Hypothalamic paraventricular nucleus mediates sodium-induced changes in cardiovascular and renal function in conscious sheep

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Frithiof R, Ramchandra R, Hood S, May C, Rundgren M. Hypothalamic paraventricular nucleus mediates sodium-induced changes in cardiovascular and renal function in conscious sheep. Am J Physiol Regul Integr Comp Physiol 297: R185–R193, 2009. First published May 13, 2009; doi:10.1152/ajpregu.00058.2008.—The contribution of the paraventricular nucleus of the hypothalamus (PVN) in mediating cardiovascular, renal, and sympathetic nerve responses to increased cerebrospinal fluid (CSF) [Na+] was investigated in conscious sheep. Intracerebroventricular hypertonic NaCl (0.5 mol/l, 20 µl/min for 60 min) increased arterial blood pressure [AP; +13.4 (SD 2.0) mmHg, P < 0.001] and central venous pressure [CVP; +2.8 (SD 1.3) mmHg, P < 0.001], but did not significantly change heart rate or cardiac output (n = 6). Elevated CSF [Na+] also lowered plasma ANG II levels [−3.3 (SD 1.6) pmol/l, P = 0.004] and increased creatinine clearance [+31.5 (SD 32.7) ml/min, P = 0.03] and renal sodium excretion [+9.2 (SD 9.2) mmol/h, P = 0.003]. Lidocaine injection (1 µl %, 2%) into the PVN prior to the ICV infusion had no apparent effect per se, but abolished the AP, CVP, creatinine clearance, and ANG II responses to hypertonic NaCl, as well as reducing the increase in renal sodium excretion (n = 6). Subsequent studies were performed in conscious sheep with chronically implanted electrodes for measurement of renal sympathetic nerve activity (RSNA). The effects of ICV hypertonic NaCl on AP and RSNA were measured before and after PVN-injection of glycine (250 nmol in 500 nl artificial CSF). ICV NaCl increased AP and decreased RSNA (P < 0.001). These effects were significantly reduced by glycine (P = 0.02–0.001, n = 5). Saline injected into the PVN (n = 5) or lidocaine injected outside the PVN (n = 6) had no effect on the response to ICV hypertonic NaCl. These results indicate that the PVN is an important mediator of cerebrally induced homeostatic responses to elevated sodium concentration/hyperosmolality.

Hypothalamic medulla; hypothalamus; hypertension; angiotensin II; intracerebroventricular; RSNA; sympathetic nerve activity

INCREASED BODY FLUID SODIUM CONCENTRATION [Na+] affects cardiovascular and renal function via actions on the brain. Intracerebroventricularly (ICV) administered hypertonic NaCl increases brain sodium concentration without concomitant peripherally elicited effects. It has been shown to induce arginine vasopressin (AVP) release from the posterior pituitary (2, 10), cause natriuresis (4, 31, 47), and increase sympathetic nerve activity (SNA) (11, 40, 45). Several studies have also demonstrated pressor responses to ICV-administered NaCl in conscious hypertensive (18), normotensive (3, 30, 44), and hypertensive (36) animals. A large increase in cerebrospinal fluid (CSF) [Na+] results in a rapid elevation of arterial blood pressure (AP), while more modest, but persistent, rises in CSF [Na+] are associated with a blood pressure increase over several days (22). In severely hypovolemic sheep, resuscitation with intravenous hypertonic NaCl increased blood pressure and regional blood flow, partly by improving cardiac function via an ANG II type 1 receptor-mediated mechanism in the periventricular area of the brain (17).

A brain region crucial for the regulation of body fluid homeostasis is the paraventricular nucleus of the hypothalamus (PVN). Located lateral to the third ventricle, it is anatomically and functionally connected to neurons residing in forebrain sensory circumventricular organs, parts of the brain lacking a functional blood-brain barrier known for their ability to sense small changes in sodium concentration/osmolality (34). It includes large vasopressin-producing magnocellular neurons and smaller parvocellular neurons that project to spinal preganglionic sympathetic neurons and premotor sympathetic neurons in the brainstem (25). The PVN is thus a plausible site for coordination of neurogenic and hormonal actions on the cardiovascular system and the kidney in response to changes in sodium concentration/osmolality. Indeed, in anesthetized rats blockade of ANG II type 1 receptors in the PVN attenuates the acute renal sympathoexcitation that occurs after intracarotid infusion of hypertonic NaCl (11). As well, in an in situ decorticate rat preparation, hyperosmolality induced an increase in lumbar SNA that was blunted by microinjection of a GABA receptor agonist or a glutamate receptor antagonist into the PVN (6). However, the influence of the PVN on homeostatic changes by elevated brain sodium has not been investigated in intact, conscious animals.

The effect of cerebral hyperosmolality on renal SNA (RSNA) varies with species and/or anesthesia. RSNA has been shown to decrease in response to ICV hypertonic NaCl in conscious sheep and anaesthetized dogs (21, 29) and to intravenous hypertonic saline in conscious rabbits and anaesthetized rats (7, 46). In sheep this effect is independent of activation of baroreceptors (28). Renin release, which is strongly regulated by RSNA (13), is consistently reported to decrease in response to elevated brain [Na+] (15, 33, 37). In contrast, intracarotid NaCl caused an increase in RSNA in anaesthetized rats (11, 40). It is unknown whether the PVN facilitates the changes in renin levels as well as the decrease in RSNA seen in many studies. Furthermore, the contribution of the PVN in mediating changes in renal and cardiac function by ICV hypertonic NaCl is largely unexplored.

In the present studies, we investigated the influence of the PVN on the integrated cardiovascular and renal responses to an acute elevation of sodium concentration limited to the brain. Based on available evidence, we hypothesized that the PVN would be crucial for the full manifestation of cerebrally mediated effects of hypertonic NaCl. To investigate this hypothesis, the effects of ICV hypertonic NaCl on cardiovascular and renal

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function, as well as plasma levels of ANG II, were examined in conscious sheep with and without lidocaine-induced inhibition of the PVN. In further studies, direct neural recordings of RSNA were carried out in conscious sheep where cell bodies in the PVN were inhibited by injections of glycine.

METHODS

The study is based on two sets of experiments. The first set was performed at Karolinska Institutet, Stockholm, Sweden, and consists of PVN injections of lidocaine in sheep equipped with arterial and venous cannulae, a pulmonary artery catheter, and urinary catheter for the measurement of various cardiovascular and renal function parameters. These experiments are referred to as the PVN lidocaine experiments. The second series of experiments took place at the Howard Florey Institute in Melbourne, Australia. Here glycine was injected in the PVN of sheep where arterial pressure and RSNA were measured. In the text, these experiments are referred to as the PVN glycine experiments.

Animals

PVN lidocaine experiments. Twelve Texel crossbred ewes with an average weight of 53.5 kg (SD 7.5) were housed individually in pens with drinking water ad libitum. Each day they were fed hay twice and 6 g of NaCl mixed with 75 g of pellets. All experiments were conducted with the animal standing, unrestrained, in an open cage within sight and hearing of other sheep. The experiments were approved in advance by the animal ethics committee in Stockholm, Sweden, and adhere to the European Union directive no. 86/609/EEG and the European Council convention no. ETS 123.

PVN glycine experiments. Five adult merino ewes weighing 36.5 kg (SD 3.5) were housed in individual metabolism cages in association with other sheep. They were fed a diet of oaten chaff (800 g/day), and water was offered ad libitum. All experiments were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute.

Surgical Procedures

At a minimum of 3 wk before the experiments, the sheep were anesthetized and subjected to unilateral exteriorization of one carotid artery into a cervical skin loop. Special care was taken not to damage or stretch the vagus nerve. Standard surgical anesthesia included premedication with acepromazin (0.3 mg/kg iv) and induction using sodium thiopental injection (10 mg/kg iv) followed by succinylcholine (1 mg/kg iv). After oral intubation, anesthesia was maintained by sodium thiopental injection (10 mg/kg iv) followed by succinylcholine (0.002 mg/kg im) and benzylpenicillin (20,000 IU/kg)/dihydrostreptomycin (0.0025 g/kg) were made routinely after all surgical procedures. To avoid the later ventricle. The other two (OD, 1.0 mm) were placed bilaterally with the tips 5 mm above the PVN (n = 6) or in other parts of the hypothalamus for comparison (n = 6, sham). Placement of guide cannulae aiming toward the PVN was done under x-ray guidance after an inner needle was lowered into the cerebral ventricles via one of the ICV-guide cannulae, and 0.6 ml iohexol contrast medium (Omnipaque; GE Healthcare Europe, Munich, Germany) was injected. Radiographs were used to identify the location of the PVN in a two-dimensional sagittal plane. With the use of a micromanipulator (David Kopf Instruments, Tujunga, CA) the cannulae were lowered into the brain in different caudal-to-cranial angles before they were fixed in place by dental acrylic. All guide tubes were blocked with an obturator until the time of the experiment. Additional radiographs were taken to assure communication between the lateral and third ventricle.

In an additional five sheep (PVN glycine experiments), with previously prepared carotid artery loops and PVN guide tubes, additional surgery was performed to implant recording electrodes into a renal sympathetic nerve (7, 29). Under general anesthesia, the right or left renal artery was exposed via a paracostal retroperitoneal approach. With the aid of a dissection microscope, the renal nerve was identified running along or parallel to the renal artery and cleared of surrounding fat. The recording electrodes consisted of tungsten wire (0.05 mm diameter) etched to a fine point, and glued into the end of Teflon-coated 25-strand silver-coated copper wires (model CZ1174SPC; Cooner, Chatsworth, CA). The exposed point of the electrode (1.5–2.0 mm in length) was inserted obliquely through the nerve sheath, ensuring that the tip was positioned in the center of the nerve. Up to five electrodes were implanted along the exposed length of nerve and fixed in place with cyanoacrylate glue. The wires were looped and exteriorized through the sutured wound. For a ground, a stainless steel plate with an attached metal loop was inserted subcutaneously close to the exit site of the electrodes, leaving the loop protruding through the skin.

Experimental Preparations and Recordings

PVN lidocaine experiments. The urinary bladder was catheterized via the urethra. A cannula (OD, 1.0 mm) was inserted in one of the exteriorized carotid arteries and connected to a saline-filled tube to measure AP. A central venous catheter was introduced through the left jugular vein and was used for blood sampling. Finally, a flow-directed, balloon-tipped thermocatheter catheter (Swan-Ganz; Edward Labs, Santa Ana, CA) was fed into the pulmonary artery via an introducer in the right jugular vein and used for measuring central venous pressure (CVP) and cardiac output (CO). Intravenous and bladder catheterizations were performed under local anesthesia (lidocaine hydrochloride injection 5 mg/ml and 2% gel, respectively) at least 60 min before starting the experiments.

The AP and CVP tubes were connected to pressure transducers (model DPT-6003; PVB Medizin Technik, Germany), and signals from the pulmonary artery catheter were fed into a Vigilance Edwards Critical Care Monitor (Baxter Healthcare, Deerfield, IL). CO was calculated by the Vigilance Monitor continuously every 30 s (thermodilution technique) as well as intermittently using three consecutive rapid infusions of ice-cooled isotonic saline (10 ml × 3). The later measurement was used for the statistical evaluation. Continuous online data acquisition was achieved by using the model MP150/ Acknowledge 3.8.2 system (BIOPAC Systems; Goleta, CA) with a sampling rate of 250 Hz. Heart rate (HR) was computed from the AP signal and displayed online.

PVN glycine experiments. On the day of the experiment, a cannula (OD, 1.0 mm) was inserted in the exteriorized carotid artery and connected to a pressure transducer (model TDXIII; Cobe) tied to the wood on the sheep’s back. RSNA was recorded differentially, amplified (×100,000) and filtered (band pass 100–1,000 Hz), displayed on an oscilloscope and passed through an audio amplifier and loud speaker. RSNA (5,000 Hz), AP (100 Hz), and CVP (100 Hz) were recorded on a computer using a micro 1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Data were analyzed on a beat-to-beat basis using custom-written routines in the Spike2 program. For each heart beat the program determined diastolic, systolic, and mean AP, heart period, and the number of discriminated spikes above threshold between the following diastolic pressures, a measure of burst size. The threshold was set just above background so that spikes from small bursts were counted. The background noise was taken as the spikes/s during an intravenous infusion of phenylephrine performed separately, which abolished RSNA, and this was subtracted from all data.

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As a measure of RSNA, the spikes above threshold minus the background were calculated for each heart beat. Burst frequency was calculated as the percentage of heart beats that included spikes above background. The accuracy of burst determination was checked by eye over the 5-min control data for each sheep. Post hoc analysis of data was conducted using custom written scripts for Spike2, and data were exported to a spreadsheet for further analysis.

Intracerebral Injections and ICV Infusions

Hypertonic NaCl (0.5 mol/l) was prepared by adding 0.35 mol/l NaCl to artificial CSF (aCSF). For the ICV-infusions, a probe (OD, 1.0 mm) of appropriate length was lowered into one of the lateral ventricles via a guide cannula. Gentle siphoning of CSF confirmed the placement and the probe was connected via a polyethylene tube to a syringe placed in an infusion pump (model 802 syringe pump; Univentor, Zejtun, Malta).

Bilateral intracerebral lidocaine injections (1 μl on each side over 2 min) were performed simultaneously by inserting probes (OD, 0.7 mm) in the appropriate guide cannulae and connecting them to two microsyringes in the infusion pump via polyethylene tubes. The exact lengths of the probes chosen were based on the radiographs and designed to reach the designated target (PVN or sham area). They usually extended 5 mm past the guide cannula. The probes were flushed with the experimental injectate before each insertion.

At the end of the experiment, 1 μl blue dye was injected bilaterally through the probe for histological verification of the cannula position after the sheep were killed. To be grouped as a PVN injection, the injection site had to be positioned inside or within 0.5 mm of the boundaries of the PVN bilaterally.

Experimental Protocol

PVN lidocaine experiments. The experimental protocol for the PVN lidocaine experiments is illustrated in Fig. 1. After a 60-min recovery period, baseline recordings were initiated. Lidocaine (1 μl, 2%) was injected into the PVN, and the effects on hemodynamic and renal parameters were evaluated for 60 min. Thereafter the lidocaine injection was repeated and an ICV-infusion of 0.5 M NaCl (20 μl/min) was started. The ICV-infusion was discontinued after 60 min. After a 2-h washout period, the protocol was repeated without the lidocaine injections. In three animals the lidocaine interventions were performed before and 60 min after the start of each ICV-infusion and before, 15, and 60 min after each lidocaine injection. At these same time points as CO was measured, blood samples (17 ml) were drawn from the left jugular vein. Additional blood (5 ml) was taken 30 min after lidocaine injection when not followed by an ICV-infusion for determination of plasma creatinine concentration. In the ICV-infusion experiments the plasma creatinine concentration was measured after 60 min. CO measurements and urine and blood sample collections were made at corresponding times in the control protocol. CSF was sampled (300 μl) before and after the ICV-infusions for determination of [Na⁺].

PVN glycine experiments. After a 15-min baseline period, while recording arterial pressure and RSNA, an ICV-infusion of 0.5 M NaCl (16.6 μl/min) was started. When AP had increased to a level comparable to that in the PVN lidocaine experiments, after ~30 min of infusion, 250 nmol glycine in 500 nl aCSF or 500 nl aCSF (control) was injected in the PVN bilaterally over 2 min. Arterial pressure and RSNA were measured for another 30 min. The glycine and control experiments were performed in the same sheep on separate days, with the order randomized.

After the experiments, the sheep were killed by an overdose of sodium barbiturate followed by decapitation. The head was instantly perfused with isotonic saline via the carotid arteries followed by 4% formaldehyde in isotonic saline. The brain was removed and fixed in formaldehyde (4%) for 48 h before a block containing the hypothalamus was frozen in isopentane mixed with dry ice. Serial transverse sections (30 μm) were cut from the block using a cryostat (Micron HM 500 M; Cellab Nordia, Nacka, Sweden), mounted on object glasses, and stained with toluidine blue. The injection sites were verified using light microscopy (Wild M8 stereozoom microscope; Heerbrugg, Switzerland).

Blood, CSF, and Urine Analysis

Venous blood samples were put immediately into prechilled tubes and centrifuged at 3,000 rpm (1,460 g). An aliquot of blood was mixed with EDTA, and the plasma was frozen (−20°C) for later determination of ANG II concentrations. The remaining blood was heparinized and used for measurement of hematocrit, plasma osmolality (Auto & Stat Om 6100 osmometer; Kagaku, Kyoto, Japan), [Na⁺] and [K⁺] (model IL 943 flame photometer; Instrumentation Labs), protein concentration by refractometry (Atago, Kyoto, Japan), and creatinine concentration by the Jaffe method (Synchron LX; Beckman Instruments, Richmond, CA). Plasma samples were analyzed for [Na⁺]. Urine osmolality, creatinine, [Na⁺], and [K⁺] were determined as above. Plasma concentrations of ANG II were quantified using commercial radioimmunooassay kits (Peninsula Laboratories, San Carlos, CA). Plasma samples (2 ml) were extracted with cold aceton and petroleum benzine prior to radioimmunoassay. The lowest detectable levels in the assays were 2.0 pmol/l. The ANG II antibody fully cross-reacts between human and sheep ANG II ([Val⁵]-ANG II).

Data Analyses

AP, CVP, and HR were averaged over 5-min intervals in the lidocaine experiments. In the glycine experiments, RSNA and AP were calculated over 2-min intervals. Total peripheral vascular resistance was calculated as [(AP−CVP)/CO]. For determination of the urinary sodium excretion and urine flow effects of ICV hypertonic...
NaCl values from two samples following the start of the infusion were averaged. Creatinine clearance was calculated as [(urine flow × urine creatinine concentration)/plasma creatinine concentration]. Endogenous creatinine clearance has shown a good correlation to inulin clearance in sheep and was thus taken as an approximation for glomerular filtration rate (GFR) in these experiments. Free water clearance was calculated as [(urine flow − (urine flow × urine osmolality/plasma osmolality)] and fractional sodium excretion as [(urine sodium concentration × plasma creatinine concentration)/(plasma sodium concentration × urine creatinine concentration)].

All statistical calculations were performed using Statistica 7.1 (Statsoft, Tulsa, OK), and the graphs were created with Sigma Plot 8.02 (SPSS, Chicago, IL). The results are presented as individual values or means and SD with a significance level set as \( P \leq 0.05 \). Cardiovascular effects of PVN lidocaine injection per se were evaluated with a Bonferroni corrected Student’s \( t \)-test for dependent samples, comparing baseline values with those obtained 15 min after PVN-injection. The responses to ICV infusion of hypertonic NaCl after intracerebral lidocaine injection were analyzed with two-way repeated-measures ANOVA (lidocaine/control × time). A significant effect of time was interpreted as an effect of ICV hypertonic NaCl and a significant interaction effect was taken as an effect of lidocaine. Sodium excretion and creatinine clearance values were transformed to follow a normal distribution by taking the logarithm of the raw data. Separate analyses were performed for PVN and sham experiments.

The effects of ICV hypertonic NaCl followed by glycine injection in the PVN on RSNA and AP and were evaluated with pairwise Bonferroni corrected \( t \)-tests (baseline vs. 0.5 M NaCl ICV and 0.5 M NaCl ICV vs. 0.5 M NaCl ICV + 250 nmol glycine in the PVN).

RESULTS

During the experiments the animals were standing quietly in their cages. They showed no signs of discomfort and the infusions/injections did not cause any change in behavior.

Effects of PVN Inhibition Per Se

Inhibition of neural activity in the PVN with lidocaine had no effects on resting AP [99.3 (SD 3.1) vs. 100.8 (SD 3.4) mmHg, \( P = 0.44, n = 6 \)], CVP [2.3 (SD 1.4) vs. 1.9 (SD 0.8) mmHg, \( P = 0.55 \)], or HR [72.0 (SD 9.0) vs. 73.1 (SD 9.2) beats/min, \( P = 0.84, n = 6 \)], measured 15 min after PVN injection. No significant changes were observed in CO [6.0 (SD 1.2) vs. 5.5 (SD 1.6) l/min, \( P = 0.50, n = 6 \)] or ANG II levels [23.5 (SD 4.0) vs. 22.5 (SD 3.1) pmol/l, \( P = 0.64, n = 6 \)] after 15 min compared with preinjection. Urine flow (\( P = 0.72, n = 6 \)) and Na\(^+\) excretion (\( P = 0.75, n = 6 \)) measured during the hour following the lidocaine injection did not differ in relation to the control period (data not shown).

Figure 2 illustrates a schematic overview of the sites of microinjections. The injection sites were distributed from the rostral to the caudal parts of the PVN, with the majority placed at midlevel. Considering the volume of the injectate and that these areas partly overlap in the sheep, the lidocaine probably affected both magnocellular and parvocellular neurons. Sham injections were placed above and/or lateral to the PVN.

Effects of PVN Lidocaine on the Responses to ICV Hypertonic NaCl

ICV infusion of 0.5 mol/l NaCl resulted in a significant increase in AP [98.2 (SD 4.0) to 111.5 (SD 5.3) mmHg, \( P < 0.001 \), Fig. 3A] and CVP [1.8 (SD 0.9) to 4.7 (SD 1.6) mmHg, \( P < 0.001 \), Fig. 3B]. Both the AP [97.8 (SD 3.1) to 97.7 (SD 2.7) mmHg, Fig. 3A] and the CVP [2.3 (SD 0.9) to 2.2 (SD 0.9) mmHg, Fig. 3B] responses were abolished after inhibition of the PVN with lidocaine. There were nonuniform changes in heart function when hypertonic NaCl was infused ICV. Some sheep had tachycardia (Fig. 4A) with an attendant increase in CO (Fig. 4B), while some animals had no change or decrease in HR and CO. Regardless of the direction, the response was usually consistent between the two ICV-infusions in the same animal. Group analysis showed no significant change in HR [70 (SD 8) to 72 (SD 11) beats/min, \( P = 0.92 \), Fig. 4A] or CO [5.3 (SD 1.2) to 5.5 (SD 1.4) l/min, \( P = 0.47 \), Fig. 4B] observed after ICV hypertonic NaCl. The lidocaine injection had no effect on the HR or CO responses (\( P = 0.12 \) for both HR and CO, Fig. 4, A–B). As the CO response was variable, so was the calculated total peripheral vascular resistance response. However, without inhibition of the PVN, the average response was still a significant increase in total peripheral vascular resistance after ICV hypertonic NaCl [19.5 (SD 3.5) to 22.1 (SD 4.4) mmHg l\(^{-1}\)·min\(^{-1}\), \( P = 0.041 \), Fig. 4C]. This change was significantly (\( P = 0.007 \)) different from when ICV hypertonic

**Fig. 2.** Distribution of all injection sites in a schematic drawing adapted from coronal sections from 1 animal. Dots show injection sites as visualized by the distribution of blue dye. White dots indicate the paraventricular nucleus of hypothalamus (PVN) lidocaine injection sites, black dots with white crosses are the PVN glycine injection sites, and black dots are the sham injection sites. OC, optic chiasm.
NaCl was preceded by lidocaine injection in the PVN [18.4 (SD 4.4) to 16.4 (SD 3.6) mmHg], but not plasma [Na⁺], was increased in all animals. CSF [Na⁺] increased from 150.4 (SD 1.7) mmol/l to 169 (SD 2.3) mmol/l (P < 0.001, n = 6) in the sheep pretreated with lidocaine and from 150.2 (SD 1.7) to 166.8 (SD 3.3) mmol/l (P < 0.001, n = 6) in the control group. Compared with preinfusion levels, urinary sodium excretion increased (P = 0.003, n = 6, Fig. 5B) during the 2 h following commencement of the ICV infusion. This response was significantly blunted by lidocaine injection in the PVN (P = 0.042, n = 6, Fig. 5B). Interestingly, fractional sodium excretion was decreased significantly by ICV hypertonic NaCl in the control experiments (Fig. 5E), indicating that the elevated sodium excretion is due to an increased filtered load of sodium and not to a reduced tubular reabsorption. The decrease in fractional sodium excretion was abolished with lidocaine injection in the PVN (Fig. 5E).

Fig. 3. Individual responses in arterial pressure (AP; A) and central venous pressure (CVP; B) to ICV infusion of hypertonic NaCl with (lidocaine, black symbols) or without (control, white symbols) lidocaine injection inside (PVN) or outside (sham) the PVN. Inhibition of the PVN with lidocaine significantly (P < 0.001) affected the AP and CVP responses to ICV hypertonic NaCl. Symbols represent individual animals.

Fig. 4. Individual responses in heart rate (HR; A), cardiac output (CO; B), and total peripheral resistance (TPR; C) to ICV infusion of hypertonic NaCl with (lidocaine, black symbols) or without (control, white symbols) lidocaine injection inside (PVN) or outside (sham) the PVN. Hypertonic NaCl ICV had no significant effect on heart rate (HR) or cardiac output (CO), regardless of lidocaine injection, but significantly increased TPR (P = 0.04) if lidocaine was injected into the PVN. Symbols represent the same individual animals as in Fig. 4.
Urine flow measured during the same period, did not change significantly over time (P = 0.39, n = 6, Fig. 5A), and PVN inhibition had no effect. GFR increased in response to hypertonic NaCl ICV (P = 0.03, n = 6, Fig. 5C) with this response being blocked by lidocaine injection in the PVN. There was a small but significant decrease in plasma ANG II levels (P = 0.004, n = 6, Fig. 5F) with ICV hypertonic NaCl, which was also blocked by PVN lidocaine injection (P = 0.57, n = 6, Fig. 5F). Free water clearance was significantly decreased, indicating elevated levels of AVP (Fig. 5D). Lidocaine into the PVN did not alter the free water clearance responses to ICV hypertonic NaCl. Neither plasma protein concentration nor plasma osmolality changed significantly in response to increased CSF [Na⁺], regardless of PVN inhibition (data not shown).

Changes in AP and RSNA in Response to ICV Hypertonic NaCl and Subsequent PVN Glycine Injection

In this second group of conscious sheep, ICV infusion of 0.5 mol/l NaCl (1 ml/h) significantly increased AP and reduced RSNA (P < 0.001 for both, n = 5, Figs. 6 and 7). After ~30 min infusion of hypertonic saline, when AP had increased by 12 mmHg and RSNA was inhibited by 56%, bilateral injection of glycine (250 nmol) in the PVN caused a rapid decrease in AP and increase in RSNA (P = 0.002 and P = 0.02, 2-min average directly after PVN-injection, n = 5,) (Figs. 6 and 7).

Effects of Lidocaine Injection Outside or Saline Inside the PVN

Lidocaine injections in regions outside the PVN caused no significant changes in the cardiovascular (Figs. 3–4), hormonal, or renal responses to ICV hypertonic NaCl (data not shown). This indicates that the results obtained after inhibiting neural activity in the PVN were not a nonspecific effect of cerebral lidocaine microinjections. Furthermore, saline injected in the PVN failed to alter the response to ICV hypertonic NaCl.

DISCUSSION

The aim of the present study was to determine the contribution of the PVN to the cardiovascular and renal responses to...
increased CSF sodium concentration. There are four main findings originating from the experiments performed. First, inhibition of neural activity in the PVN abolished the increase in arterial and CVPs seen after selectively elevating [Na+] in the CSF compartment. Second, blockade of the PVN inhibited the fall in plasma ANG II observed after ICV hypertonic NaCl. Third, the increased urinary sodium excretion and glomerular filtration rate evoked by the increase in central [Na+] was blunted by lidocaine injection in the PVN. Finally, inhibition of cell bodies in the PVN with glycine substantially reversed the reduction in RSNA induced by ICV hypertonic NaCl. These observations support the hypothesis that the PVN is a key structure in sodium-induced alterations in cardiovascular and renal function mediated by the brain.

The PVN has long been known to be involved in the control of a variety of autonomic and endocrine functions (43). AVP is produced in the PVN by magnocellular neurosecretory neurons that project to the posterior pituitary. Surrounding the group of magnocellular cells are smaller parvocellular neurons that innervate “presympathetic” neurons in the brainstem, but also project directly to sympathetic preganglionic motor neurons in the intermediolateral cell column of the spinal cord (25). Besides the anatomical connections, functional studies support a role for the PVN in controlling sympathetic outflow (8, 23). The magnitude of contribution of the PVN to the control of AP in healthy conscious animals is, however, still not clear. In the present experiments, we were not able to detect any changes in AP, or HR after bilateral PVN inhibition with lidocaine in normotensive sheep.

In the brain, plasma and CSF [Na+] are monitored via sodium/osmo receptors in sensory circumventricular organs. One of the most important areas in this respect is the lamina terminalis in the anterior wall of the third ventricle (34). The lamina terminalis is composed of the organum vasculosum laminae terminalis (OVLT), median preoptic nucleus (MnPO), and the subfornical organ (SFO) and is of crucial importance for osmotically induced thirst, vasopressin release, and natriuresis (34). In conscious sheep, lesion of this area abolished the pressor effect and attenuated the inhibition of renin release induced by ICV hypertonic NaCl, as well as preventing the decrease in RSNA (30). This is consistent with the recent finding that chemical inhibition or electrolytic lesion of the OVLT inhibited the changes in RSNA seen after injections of hypertonic NaCl in the internal carotid artery of anesthetized rats (40).

There is extensive evidence of anatomical and functional links between the lamina terminalis and the PVN. Both parvo- and magnocellular PVN neurons are densely innervated by neurons originating from the OVLT, MnPO, and SFO (32). There is also evidence from electrophysiological and c-fos expression studies that neurons in the OVLT, MnPO, and SFO projecting to the PVN are activated by increased extracellular osmolality (1, 24, 34, 39, 42). A role for the PVN is supported by the finding that the increase in lumbar SNA, in response to intravenous hypertonic NaCl, was attenuated by ionotropic glutamate receptor blockade of the PVN, in an in situ decorticating preparation (6). In the present study, lidocaine injected into the PVN would have had the same effect as an electrolytic lesion, blocking fibers of passage as well as neuronal cell bodies. From this experiment it is therefore unclear whether the actions of lidocaine were due to inhibition of cell bodies in the PVN, or due to interference with transmission in fibers. The PVN injection of glycine, which inhibits cell bodies but does not affect fibers of passage, caused a rapid reduction of the sodium-induced pressor response and attenuated the renal sympathoinhibition, confirming that neurons located in the PVN are part of the pathway mediating the homeostatic responses to elevated CSF [Na+]. By the end of the ICV infusion, CSF [Na+] had reached levels higher than is expected during normal physiological conditions, but the attenuation of the effects by inhibition of the PVN persisted. It is acknowledged that the CSF [Na+] reaching almost 170 mmol/l is rarely seen even during pathophysiological conditions. However, this level was obtained at the end point of the protocol, and PVN inhibition with lidocaine also blocked effects of more subtle changes in CSF compositions, occurring earlier during the infusion. These findings in conscious animals indicate that it is likely that the pressor response, change in renal function, and inhibition of RSNA are mediated by neurons in the PVN. These responses are also abolished by lesions of the lamina terminalis (30, 35), suggesting that changes in CSF composition are detected by neurons in the lamina terminalis that project to the PVN.

There is evidence from a number of studies that the SNA plays the major role in determining the pressor response to central hypertonicity. A recent study showed that in conscious rats the increase in AP after an intravenous bolus injection of hypertonic NaCl was reversed by the α1-adrenoceptor antagonist prazosin (6). Further evidence that the increase in AP is coupled to an augmented discharge in SNA is the finding that in sino-aortic baroreceptor-denervated, anesthetized rats, inhib-
bition of ANG II type-1 receptors in the PVN attenuated the increase in RSNA and AP in response to intracarotid administration of hypertonic NaCl (11). In addition, physiologically produced hypertonicity, by dehydration, induced higher renal and lumbar sympathetic output originating from the PVN in anesthetized rats (16, 41).

It remains unclear, however, which sympathetic outflows contribute to the pressor response due to the variable changes in SNA reported. Differences in the use of anesthesia, species, baroreceptor function, and ways of inducing hypertonicity renders direct comparisons difficult. A number of studies have reported an increase in RSNA, which will have a direct pressor action and will also induce formation of ANG II. This will have a pressor effect per se as well as acting in conjunction with the elevated SNA to further increase pressure (20). In contrast, the present and other studies have found that increases in brain [Na⁺] cause a decrease in RSNA (7, 21, 29, 46), which in conscious sheep is baroreceptor independent (28), suggesting that the pressor response is due to stimulation of SNA to other vascular beds. Indeed, renal sympathoinhibition would appear to be the logical step to facilitate a rapid excretion of excess sodium, and functional parameters measured did change in accordance with a decreased RSNA (increased sodium excretion and GFR as well as decreased plasma ANG II).

Studies of changes in other sympathetic outflows indicate that hypernatremia increased lumbar but decreased splanchnic and renal SNA in anesthetized baroreceptor-intact rats (46), and studies in conscious sheep found a decrease in RSNA but an increase in cardiac SNA (CSNA) in response to ICV hypertonic saline (7, 29, 45).

Although variable changes in heart function were observed, the pressor effect of ICV hypertonic NaCl was mainly via peripheral vasoconstriction. In normotensive conscious sheep, ICV hypertonic NaCl has been shown to increase CSNA (45). This small increase in CSNA probably accounted for the maintained or slightly increased CO induced by hypertonic saline in the present study, whereas if there had been a baroreceptor-mediated fall in CSNA, a fall in CO would probably have occurred. These findings are in contrast to hypovolemic sheep where cardiac stimulatory effects of cerebral increase in [Na⁺] are essential for an increase in AP (17).

The development of spontaneous hypertension in rats is characterized by an initial autonamically mediated venoconstriction (27). Interestingly, in this study CVP was increased by hypertonic saline in the present study, whereas if there had been a baroreceptor-mediated fall in CSNA, a fall in CO would probably have occurred. These findings are in contrast to hypovolemic sheep where cardiac stimulatory effects of cerebral increase in [Na⁺] are essential for an increase in AP (17).

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The increased loss of sodium via the kidneys in response to ICV hypertonic NaCl appears to be mainly due to augmented GFR as indicated by the reduced calculated fractional sodium excretion. Several factors may have contributed to the attenuation of this effect after PVN inhibition. The most obvious is the lack of increase in AP after ICV hypertonic NaCl, preventing a pressure natriuresis. This likely involved the renal sympathetic nerves since renal denervation prevents increased renal sodium excretion by ICV hypertonic NaCl in conscious rats (19). Also, since PVN inhibition prevented the elevation in CVP by ICV hypertonic NaCl, stretch-sensitive mechanoreceptors in the veno-atrial junction of the heart subservient to the plasma volume expansion reflex (38), may have been less activated and not induced by increased GFR and sodium loss via the kidneys. Especially the reduction in RSNA by ICV hypertonic NaCl may be partly attributed to an increase in CVP as it has been demonstrated that sympathetic activity to the kidney is sensitive to changes in central pressures and volume (13). However, it is likely that the increased renal sodium excretion by ICV hypertonic NaCl is partly due to a reduction in RSNA, originating directly from the lamina terminalis and involving the PVN.

Perspectives and Significance

Both experimental (12) and epidemiological (19a) data indicate a strong correlation between sodium intake and increased blood pressure. A reduction in excessive sodium consumption has also been shown to mitigate hypertension (14). Increased brain sodium concentration per se has been proposed to cause hypertension via activation of the sympathetic nervous system resulting in increased peripheral vasoconstriction (9). The present study in conscious, normovolemic sheep demonstrates that an increase in brain [Na⁺] stimulates the PVN from a resting state where it has no tonic effect on short-term cardiovascular control to an excited state leading to an increase in blood pressure. This is due to peripheral vasoconstriction of both arteries and veins independent of circulating AVP and ANG II levels. Together with a decrease in circulating ANG II and RSNA this pressor effect aids in acutely removing excess sodium via the urine. In accordance with studies performed in rats (5, 11), these results suggest that the PVN may be part of the neural circuitry stimulated by increased brain sodium concentration that is responsible for slowly developing sodium-sensitive hypertension.

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