies in salt-sensitive models of hypertension. Hatton et al. (8) reported that electrolytic ablation of the AV3V in borderline hypertensive rats fed a high-salt diet did not affect cardiovascular responses to air stress. In spontaneously hypertensive rats on a high-salt diet, enhanced cardiovascular responses to air jet stress can be prevented by intracerebroventricular administration of Fab fragments but not by blockade of ouabain-like compound in the MnPO (12, 34). Although various brain areas have been proposed to mediate the cardiovascular responses to air jet stress (19, 21, 32), it appears that neurons releasing ouabain-like compounds in area(s) other than the AV3V are involved in mediating the responses to sodium loading.

In conclusion, this study demonstrates that the vAV3V is essential for hypertension that is induced by chronic intracerebroventricular hypotonic saline and subcutaneous ouabain possibly via sympathetic activation. Because both of these models of hypertension exhibit an elevation in central ouabain and can be prevented by blockade of central ouabain (11, 13), it is tempting to speculate that the vAV3V mediates the pressor response to intracerebroventricular hypotonic saline via a release of ouabain-like compounds in the vAV3V. In this regard, neurons in the vAV3V may act as the primary sodium sensor or may act as a “relay” in mediating these effects, and the primary sodium sensor may be located in the SFO or elsewhere.

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