Cardiovascular changes induced by central hypertonic saline are accompanied by glutamate release in awake rats

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Jin, Qing-Hua, Yuto Ueda, Yuta Ishizuka, Takato Kunitake, and Hiroshi Kannan. Cardiovascular changes induced by central hypertonic saline are accompanied by glutamate release in awake rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R1224–R1231, 2001.—To elucidate neurochemical mechanisms responsible for cardiovascular responses induced by central salt loading, we directly perfused the paraventricular nucleus (PVN) of the hypothalamus region with hypertonic saline (0.3 or 0.45 M) by using an in vivo brain microdialysis technique. We then measured the extracellular concentrations of glutamate in the PVN region in conscious rats along with the blood pressure and heart rate. Blood pressure, heart rate, and glutamate levels were increased by perfusion of 0.45 M saline; however, they did not change by perfusion of 0.3 M saline. Next, we examined the possible involvement of glutamate in the cardiovascular responses induced by hypertonic saline. Dizocilpine, a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, attenuated the increases of blood pressure and heart rate, although 6-cyano-7-nitroquinoxaline-2,3-dione, an antagonist of the non-NMDA receptor, did not affect the blood pressure and heart rate. Our results show that local perfusion of the hypotalamic PVN region with hypertonic saline elicits a local release of glutamate, which may act via NMDA-type glutamate receptors to produce cardiovascular responses.

paraventricular nucleus; blood pressure; heart rate; microdialysis

THE OSMOTIC HOMEOSTASIS and ionic balance of the body are mainly regulated by neural and humoral factors through the central nervous system (CNS). Despite its clinical and physiological significance, however, little is known about the underlying cellular and molecular mechanisms by which the osmotic and ionic balances of the CNS are maintained in conscious and freely moving animals. Salt loading is a potent stimulus for drinking, vasopressin (AVP) secretion, and increasing arterial blood pressure. Acute saline injection seems to activate osmoreceptive structures, including those in the subfornical organ and the anteroventral third ventricle (AV3V region) (17, 39), subsequently stimulating AVP secretion and neurons related to cardiovascular and behavioral responses in the hypothalamus and brain stem (50). In addition, a number of studies have pointed to the importance of the hypothalamic paraventricular nucleus (PVN) in the regulation of body fluid homeostasis and in the central control of cardiovascular function (36, 46). The PVN is involved in the control of not only the neurohypophysial system but also the adenohypophyial system and autonomic nervous outflow (21). Several authors, employing electrophysiological methods, have shown that, in addition to the supraoptic nucleus (SON), the PVN is a region where neurons sensitive to changes in osmolality and/or NaCl concentration are located (9, 13, 26, 31, 40). Recent research has also shown that systemic administration of hypertonic saline activates cells in the PVN and SON (16, 28, 41). Moreover, an increased c-Fos expression in the PVN after dehydration and salt loading further confirmed the role of this area in the regulation of body fluid balance (12, 39). Thus the PVN appears to be an important regulatory region of the hypothalamus that plays a critical role in the modulation of cardiovascular, neuroendocrine, and behavioral responses during osmotic challenges.

It has been reported that direct stimulation of the PVN and SON with a high-NaCl perfusion medium via microdialysis probes resulted in increased AVP and oxytocin release both locally into extracellular fluid and into systemic circulation (14, 15, 25, 29, 38). Similar releases of angiotensin (43), amino acids, and dopamine (18) after local perfusion of hypertonic saline have also been reported, and the findings have been interpreted as evidence for the roles of these substances in the central osmotic and ionic balance. However, the roles of endogenous neurochemicals in the PVN responsible for cardiovascular responses induced by salt loading remain to be elucidated. Glutamate (Glu) has been recognized as an important neurotransmitter/neurmodulator in the CNS, mediating most of the excitatory synaptic transmission. An injection of Glu into the PVN raised blood pressure and heart rate (HR) accompanied with increases in plasma catecholamines (30). These findings raise the possibility...
that Glu may be involved in the responses induced by central hypertonic saline stimulation.

In some cases, anesthesia, well known for its profound effect on cardiovascular and autonomic function, reverses the responses (21, 32). Microdialysis is a useful method for pharmacological assessment and manipulation of the microenvironment in the brain of conscious and freely moving animals, in which changes in osmolality or NaCl concentration, extracellular amino acids, and related mediator levels can be manipulated and measured (2). Therefore, in the present study, we have used a microdialysis technique to investigate the release of Glu in response to local stimulation with hypertonic saline and to monitor cardiovascular responses in conscious and freely moving rats. In addition, to test whether the observed Glu release is involved in the cardiovascular responses, N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists were co-administered with hypertonic saline stimulation, and the cardiovascular responses that were induced by hypertonic saline were examined.

METHODS AND MATERIALS

Animals and surgical procedures. Male Wistar rats, weighing 350–450 g at the start of the experiments, were used in our study. The experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals [DHENV Publication No. (NIH) 85–23, Revised 1985, Bethesda, MD] and were approved by the Committee on Animal Care of Miyazaki Medical College. The rats were anesthetized with pentobarbital sodium (50 mg/kg ip) 5 days before the experiments. An arterial catheter (SP-31 polyethylene tubing heat-coupled to SP-50) was inserted into the surgically exposed abdominal aorta for measurement of arterial blood pressure (BP) and HR and tunnelled under the skin to exit at the nape of the neck. A guide cannula (0.50-mm ID, 0.70-mm OD) for the placement of a microdialysis probe was stereotaxically implanted 2.0 mm above the PVN according to the atlas of Paxinos and Watson (42) and sealed with a dummy cannula after implantation. The stereotaxic coordinates were 1.7 mm posterior to the bregma, 0.3 mm lateral to the midline, and 6.7 mm ventral to the dural surface. To determine whether the direct effects of hypertonic saline (HTS) were specific to the PVN, the guide cannula was implanted into the outside of the PVN region (1 mm rostral to the PVN, n = 2; 1 mm lateral to the PVN, n = 3) as an anatomic control group. A head plate for the fixation of the arterial catheter and a guide cannula were fitted to the skull by dental cement.

Experimental procedure. On the day before the experiment, the animals were anesthetized with isoflurane (2.5–3.0% in 100% oxygen), and the dummy cannulas were replaced with microdialysis probes. To reach the PVN region, the tip of the probe covered with a 2.0-mm length of hollow fibers (200–μm OD, cellulose acetate membrane, cut-off 4.8 × 10^4 mol wt; Terumo, Japan) was set to extend 2 mm beyond the guide shaft, and a similar operation was performed in the anatomic control group. So that they would get used to the environment in which the experiment would later be performed, the animals were placed in cylindrical metabolic cages (28-cm diameter × 28-cm height) that were controlled by computers. This system can protect the arterial catheter and tubes connected to the probe from the twist induced by the movement of rats, allowing for long-term recording without twisting. On the day of the experiments, the collection of dialysates for the Glu and cardiovascular measurements were carried out while the animals were permitted to move about freely in their cages. The microdialysis probe was perfused with modified Ringer solution (147 mM NaCl, 4 mM KCl, 2.3 mM CaCl₂; pH 6.5) at a constant rate of 1 μl/min using a microinfusion pump. The perfusate from the PVN region was collected manually in an Eppendorf tube every 20 min. After a 2-h stabilization period, two consecutive dialysate samples were collected to measure the baseline Glu levels. All samples of collected dialysates were kept at −80°C for later analysis. The arterial catheter was connected to a pressure transducer (P23 ID, Gould, Singapore) to monitor BP, while the HR was counted by BP wave. The BP and HR were monitored simultaneously and recorded on a thermal pen-writing recorder (RJG-4122, Nihon Kohden, Japan) and on an FM magnetic tape recorder (RM-7000, Sony, Tokyo, Japan). The mean arterial pressure (MAP) was obtained by electronic averaging of the pulsatile arterial pressure signal.

The Glu levels were measured by using high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Before applying the HPLC-ECD method, an o-phthalaldehyde (OPA) solution (40 mM) was made by adding 13.5 mg of OPA and 10 μl of 2-mercaptoethanol to 2.5 ml of 0.1 M K₂CO₃ buffer (pH 9.5) with 10% ethanol. The solution was then stored at −4°C and diluted in 0.1 M K₂CO₃ to yield a 4 mM OPA solution just before detection. The dialysate (18 μl) was mixed with 5 μl of the 4 mM OPA solution and allowed to react for 3 min at 25°C incubation. After completing the reaction, 20 μl of the reaction mixture was applied to HPLC with an Eicom MA-5 ODS column (4.6-mm ID × 150 mm; Eicom, Tokyo, Japan). Detection was accomplished with an ECD (Eicom) with +700 mV Ag/AgCl electrodes. The elution buffer for the Glu was 0.1 M phosphate buffer, 30% methanol, and 0.5 mM EDTA (pH 6.5).

At the end of each experiment, the rats were killed with an overdose injection of pentobarbital sodium, and their brains were fixed in 10% neutral-buffered formalin. The implantation site of the dialysis probe was verified histologically in 40-μm coronal sections after cresyl violet staining.

Reagents. HTS with two different toxicities, 0.3 and 0.45 M (the osmolalities were 591 and 900 mosmol/kgH₂O, respectively), was used in this experiment to observe the responses. The PVN region was perfused with the HTS through the microdialysis probe at a constant rate of 1 μl/min for 20 min. Physiological saline (0.15 M NaCl) was used in this experiment as a control. In addition, 0.6 M mannitol (Nacalai Tesque, Kyoto, Japan) dissolved in the physiological saline (the osmolality is 904 mosmol/kgH₂O) was perfused in the PVN via the microdialysis probe for 20 min, and 1 mM NMDA (Nacalai Tesque) dissolved in the modified Ringer solution was perfused for 10 min.

Dizocilpine (MK-801; Sigma), an NMDA-receptor antagonist, was dissolved in the modified Ringer solution (1 mM), and 6-cyano-7-nitroquininaline-2,3-dione (CNQX; Sigma), a non-NMDA-receptor antagonist, was dissolved in a 0.1 M NaOH solution (1 mM). The drugs were then stored at 4°C. To measure the effect of these drugs on the responses induced by HTS stimulation, both drugs were diluted with 0.45 M NaCl to 100 μM, and the mixtures were administered via the microdialysis probe. The same vehicles used to dilute the drugs were given at the same concentration in the 0.45 M saline, and this solution served in this experiment as the vehicle control.

Statistical analysis. All data are expressed as means ± SE, and statistical analysis was performed using ANOVA followed by Fisher’s protected least significant difference test.
To provide a temporal description of the cardiovascular responses, the area under the curve (AUC) was also calculated (1). A comparison of the maximal changes in BP and HR and in AUC between the PVN group and the anatomic control group was carried out by a paired Student’s t-test. A probability level of \( P < 0.05 \) was considered statistically significant.

RESULTS

In the present study, the baseline levels are the average of baseline values before stimulation in each parameter. The baseline levels for MAP, HR, and Glu are shown in Table 1, with no significant differences between the groups. (See Fig. 2, top, for the exact positioning of the probe.)

Figure 1 shows the effect on MAP and HR of direct infusion of HTS into the PVN region for 20 min through a dialysis probe. The local perfusion of 0.45 M saline elicited significant increases in MAP and HR from the baseline levels of 96.7 ± 3.6 mmHg and 285.3 ± 3.5 beats/min to the maximums of 114.4 ± 2.4 mmHg and 394.0 ± 10.7 beats/min, respectively. The MAP and HR began to increase 12 min after starting the perfusion and returned to the basal levels ∼15 min after the perfusion was stopped. However, the 0.3 M saline did not cause significant increases in MAP and HR, although the maximums reached 99.8 ± 3.7 mmHg and 336.5 ± 18.1 beats/min, respectively (baseline levels in this group were 93.6 ± 1.9 mmHg and 297.6 ± 9.7 beats/min, respectively).

To investigate whether the increases in BP and HR in response to local administration of HTS were specific to the PVN region, perfusion of 0.45 M saline into a region outside the PVN [−1 mm rostral (n = 2) or lateral (n = 3)] was performed (Fig. 2, middle and bottom). In this anatomic control group, BP and HR did not show significant increases after 0.45 M saline perfusion, although the maximal changes reached 7.2 ± 2.7 mmHg and 39.2 ± 22.2 beats/min, respectively. The maximal changes in the HTS perfusion of the PVN region were 20.9 ± 2.6 mmHg and 115.9 ± 8.7 beats/ min, respectively, which were strikingly larger than the anatomic control group.

In addition, a 0.6 M mannitol solution dissolved in the 0.15 M saline was used to determine whether the cardiovascular responses to direct HTS perfusion into the PVN region were caused by a change in ions, sodium chloride, or osmolality. The mannitol solution, its osmolality approximately equivalent to 0.45 M saline, caused little increase in BP and HR after the perfusion was stopped (Fig. 3). The maximal changes were only 4.7 ± 2.5 mmHg and 42.1 ± 14.9 beats/min, respectively. As shown in Fig. 3, the responses of BP and HR to the mannitol were markedly less than they were to NaCl, and their durations were also shorter than they were in the NaCl group.

The effect of local stimulation of PVN with HTS on Glu levels in the PVN region is summarized in Fig. 4. The Glu levels in the PVN region showed an immediate increase by the local administration of 0.45 M saline, which reached to 249.8 ± 54.7% of the basal level. The elevated Glu levels remained after the perfusion and almost returned to the basal levels at 40 min after the perfusion was stopped. The direct perfusion of 0.3 M saline did not cause significant increases in MAP and HR (0.15 M saline did not cause significant increases in MAP and HR). However, the 0.3 M saline did not cause significant increases in MAP and HR, although the maximums reached 99.8 ± 3.7 mmHg and 336.5 ± 18.1 beats/min, respectively (baseline levels in this group were 93.6 ± 1.9 mmHg and 297.6 ± 9.7 beats/min, respectively).

Table 1. Baseline levels of MAP, HR, and Glu concentration in the PVN

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>Glu, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15 M</td>
<td>5</td>
<td>95.9 ± 2.7</td>
<td>295.3 ± 8.5</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>0.3 M</td>
<td>6</td>
<td>93.6 ± 2.0</td>
<td>297.5 ± 9.7</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>0.45 M</td>
<td>6</td>
<td>97.6 ± 3.6</td>
<td>288.3 ± 5.5</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Anatomic control</td>
<td>5</td>
<td>99.0 ± 4.1</td>
<td>303.8 ± 12.4</td>
<td></td>
</tr>
<tr>
<td>Mannitol</td>
<td>5</td>
<td>96.9 ± 2.4</td>
<td>303.7 ± 9.5</td>
<td></td>
</tr>
<tr>
<td>Vehicle of MK-801</td>
<td>6</td>
<td>90.5 ± 4.3</td>
<td>297.8 ± 10.8</td>
<td></td>
</tr>
<tr>
<td>MK-801</td>
<td>6</td>
<td>99.8 ± 5.4</td>
<td>300.8 ± 8.2</td>
<td></td>
</tr>
<tr>
<td>Vehicle of CNQX</td>
<td>6</td>
<td>96.9 ± 4.4</td>
<td>301.0 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>CNQX</td>
<td>6</td>
<td>95.0 ± 2.2</td>
<td>295.3 ± 7.8</td>
<td></td>
</tr>
<tr>
<td>Vehicle of NMDA</td>
<td>5</td>
<td>92.9 ± 3.9</td>
<td>283.8 ± 10.1</td>
<td></td>
</tr>
<tr>
<td>NMDA</td>
<td>6</td>
<td>93.1 ± 4.1</td>
<td>285.1 ± 8.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE. MAP, mean arterial pressure; HR, heart rate; Glu, glutamate; PVN, paraventricular nucleus; HTS, hypertonic saline; NMDA, N-methyl-D-aspartate.
saline increased the Glu levels to 118.4 ± 7.4% of the basal level, but there was no significant difference compared with the control group with 0.15 M NaCl.

Figure 5 shows the effects of MK-801 on the responses of MAP and HR that were induced by 0.45 M saline. Coadministration of MK-801 (100 μM) with HTS significantly attenuated the increased responses of MAP and HR. The AUC in the MAP and HR was changed from 314.0 ± 70.7 mmHg·m and 1,791.6 ± 465.4 beats/min·m of the vehicle control group to 85.8 ± 21.1 mmHg·m and 533.3 ± 206.6 beats/min·m (both \( P < 0.05 \)), respectively. On the other hand, the responses of MAP and HR, induced by direct perfusion of 0.45 M saline into the PVN, were not influenced by CNQX (100 μM; Fig. 6). The AUC in the MAP and HR in the CNQX group was 209.9 ± 23.7 mmHg·m and 1,119.8 ± 149.9 beats/min·m, and in the vehicle group it was 295.0 ± 73.7 mmHg·m and 1,320.3 ± 396.2 beats/min·m, respectively (both \( P > 0.05 \)).

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Fig. 2. Photomicrographs of a typical placement of the unilateral microdialysis probe in the PVN (top) and rostral (middle) and lateral to the PVN (bottom). Arrows indicate tip of the microdialysis probe. SCh, suprachiasmatic nucleus.

Fig. 3. Comparison between 0.45 M NaCl and 0.6 M mannitol groups in responses of MAP (A) and HR (B) induced by direct perfusion of the PVN. Data are means ± SE. * \( P < 0.05 \) compared with baseline levels; † \( P < 0.05 \) compared with 0.6 M mannitol group.

Fig. 4. Effects on glutamate (Glu) level in the PVN by direct infusion of HTS into the PVN through microdialysis probes. Glu levels are expressed as percentages of baseline levels. Horizontal bar shows period of perfusion. Data are means ± SE. * \( P < 0.05 \) compared with 0.15 M NaCl group; † \( P < 0.05 \) compared with 0.3 M NaCl group.
Finally, effects of direct perfusion of NMDA into the PVN region on BP and HR were examined. BP and HR increased strikingly by perfusion of 1 mM NMDA. The maximal changes reached
\[25.0 \pm 3.6\, \text{mmHg}\] and 
\[132.4 \pm 11.8\, \text{beats/min},\] respectively, and the increases remained at high levels for 15 min after the NMDA perfusion was stopped (Fig. 7).

**DISCUSSION**

This study demonstrates that dialysis with hypertonic saline in the PVN region results in increased BP and HR accompanied with an increase in the Glu level in the dialysates obtained from the PVN. These changes are related to the tonicity of the hypertonic saline. Any consideration of the effects of hypertonic saline solution requires recognition of whether either osmotic or ion-specific factors are involved. In the PVN, some neurons show excitation and others show inhibition in response to hypertonic saline and other osmotic stimuli in vitro and in vivo (11, 13, 36, 40, 52). On the other hand, in vivo local microdialysis of 1 M hypertonic saline elicited oxytocin release in the PVN, whereas dialysis with equimolar mannitol did not (14). Present work showed that equiosmotic mannitol perfusion in the PVN showed a much lesser cardiovascular effect than the hypertonic saline solution in conscious rats (Fig. 3). Therefore, the results observed in the present studies with hypertonic saline solution might be much more dependent on an ion effect, i.e., Na\(^{\text{+}}\) or Cl\(^{-}\), than on an osmotic effect. In this regard, Eriksson and colleagues (10) found in the goat that antidiuretic and thirst responses could be induced by intracerebroventricular (icv) infusion of hypertonic NaCl but not by solutions of equivalent osmolarity made from sucrose in water. They proposed that a periventricular sodium-sensitive receptor system, located in the vicinity of the third ventricle, was responsible for the regulation of antidiuresis, thirst, and renal Na excretion. However, McKinley and colleagues (33) revealed that icv infusion of hypertonic sucrose in sheep can elicit thirst and antidiuresis if the solution contains sufficient amounts of NaCl to prevent the osmotic dilution of cerebrospinal fluid (CSF) Na. These results provided support for the involvement of osmoreceptors. Whether sodium and/or an osmoreceptor is involved in the cardiovascular responses that are induced by salt loading remains to be elucidated. Because cardiovascular responses induced by local perfusion of hypertonic saline in the PVN region were more prominent than those induced by mannitol in the present study, activation of NaCl receptors rather than osmoreceptors might be involved in

![Fig. 5. Effects of MK-801 (100 \(\mu\)M) on HTS-induced increases in MAP (A) and HR (B). Data are means \(\pm\) SE. *\(P\) < 0.05 compared with baseline levels; +\(P\) < 0.05 compared with vehicle group.](image1)

![Fig. 6. Effects of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 100 \(\mu\)M) on HTS-induced increases in MAP (A) and HR (B). Data are means \(\pm\) SE. *\(P\) < 0.05 compared with baseline levels.](image2)
the cardiovascular responses induced by hypertonic saline.

Considerable evidence exists that extracellular concentrations of putative amino acid neurotransmitters are also affected by osmotic and/or NaCl changes in the microenvironment (4, 47, 49). It is known from immunocytochemical and physiological studies that glutamatergic synaptic terminals innervate neurons in the PVN and control their activity (7, 8, 34, 48). In addition, the injection of Glu into the PVN evoked the increases of MAP, HR, and plasma catecholamines (30), and the unilateral infusion of the PVN region with Glu produced a great increase of plasma oxytocin level (15).

Therefore, we asked whether the release of Glu on local stimulation with hypertonic saline solutions is involved in the cardiovascular responses. If so, blockade of glutamatergic synaptic transmission with antagonists could modify the cardiovascular responses. We found an increase in extracellular Glu concentration after local perfusion of hypertonic saline. Furthermore, the NMDA-receptor blocker MK-801, but not the non-NMDA-receptor antagonist CNQX, attenuated cardiovascular responses after local perfusion of the PVN with hypertonic saline, and local injection of NMDA into the PVN region evoked increases in BP and HR. Glu excites parvocellular neurons in the PVN (8), some of which project directly to the preganglionic sympathetic neurons in the intermediolateral cell column of the spinal cord (44, 46). Electrical and chemical stimulation of the PVN increases BP and renal sympathetic nerve activity in conscious rats (21). Therefore, released Glu in the PVN might be involved in the cardiovascular responses induced by hypertonic saline.

Hypertonic saline stimulation of the PVN and SON was reported to increase AVP and oxytocin in the nuclei, with a maximum during poststimulation (14, 25, 28, 37, 38). In contrast to these studies on hypothalamic oxytocin and AVP release, we observed that the stimulated release of Glu in the PVN was quantitatively related to the increase in tonicity, which increased immediately during the hypertonic saline stimulation and returned gradually to baseline when the stimulus was withdrawn. These findings suggest that a mechanism different from the one operating for AVP and oxytocin release is responsible for Glu release in the PVN region under hypertonic saline loading.

The mechanism that triggers the hypertonic saline-induced release of amino acids is not known. One plausible explanation is that the amino acids are released as neurotransmitters in response to direct NaCl and/or osmotic stimulation of the neurons of their origin (18). In this regard, Glu, γ-aminobutyric acid, and glycine can be released from nerve terminals through their carrier by exchanging with extracellular amino acids sharing the same transport system (22, 27). Therefore, it is unclear which of the released substances may trigger a possible secondary amino acid substance. Another possible explanation could involve pH changes due to activation of the plasmalemmal sodium/hydrogen exchanger (20) or sodium/calcium exchange mechanisms, causing the release of intracellular calcium from internal stores such as mitochondria (6), resulting in facilitation of exocytosis. It has been known for many years that increases in the frequency of spontaneous miniature end-plate potentials at a neuromuscular junction occur after exposure to high osmotic pressure (35). Despite continued investigation, an explanation for this mechanism remains to be revealed (19, 23). However, the question arises whether the measured extracellular concentrations of Glu within the PVN originated from neuronal or glial release. Both glial cells and neurons contain Glu, and the release could have come from either cellular compartment. The possible release of Glu from glia rather than, or in addition to, that from neuronal axon terminals raises the possibility of the reversal of normal glial Glu uptake by transporters due to disturbance of the ionic concentration of the extracellular milieu. A recent study by Landgraf and colleagues (18) demonstrated that the changes in extracellular amino acid concentration within the PVN are calcium dependent for some amino acids but not for others. Thus it can be presumed that local perfusion with hypertonic saline solution evokes an increase in Glu release in the PVN through multiple pathways.
Excitatory amino acid (EAA) receptors have been implicated in cardiovascular and body fluid regulation at the brain stem and hypothalamus levels (24, 45, 50). These ionotropic EAA receptors are classified as NMDA and non-NMDA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid and kainate) (5). The non-competitive NMDA-receptor antagonist MK-801 could significantly suppress the increases in BP and HR induced by local application of hypertonic saline. This suggests that Glu transmission through an NMDA receptor in the PVN is involved in the neural mechanism whereby hypertonic saline stimulates provoke cardiovascular activation. The non-NMDA-receptor antagonist CNQX had no effect on cardiovascular responses, suggesting that the effects observed were specific for an NMDA subtype of the Glu receptor. Other findings also point to NMDA being involved in the central regulation of body fluid balance (50, 51), such as, for example, ivc administration of NMDA-stimulated dipsogenic responses in pigeons (3).

Perspectives

The phenomenon of hypertonic saline-induced release of amino acids such as Glu appears to be constant throughout many areas of the CNS (18), although the sensitivity to hypertonic saline solution might be different on the basis of areas examined. However, cardiovascular responses induced by injection of hypertonic saline through microdialysis probes were presumed to be site specific. This assumption can be supported by the following. Cardiovascular responses were not induced or greatly attenuated after hypertonic NaCl perfusion into areas 1.0 mm rostral to or 1.0 mm lateral to the PVN, suggesting a localized site of action. However, using microdialysis probes to administer or collect substances, we cannot be certain that hypertonic saline stimulates cardiovascular activation. The non-NMDA-receptor antagonist CNQX had no effect on cardiovascular responses, suggesting that the effects observed were specific for an NMDA subtype of the Glu receptor. Other findings also point to NMDA being involved in the central regulation of body fluid balance (50, 51), such as, for example, ivc administration of NMDA-stimulated dipsogenic responses in pigeons (3).

REFERENCES


