Glutamatergic input in the PVN is important in renal nerve response to elevations in osmolality

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Submitted 15 October 2002; accepted in final form 2 June 2003

Badoer, Emilio, Chi-Wai Ng, and Robert De Matteo.
Glutamatergic input in the PVN is important in renal nerve response to elevations in osmolality. Am J Physiol Renal Physiol 285: F640–F650, 2003; 10.1152/ajprenal.00372.2002.—Elevations in plasma osmolality elicit reflex humoral and neural responses. The hypothalamic paraventricular nucleus (PVN) is important in humoral responses. We have investigated whether the PVN contributed to the renal nerve reduction that is normally elicited by increased plasma osmolality in the conscious rabbit. Renal sympathetic nerve activity (RSNA) was monitored after an intravenous infusion of hypertonic saline (1.7 M NaCl, 2 ml/min for 7 min). The responses were examined in animals microinjected with muscimol (10 nmol) into, and outside, the PVN to acutely inhibit neuronal function or with kynurenate (25 nmol) to block glutamate receptors. Compared with vehicle, the maximum reduction in RSNA elicited by hypertonic saline was significantly less with muscimol or kynurenate pretreatment into the PVN. A similar study with kynurenate was also performed in sinoaortically denervated rabbits, and similar effects were observed. The effect was specific to the PVN because microinjections of the drugs outside the PVN had no effect on the response. The findings suggest that excitatory inputs into the PVN may be important in the neural responses elicited by elevations in plasma osmolality.

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AN INCREASE IN PERIPHERAL osmolality elicits reflex changes in sympathetic nerve activity, including a marked reduction in renal sympathetic nerve activity (RSNA) and changes in vasopressin and plasma renin levels (40). The renal excretion of sodium and the increase in urinary flow rate that accompany an intravenous infusion of hypertonic saline are heavily dependent on intact renal nerves (30). Thus the reflex reduction in RSNA invoked by an elevation in plasma sodium is an important homeostatic mechanism designed to reduce the sodium load.

Surprisingly, we know very little about the central pathways mediating the reflex reduction in RSNA after an intravenous infusion of hypertonic saline. However, the detection of the protein Fos, a marker of neuronal activation, after this stimulus suggests that the hypothalamic paraventricular nucleus (PVN) is likely to be an important integrative site in mediating the responses (16, 32, 36, 41).

The PVN contains distinct subdivisions of magnocellular and parvocellular neurons. Vasopressin-containing magnocellular neurons project to the posterior pituitary and release the hormone into the bloodstream. As expected, these magnocellular neurons in the PVN were activated by the hypertonic saline stimulus. Additionally, many neurons in the parvocellular PVN were also activated by the stimulus (16, 32, 36, 41).

Parvocellular PVN neurons project to different brain regions that are known to be important in the regulation of the sympathetic nervous system (2, 34, 39). These include the intermediolateral cell column of the thoracolumbar spinal cord, where the sympathetic preganglionic motoneurons are located (26). Additionally, parvocellular PVN neurons have been shown to have direct connections to the rostral ventrolateral medulla, where the tonic generation of sympathetic nerve activity is believed to originate (15, 33, 35). These pathways are likely to mediate the changes in sympathetic nerve activity in which the PVN is involved.

On the basis of these anatomic connections and on the previous work that suggested the parvocellular neurons were excited after peripheral hypertonic saline (18, 32, 41), we hypothesize that excitation of the PVN is involved in the sympathetic nerve activity responses evoked by peripheral administration of hypertonic saline. Furthermore, we hypothesize that excitation of parvocellular neurons in the PVN is important in mediating those responses. Surprisingly, the role of the PVN in mediating the renal nerve responses elicited by hypertonic saline has not been examined previously in conscious animals.

Thus the aim of the study was to determine the effect of neuronal inhibition of the PVN and the effect of blocking excitatory glutamatergic input in the PVN on the RSNA response elicited by an intravenous infusion of hypertonic saline. In the study, we microinjected into the PVN 1) muscimol, the GABA agonist, to acutely inhibit neuronal function and 2) the glutamate antagonist kynurenate and determined the effect on the reflex RSNA. In separate experiments, we investi-
gated whether the drugs acted specifically within the PVN by also examining the effects of administering the drugs into regions adjacent to, but outside, the PVN. To further study the role of the excitatory inputs into the PVN, we also performed experiments in rabbits that underwent sinoaortic denervation (SAD), in which we investigated the effects of kynurenate on the reflex RSNA elicited by hypertonic saline infusion.

The experiments were performed in conscious rabbits, alleviating the interference of anesthesia that is known to dampen reflex function.

**MATERIALS AND METHODS**

New Zealand White rabbits of either sex (2.5–3.0 kg) were used in this study and were obtained from Monash Animal Services (Monash University, Clayton, Victoria, Canada) or Nanawie Stud Farm (Geelong, Victoria, Canada). All experimental protocols were approved by the Royal Melbourne Institute of Technology University Animal Ethics Committee and conform to the **Guiding Principles for Research Involving Animals and Human Beings** (1) and guidelines set up by the National Health and Medical Research Council of Australia.

**Surgical Procedures**

Before the experiment, the animals underwent surgical procedures that were performed under general anesthesia using ketamine hydrochloride (40 mg/kg im) and xylazine hydrochloride (5.0 mg/kg im), with additional ketamine (20 mg/kg im) administered every 30 min. Diazepam (5 mg) was injected intramuscularly as a premedication. Analgesia (buprenorphine HCl, 60 μg im) was administered after each surgical procedure to alleviate any postoperative pain, and an antibiotic (chloramphenicol, 100 mg sc) was routinely administered after each operation. At least 2 wk separated each surgical procedure.

**Implantation of PVN guide cannulas**. Under general anesthesia, the head of the rabbit was placed in a David Kopf stereotaxic apparatus. The head clamps were modified so that a small pin protruded from each clamp to allow the clamps to fix onto the zygomatic arch. A longitudinal incision was made on the head to expose both the bregmoid and lambdoid sutures. The head was positioned so that bregma and lambda were on the same horizontal plane. The head was vertically aligned by dividing the skull into quadrants at the level of bregma using the bregmoid and midsagittal sutures. The head was considered level when the dorsoventral coordinate was equidistant from the midsagittal separation of the bregmoid and lambdoid sutures. The head was positioned so that bregma was removed by drilling the burr hole to expose both the bregmoid and midsagittal sutures as the dividing lines. A reference point in each quadrant of the skull centered at 1.7 mm caudal to bregma was considered the anterior-posterior reference point because bregma was removed by drilling the burr hole to enable placement of our guide cannula.

A small burr hole (~5 mm in diameter) was made in the skull center at 1.7 mm caudal to bregma. Three very small drill holes, in separate quadrants and ~1 cm from the larger burr hole, were also made to allow small jeweler's screws to be screwed into the bone to act as anchors. The guide cannulas were positioned bilaterally, at 1.7 mm caudal to bregma (i.e., 4.7 mm caudal to our reference point) and 0.8 mm lateral to the midline, identified as the sagittal separation of the brain hemispheres. Each guide cannula was lowered 5.9 mm from the brain surface so that the tip lay 5 mm dorsal to the anticipated position of the PVN. Dental cement was used to secure the guide cannula. Protection from accidental damage was provided by a small plastic cylinder that was embedded into the dental cement and encircled the guide cannulas. Stainless steel stoppers were placed into each guide cannula.

**Renal nerve electrode**. The implantation of the renal nerve electrode was performed as described previously (5). Briefly, the left kidney was exposed via a lumbar incision. The sympathetic nerve going to the kidney was cleared from the surrounding tissue and placed onto bipolar spiral electrodes, which were sutured to the adventitial wall of the renal artery. Wacker Sil-Gel (Wacker Chemie, Munich, Germany) was used to cover the electrode and nerve to insulate them from surrounding tissue. The free ends of the electrode wires were buried subcutaneously on the back, and the wound was closed. The rabbits were used 3–4 days later.

**SAD**. In a separate group of rabbits (n = 12 total), we performed SAD to remove the arterial baroreceptor afferent input. This procedure was performed immediately after implantation of the guide cannulas. The procedure for SAD has been described at length previously (9, 11, 17). Denervation was considered satisfactory when an intravenous bolus infusion of phenylephrine, which raised mean arterial pressure (MAP) by 20–30 mmHg, evoked changes in heart rate (HR) of <10 beats/min.

**Minor surgical procedures**. On the day of the experiment, under local anesthesia (0.5% lignocaine), an ear artery and vein were catheterized, and the free ends of the electrode wires were exposed. After the completion of the minor surgical procedures, at least 1 h was allowed to elapse before the start of the experiment.

**Experimental Protocols**

On the experimental day, after a quiet rest period of at least 1 h, muscimol (a GABA agonist), kynurenate (a glutamate-receptor antagonist), or appropriate vehicles were microinjected (200 nl) intracerebrally into the conscious rabbit. Five to fifteen minutes later, hypertonic saline was infused intravenously (1.7 M NaCl, 2 ml/min for 7 min). MAP, HR, and RSNA were monitored before the intracerebral injections and for 40 min after the start of the infusion. A 1-ml blood sample was taken before the infusion and at 15 and 45 min postinfusion.

Muscimol (10 nmol, 200 nl/side) was injected bilaterally into the PVN of six rabbits 15 min before the start of the hypertonic saline infusion on 1 experimental day. On another day, vehicle (Ringer solution) replaced muscimol. The experimental days were separated by 48 h, and the administration of vehicle or muscimol was randomized. In a separate series, performed in eight rabbits, the same protocol was followed, except the injections were centered outside the PVN (defined as 0.5 mm or more from the PVN boundary).

Similar experimental procedures were performed with kynurenate (25 nmol, 200 nl/side; n = 7 normal, n = 5 SAD; pH adjusted to 7.4 with 0.1 M HCl and 1.0 M NaOH) microinjected into the PVN of the conscious rabbits. In these experiments, kynurenate was administered 5 min before the start of the hypertonic saline infusion. On a separate day in these same animals, vehicle (Ringer solution containing 0.1 M HCl and 1.0 M NaOH, pH = 7.4) replaced kynurenate. In separate rabbits, microinjections of kynurenate and vehicle were made into areas adjacent to the PVN (n = 7 normal, n = 7 SAD). The dose of kynurenate was chosen on the basis of previous work in the rabbit (22).
The intracerebral injections were performed using a stainless steel injection needle, which extended 5 mm past the end of the fixed guide cannula. The injection needle was connected by thin tubing to a 100-µ Hamilton syringe and a micromanipulator, which enabled the accurate injection of the volume required.

**Monitoring Cardiovascular Variables**

Blood pressure was monitored using an indwelling arterial catheter connected to a pressure transducer. The signal was recorded using a MacLab data-acquisition system (AD Instruments). MAP and HR were determined electronically using the blood pressure signal.

Raw RSNA was amplified using a low-noise differential amplifier (ENG models 187B and 133, Baker Institute, Melbourne, Australia), filtered (band-pass, 100–5,000 Hz), rectified, and integrated at 0.5-s intervals. The threshold was set visually to cut out background nerve activity during quiet periods between bursts. We have found that this method provides a similar estimate of noise level as after a maximum pressor response. The average integrated RSNA over 1- to 2-min periods was calculated and expressed as a percentage of the resting period before each stimulus (5).

**Brain Histology**

After the completion of all experiments, 200 nl of rhodamine or fluorescein beads were injected intracerebrally as an aid in determining the site of injection. Rabbits were deeply anesthetized with pentobarbitone sodium (60 mg/kg iv), injected with 1,000 U heparin (iv), then perfused transcardially with 1 liter of 0.1 M phosphate-buffered saline (pH 7.2) followed by 1 liter of 4% paraformaldehyde in phosphate buffer. The brain was removed and stored in fixative solution containing 20% sucrose. The hypothalamus was cut into sections (40 mm thick), and every fourth serial section was taken for histological examination. The sections were mounted onto subbed slides and allowed to dry before being counterstained with cresyl violet and coverslipping with Deflux mounting medium (BDH Laboratory Supplies). The sections were examined using light microscopy, and the site of the dye injection was recorded in relation to the PVN. An injection site was categorized as "in the PVN" if it was <0.5 mm from the boundary of the PVN. The positions of the injections sites relative to the PVN were drawn onto maps.

**Plasma Electrolytes**

Arterial blood samples were taken to determine the plasma osmolality and the concentration of sodium. Samples were spun at 3,000 rpm for 10 min, the plasma was collected, and the concentration of sodium was determined by indirect ion-selective electrodes using a DADE Dimension random access analyzer. Osmolality was determined by freezing-point depression using an ADVANCED Osmometer.

**Statistical Analysis**

The basal resting MAP and HR levels were compared using a paired or unpaired Student’s t-test as appropriate. Data were expressed as the change from resting levels, and comparisons between treatments were made using a two-way ANOVA with repeated measures. Comparisons between time points were made using Student’s t-test and applying Bonferroni’s modification to compensate for multiple comparisons.

In the case of RSNA, it is well known that a comparison of absolute levels of the sympathetic nerve activity between animals and between days is inappropriate because of technical reasons. Thus data were expressed as a percentage of the resting level before hypertonic saline, and the changes were compared between treatments as described above.

**RESULTS**

**Effect of Muscimol on Responses Elicited by Hypertonic Saline**

*Inside the PVN.* In the conscious rabbits pretreated with vehicle (n = 6), intravenous hypertonic saline infusion was accompanied by an acute increase in MAP of 13.1 ± 1.5 mmHg, which was maximal within 5 min and returned toward resting levels over the observation period (Fig. 1). This was not observed on the muscimol pretreatment day. Comparison of the individual time points between the treatment days indicated that when muscimol was microinjected into the PVN, there were significant differences at the 5- and 20-min time points (P < 0.05 compared with vehicle for each time point) (Fig. 1). There was no statistically significant difference in the HR responses observed...
after hypertonic saline between the muscimol and vehicle treatment days (Fig. 1). RSNA was reduced by 73% at 5 min and 60% at 10 min after the start of the intravenous hypertonic saline infusion into the conscious rabbits that had been microinjected with vehicle into the PVN (Fig. 1). This effect was maximal within 5 min of the start of the infusion and slowly returned toward the resting level during the observation period (Fig. 1). In contrast, with muscimol pretreatment, the maximum reduction in RSNA was only 43% at 5 min and 41% at 10 min. These were significantly different from the changes seen on the vehicle treatment day ($P < 0.05$ at both time points) (Fig. 1).

Outside the PVN. When vehicle was microinjected into regions adjacent to, but outside, the PVN ($n = 8$), the intravenous hypertonic saline infusion was accompanied by an increase in MAP of $13.6 \pm 2.7$ mmHg. The increase was maximal at 5 min after the start of the infusion (Fig. 2). A similar acute increase in MAP also occurred when muscimol was microinjected before the hypertonic saline (MAP increased by $11.1 \pm 2.8$ mmHg) (Fig. 2). There was no significant difference between the time points. In addition, there was no significant difference between the 2 treatment days in the HR response after hypertonic saline infusion (Fig. 2).

Figure 2 also shows there was a marked reduction in RSNA in response to hypertonic saline in the rabbits in which intracerebral microinjections were made into regions adjacent to the PVN. The RSNA fell by $\sim 65\%$ within 5 min of the start of the hypertonic saline infusion on the vehicle treatment day (Fig. 2). When muscimol was microinjected, the maximal reduction in RSNA in response to the hypertonic saline infusion was $62\%$ at 5 min, which was similar to that seen on the vehicle treatment day (Fig. 2).

**Effect of Kynurenate on Responses Elicited by Hypertonic Saline in Normal Intact Rabbits**

**Inside the PVN.** In animals pretreated with vehicle microinjected into the PVN, the hypertonic saline infusion was not accompanied by any marked change in MAP (Fig. 3). HR was increased by $\sim 50$ beats/min at 5 and 10 min after the start of the hypertonic saline infusion (Fig. 3). In these rabbits, RSNA was dramatically reduced by $\sim 64\%$ at both 5 and 10 min after the start of the hypertonic saline infusion (Fig. 3). The magnitude and the time course of the change in RSNA were similar to that observed in the animals pretreated with the muscimol vehicle.

![Fig. 2](http://ajprenal.physiology.org/)

![Fig. 3](http://ajprenal.physiology.org/)
MAP and HR responses that accompanied the hypertonic saline infusion in rabbits that were pretreated with kynurenate in the PVN were similar to those observed on the vehicle pretreatment day (Fig. 3). However, the reduction in RSNA elicited by the hypertonic saline infusion was significantly attenuated after kynurenate pretreatment. RSNA fell by only 19% at 5 min after the start of the infusion and by only 30% at 10 min after the start of the hypertonic saline infusion ($P < 0.01$ for each time point compared with vehicle) (Fig. 3).

**Outside the PVN.** When vehicle was microinjected outside the PVN, hypertonic saline was accompanied by an increase in MAP that was maximal at 5 min after the start of the infusion (11.7 ± 3.0 mmHg) (Fig. 4). HR was also elevated by over 50 beats/min at 5 and 10 min after the start of the infusion (Fig. 4). RSNA was reduced by ~53% within 5–10 min after the start of the infusion (Fig. 4).

On the day in which kynurenate was microinjected outside the PVN, the hypertonic saline infusion was not accompanied by an increase in MAP at 5 min, and this was significantly different from the vehicle pretreatment day ($P < 0.005$) (Fig. 4). An increase in HR accompanied the hypertonic saline infusion, and there was no significant difference in the magnitude or the time course of the HR responses observed between the kynurenate and vehicle pretreatment days (Fig. 4).

The reduction in RSNA that was elicited by the hypertonic saline infusion on the kynurenate pretreatment day was of similar magnitude to that observed on the vehicle pretreatment day (Fig. 4). However, the time course of the response appeared longer with kynurenate pretreatment, such that the magnitude of the reduction at 40 min after the start of the hypertonic saline infusion was ~60%. This was significantly greater than the reduction that was observed at that time on the vehicle pretreatment day (30% reduction; $P < 0.001$) (Fig. 4).

**Effect of Kynurenate on Responses Elicited by Hypertonic Saline in SAD Rabbits**

### Inside the PVN.

MAP and HR responses elicited by intravenous hypertonic saline infusion were not significantly different between vehicle and kynurenate pretreatment days in the conscious SAD rabbits (Fig. 5). In contrast, the RSNA response differed markedly between the two pretreatments (Fig. 5). With vehicle pretreatment, RSNA fell by 50–60% after the hypertonic saline infusion, whereas with the kynurenate pretreatment, there was no marked reduction in RSNA. This difference was significantly different from the response observed with vehicle pretreatment (Fig. 5) ($P < 0.05$ at 5 min).

**Outside the PVN.** MAP and HR responses elicited by intravenous hypertonic saline infusion were not significantly different between vehicle and kynurenate pretreatment days in the conscious SAD rabbits (Fig. 5). Before infusion, the animals were bilaterally microinjected into areas outside the hypothalamic PVN with the glutamate antagonist kynurenate (25 nmol/side) or vehicle (E) on separate days. $*P < 0.05$ at 5-min and $P < 0.001$ at 40-min time points.

**Effect of Kynurenate on Responses Elicited by Hypertonic Saline in SAD Rabbits**

### Inside the PVN.

MAP and HR responses elicited by intravenous hypertonic saline infusion were not significantly different between vehicle and kynurenate pretreatment days in the conscious SAD rabbits (Fig. 5). In contrast, the RSNA response differed markedly between the two pretreatments (Fig. 5). With vehicle pretreatment, RSNA fell by 50–60% after the hypertonic saline infusion, whereas with the kynurenate pretreatment, there was no marked reduction in RSNA. This difference was significantly different from the response observed with vehicle pretreatment (Fig. 5) ($P < 0.05$ at 5 min).

**Effect of Kynurenate on Responses Elicited by Hypertonic Saline in SAD Rabbits**

### Outside the PVN.

When vehicle was microinjected outside the PVN, hypertonic saline was accompanied by an increase in MAP that was maximal at 5 min after the start of the infusion (11.7 ± 3.0 mmHg) (Fig. 4). HR was also elevated by over 50 beats/min at 5 and 10 min after the start of the infusion (Fig. 4). RSNA was reduced by ~53% within 5–10 min after the start of the infusion (Fig. 4).

On the day in which kynurenate was microinjected outside the PVN, the hypertonic saline infusion was not accompanied by an increase in MAP at 5 min, and this was significantly different from the vehicle pretreatment day ($P < 0.005$) (Fig. 4). An increase in HR accompanied the hypertonic saline infusion, and there was no significant difference in the magnitude or the time course of the HR responses observed between the kynurenate and vehicle pretreatment days (Fig. 4).
Outside the PVN. When kynurenate was microinjected outside the PVN, the MAP response elicited by the hypertonic saline infusion was not different from that observed on the vehicle pretreatment day (Fig. 6). This was similar to the HR response, although HR appeared to remain higher longer with kynurenate pretreatment (see Fig. 6). Hypertonic saline elicited a marked reduction in RSNA on both the kynurenate and vehicle pretreatment days (Fig. 6). Thus, as was observed in nondenervated animals, kynurenate microinjected into the PVN attenuated the reflex reduction in RSNA elicited by the hypertonic saline infusion, and this effect was specific to the PVN.

Cardiovascular Levels Before Hypertonic Saline Infusion

Effect of muscimol. INSIDE THE PVN. MAP levels before hypertonic saline were similar on the vehicle and muscimol pretreatment days (Table 1). HR before the intravenous infusion of hypertonic saline was significantly lower on the muscimol pretreatment day compared to the vehicle pretreatment day (Fig. 6). This difference in HR level was due to a significant reduction in HR elicited by the hypertonic saline infusion, and this effect was specific to the PVN.

<table>
<thead>
<tr>
<th>Inside (n = 6)</th>
<th>Outside (n = 8)</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>Muscimol</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>68.0 ± 3.7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>257 ± 12</td>
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Values are means ± SE. n, No. of rabbits (n = 5 and 7 sinoaortic denervated rabbits injected with kynurenate inside and outside the paraventricular nucleus, respectively); MAP, mean arterial pressure; HR, heart rate. *P < 0.05, †P < 0.002 compared with respective vehicle.

Although it is well accepted that it is difficult for technical reasons to compare absolute levels of sympathetic nerve activity between animals, RSNA before hypertonic saline was always higher with muscimol treatment. This was due to the significant increase in RSNA observed after muscimol (mean difference pre- vs. post-muscimol treatment = −54 ± 16 beats/min; P < 0.02; n = 6).

Effect of Kynurenate

Inside the PVN. Before hypertonic saline, there were no statistically significant differences in the levels of MAP and HR between the kynurenate and vehicle treatment days (Table 1). RSNA before hypertonic sa-
line infusion was higher after kynurenic acid pretreatment compared with the vehicle treatment, because RSNA was significantly elevated by kynurenic acid microinjected into the PVN (mean difference pre- vs. post-kynurenic acid treatment, 24 ± 9%; P < 0.02). Vehicle did not significantly affect the cardiovascular variables.

In SAD animals, MAP and HR before hypertonic saline infusion were significantly lower on the kynurenic acid pretreatment day than on the vehicle pretreatment day (Table 1). As in intact rabbits, RSNA was elevated by kynurenic acid (mean difference pre- vs. post-kynurenic acid treatment = 22 ± 8%).

Outside the PVN. There were no significant differences in the levels of MAP and HR in both intact and SAD animals before hypertonic saline between the vehicle and kynurenic acid treatment days (Table 1). In intact rabbits, RSNA was elevated by kynurenic acid (mean difference pre- vs. post-kynurenic acid treatment = 18 ± 10%). In addition, this increase was not significantly different from that observed after kynurenic acid microinjected into the PVN. In SAD animals, RSNA was not significantly affected by kynurenic acid (mean difference pre- vs. post-kynurenic acid treatment = 13 ± 11% ). Vehicle did not affect the cardiovascular variables (Table 1).

Effect of Hypertonic Saline on Plasma Sodium and Osmolality

There was no significant difference in the size of the increase in osmolality elicited by hypertonic saline observed in rabbits pretreated with vehicle (average increase over 45 min = 22 ± 1 mosmol/kgH₂O) or muscimol microinjection into the PVN (average increase over 45 min = 23 ± 3 mosmol/kgH₂O) (Fig. 7). Plasma sodium was elevated slightly more on the muscimol treatment day (P < 0.05 between days), and this was due to plasma levels of sodium remaining significantly elevated longer compared with the Ringer solution day (P < 0.01 at t = 45 min after the start of the infusion). Resting levels were not significantly different between the 2 treatment days.

When muscimol was injected into brain regions adjacent to the PVN, there was a significantly greater increase in osmolality after hypertonic saline compared with intracerebral vehicle pretreatment (average increase over 45 min = 25 ± 2 vs. 20 ± 1 mosmol/kgH₂O, muscimol vs. vehicle; P < 0.05 between days) (Fig. 7). This was predominantly due to the greater increase in osmolality at 15 min after the start of the infusion (P < 0.05). There was also a slightly greater increase in plasma sodium after muscimol pretreatment, which was significant at the final time points examined after the start of the infusion (Fig. 7). Basal levels of osmolality and sodium were not significantly different between the 2 treatment days.

Unfortunately, technical problems prevented us from determining the plasma osmolality and sodium levels in the majority of the rabbits used in the kynurenic acid study. We were able to gather results from four animals: two had microinjections into the PVN, and two had microinjections outside the PVN. Similar levels of osmolality and sodium were reached after the hypertonic sodium infusion in those rabbits as in the animals that received muscimol/vehicle.

Fig. 7. Effect of hypertonic saline infusion (1.7 M NaCl, 2 ml/min for 7 min) on plasma osmolality and sodium concentration. Plasma samples were taken before (t = 0) and 15 and 45 min after the start of the infusion. Open symbols represent data from animals microinjected with vehicle, and solid symbols represent data from the animals microinjected with muscimol. Microinjections were made into (A; n = 6) or outside the PVN (B; n = 8). *P < 0.01 between treatments.
Intracerebral Microinjection Sites

For the muscimol studies, microinjections were distributed from the mid- to caudal levels of the PVN, with the majority located at the midlevel of the PVN (Fig. 8). Microinjections that were found to be outside the PVN were located either dorsal or caudal to it, predominantly in the reuniens nucleus. One site was found to be in the dorsomedial hypothalamic nucleus (Fig. 8D).

For the kynurenate studies, microinjections into the PVN were spread throughout the rostral-caudal extent (Fig. 9) but were predominantly located at the midlevel, at similar levels to the muscimol injections. Microinjections of kynurenate that were outside the PVN were located dorsal and caudal to it, but the majority were rostral.

DISCUSSION

The present study highlights for the first time an important role for the PVN in the renal nerve response elicited by elevations in plasma osmolality in the conscious animal. We found that administration of kynurenate into the PVN to block glutamatergic inputs resulted in an attenuation of the renal sympathoinhibition elicited by the hypertonic saline infusion in both normal intact and SAD rabbits. Inhibition of neuronal activity in the PVN with muscimol had a similar effect (see below). Changes in HR accompanying the elevation in plasma osmolality were not markedly affected by blockade of glutamatergic function nor the inhibition of neuronal activity within the PVN. The study shows that the PVN plays an important role in the neural contribution to the response elicited by hypertonic saline, and our novel findings indicate that excitatory amino acids within the PVN are involved.

In the present study, we investigated the role of endogenous glutamatergic input in the PVN in the renal sympathoinhibition elicited by an infusion of hypertonic saline. We found that kynurenate microinjected into the PVN markedly attenuated the reduction in RSNA. This effect was restricted to microinjections into the PVN because it was not observed when kynurenate was microinjected into areas adjacent to, but outside, the PVN. Similarly, in animals in which the arterial baroreceptor afferents had been removed (i.e., SAD), kynurenate microinjected into the PVN attenuated the reduction in the RSNA normally observed after hypertonic saline infusion. Thus these findings suggest that the PVN plays an important role in the renal nerve inhibition elicited by intravenous hypertonic saline. This concurs with studies using Fos as a marker of activated neurons, which suggest that PVN neurons are activated by intravenous hypertonic saline (8, 32, 36, 41). The present findings suggest for the first time that excitatory glutamatergic inputs...
within the PVN mediate the contribution this nucleus makes to the renal nerve response. In light of this, it is interesting to note that subunits of glutamate receptors are differentially distributed in the PVN. The NMDA subunits NMDAR1 and NMDAR2 and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunits, especially GluR1, appear to be particularly concentrated in regions of the PVN where neurons that project to the brain stem and spinal cord are predominantly located (3, 4, 21, 39).

It is not possible from our present work to determine the origin of the glutamatergic input into the PVN. Glutamatergic terminals within the PVN have been found to arise from neurons within the PVN, within adjacent hypothalamic nuclei, and from nuclei farther afield (14). Neurophysiological studies provide evidence for a physiological role of local excitatory glutamatergic inputs in the hypothalamus (10). Thus the glutamatergic input contributing to the RSNA response elicited by hypertonic saline might arise from neurons outside the PVN or from interneurons within the PVN. Further investigations will be required to examine this question.

The microinjection of muscimol into the PVN attenuated the reduction in RSNA elicited by the intravenous hypertonic saline infusion. Muscimol inhibits neuronal function; thus the results suggest that neurons within the PVN are important in mediating the renal nerve inhibition elicited by hypertonic saline. This effect of muscimol was specific to the PVN because microinjections of muscimol outside the nucleus did not result in an attenuation of the response. However, there are two issues that may interfere with the interpretation of the muscimol data. First, there were marked changes in the resting levels of RSNA induced by muscimol. The increase in RSNA elicited by the administration of muscimol into the PVN was almost a doubling of the resting RSNA. We have reported this interesting observation in a recent publication (6). Such a change in resting RSNA may make it difficult to interpret the effect of muscimol on the responses elicited by the infusion of hypertonic saline. However, the marked attenuation of the hypertonic saline-induced RSNA response that was observed after kynurenate microinjection into the PVN, in both intact and SAD rabbits, strongly supports the view that the PVN is important in mediating the reduction in RSNA elicited by the infusion of hypertonic saline.

The second issue is related to the observation that in the muscimol series of experiments there was a brief increase in MAP that accompanied the hypertonic saline infusion in the rabbits that were microinjected into the PVN with vehicle. This response was not observed in the animals in which muscimol was microinjected into the PVN. Thus the attenuation in the renal sympathoinhibitory response elicited by hypertonic saline that was seen in these animals could be interpreted as resulting from the attenuation of the acute pressor response and the resultant lack of baroreceptor-mediated renal nerve inhibition. However, several important observations in the present study argue against this explanation. First, in the series of experiments in which kynurenate was injected into regions outside the PVN, there was no pressor response accompanying the hypertonic saline infusion, but RSNA fell as in control animals (see Fig. 4). Second, hypertonic saline was not accompanied by an acute pressor response when vehicle was administered into the PVN, but there was the normal fall in RSNA in that group (see Fig. 3, vehicle curve). Thus it appears that the pressor response is not a consistent observation after hypertonic saline, but the reduction in RSNA occurs irrespective of a change in blood pressure. This has been observed previously (8, 30). Third, and importantly, we found that in SAD rabbits, in which arterial baroreceptor afferents had been removed, there was still a marked reduction in RSNA elicited by hypertonic saline infusion (see Figs. 5 and 6). Finally, the ability of kynurenate microinjected into the PVN to attenuate the renal sympathoinhibition elicited by intravenous hypertonic saline infusion in SAD rabbits suggests that the contribution of the PVN to the renal nerve response elicited by hypertonic saline was not dependent on an intact arterial baroreceptor reflex.

The pathways involved in the transmission of information about plasma osmolality to the PVN and relaying the appropriate responses from the PVN to the peripheral sympathetic nerves are not known. However, there is a considerable body of evidence supporting the view that peripheral osmolality changes are detected by sensors in the anterior wall of the third ventricle, in nuclei that lack a blood-brain barrier and that form part of the lamina terminalis (28, 29). These nuclei are activated by elevations in plasma osmolality and are known to project directly to the PVN (29, 32). The PVN projects to nuclei known to be important in regulating sympathetic nerve activity, including the sympathetic preganglionic motoneurons in the spinal cord and the pressor region of the rostral ventrolateral medulla (3, 4, 34, 35, 38). Whether these pathways are mediating the renal nerve responses elicited by elevations in osmolality awaits further study. However, recent evidence shows that sympathetic innervation of the kidney includes polysynaptic pathways from the lamina terminalis (37), suggesting that these osmosensitive nuclei can influence RSNA.

Some studies also suggest that hepatic osmoreceptors may be activated by elevations in plasma osmolality and contribute to the renal nerve response (23, 31). These may be important in postprandial osmolality changes. It should also be noted that the aforementioned studies suggest that the reduction in renal nerve activity is mediated by a complex combination of afferents traveling in the vagus, carotid, and hepatic nerves, in which removal of all afferents was required to block the response (23, 30, 31).

Thus there may be several afferent pathways relaying information from the peripheral osmoreceptors to the central nervous system. Our present data provide evidence for a major role of the PVN in the central pathways utilized in the neural responses elicited by...
elevations in plasma osmolality and that glutamatergic inputs within the PVN are involved. Accompanying the intravenous infusion of hypertonic saline was an increase in HR. This effect was not markedly altered by the administration of muscimol or by the microinjection of kynurenate into the PVN. This contrasts with the effects of these drugs on the RSNA response. The results suggest that the role of the PVN in the responses elicited by an infusion of hypertonic saline is not generalized. Elevations in plasma osmolality can elicit nonuniform changes in sympathetic nerve activity (40), and it would be of great interest in future studies to determine the role of the PVN in the responses of sympathetic nerve activity to organs other than the kidneys.

HR changes elicited by hypertonic saline appear to vary according to species and protocols used. In the rat, HR decreases, and this appears to be entirely mediated by arterial baroreceptors (7). In the rabbit, 1.5 M NaCl infusion has been reported previously to have no effect on HR, but higher concentrations (3.3 M) reportedly lowered HR, and this effect was not mediated by arterial baroreceptors (30). In the present study, we found that HR increased after hypertonic saline infusion in intact and SAD conscious rabbits.

The PVN is known to be important in the humoral responses to stress and body fluid disturbances. The PVN can also influence sympathetic nerve activity (2, 13, 24, 25), but there is little knowledge of the functional role of the PVN in the regulation of sympathetic nerve activity. The present study is the first to show that the PVN plays an important role in the reduction in RSNA elicited by an elevation in plasma osmolality in conscious animals. Additionally, in anesthetized SAD rats, endogenous angiotensin II within the PVN has been reported recently to contribute to the increase in RSNA elicited by intracarotid hypertonic saline in that preparation (12). Thus evidence suggests that the PVN is important in the sympathetic nerve activity responses elicited by disturbances in blood volume (20). Taken together, the findings suggest that the PVN may have a broad integrative role in the responses elicited by perturbations in blood volume and osmolality by mediating the neural components as well as the humoral components of the responses involved in maintaining body fluid homeostasis.

The present study was performed in conscious animals. This is a major advantage in the examination of reflex responses that can be adversely influenced in the presence of anesthesia. Additionally, by performing these experiments in conscious animals we were able to observe the effect of drugs on rabbit behavior. We found that muscimol produced a hypnotic type of effect, in which the animals remained conscious but in a state of relaxation from which they could easily be aroused if disturbed (e.g., by light prodding). This behavioral effect could not account for the effects of muscimol, microinjected into the PVN, on the responses elicited by hypertonic saline, because there was no influence on the RSNA response when muscimol was administered outside the PVN, but there was a similar behavioral response.

Finally, we have used hypertonic saline to raise plasma osmolality. This results in an increase in plasma sodium and chloride ions and in osmotic pressure. It was not possible in the present study to determine the contribution each makes to the effects observed.

Conclusions

We have found that the glutamate antagonist kynurenate microinjected into the PVN of the conscious rabbit antagonizes the normal renal sympathetic nerve response evoked by an intravenous infusion of hypertonic saline in both intact and SAD animals. The findings suggest that endogenous glutamatergic inputs in the PVN are important mediators of the renal sympathoinhibition initiated by the elevation of plasma osmolality.

The present findings, taken together with evidence in the literature (19, 27), suggest that the PVN is important in the neural responses that are elicited by volume expansion and altered plasma osmolality. Thus the PVN appears to be an important integrative site that is involved in the neural as well as the hormonal responses initiated by perturbations in body fluid homeostasis.

DISCLOSURES

This work was supported by the National Health and Medical Research Council of Australia, the National Heart Foundation of Australia, and the Royal Melbourne Institute of Technology.

REFERENCES


