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Water Intake Reduces the Noradrenaline Release in the Median Preoptic Nucleus Area Caused by Angiotensin II Injected into the Subfornical Oragn in the Rat

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(Key words : Drinking, Subfornical organ, Median preoptic nucleus, Angiotensin II, Noradrenaline)

Introduction

It has been known that the subfornical organ (SFO), one of circumventricular structures lacking normal blood–brain barrier (13), is an important site in the brain for dipsogenic responses (5, 7, 8, 10, 18, 19, 22, 23). Ablation of the SFO (18) or transection of SFO efferent projections (5) reduces or abolishes the drinking response produced by an increase in the plasma level of angiotensin II (ANG II). Experimental findings in several lines have revealed neural connections from the SFO to the hypothalamic nuclei thought to be implicated in the mediation of drinking behavior, such as the median preoptic nucleus (MnPO) (5, 10-12, 17, 19, 20, 23-25), the paraventricular nucleus (PVN) (7) and the lateral hypothalamic area (22).

Previous studies have demonstrated that catecholamine depletions in the anteroventral third ventricle (AV3V) region including the MnPO attenuate drinking and pressor responses and vasopressin release to many dipsogenic and pressor stimuli (1, 2, 4), indicating that catecholaminergic inputs from the brain stem to the AV 3V region may play vital roles in the regulation of normal fluid balance and cardiovascular function. Immunohistochemical tracing studies have shown that SFO neurons send angiotensinergic fibers to the MnPO (11, 12). It has been reported that destruction of the MnPO (10) or inactivation of either angiotensinergic (19, 23) or noradrenergic (19) systems in the MnPO diminishes the ANG II–induced drinking response elicited by ANG II injected into the SFO. These findings provide the proposition that the NA system in the MnPO may be involved in the elicitation of the drinking response to ANG II acting at the SFO.

The purpose of the present study was to explain more precisely the role of the noradrenergic system in the MnPO in the dipsogenic response induced by ANG II activation of the SFO. We investigated the effects of microinjection of ANG II into the SFO on the release of NA in the MnPO area and the effects of water ingestion on the NA release using in vivo microdialysis techniques in freely moving rats. In an attempt to determine whether an increase in arterial pressure produces changes in the NA release in the MnPO area, we also examined the alteration in the release of NA in response to an elevation in arterial pressure elicited by injections of ANG II into the SFO and intravenous injections of the α–agonist metaraminol under urethane anesthesia.

Materials and methods

Animals

All experiments were performed on male Wistar rats (n＝32) weighing 290–390g. The animals were housed

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individually in hanging wire cages for at least 2 weeks before testing. Water and food were available ad libitum except where noted. Lights were on in the animal rooms for 12 h per day (light at 7:00–19:00), and the temperature was maintained at 23–25 ℃.

Surgery

The animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and placed in a stereotaxic frame. A microdialysis probe guide cannula (AG–12, Eicom Co.) was lowered to coordinates which were 1mm dorsal to the MnPO since the probe assembly protrudes 1 mm below the ventral tip of the guide cannula when inserted. A 26–gauge stainless steel cannula was placed in the SFO, at a caudal angle of 15°. The guide and injection cannulae were then fixed to the skull with acrylic dental cement and small stainless steel screws. The 26–gauge cannula served as a guide for a 33–gauge stainless steel injector cannula, which was inserted just before injections. Each guide cannula was filled with an obturator of the same gauge as the injector cannula when the animals were not being tested.

Drugs

For intracerebral injection, ANG II (Asp^1–Ile^5–ANG II) salt (Sigma) was dissolved in isotonic saline vehicle and frozen in aliquots. Aliquots were thawed immediately before being used. For intravenous injection, the α–agonist metaraminol (Aldrich) was dissolved in saline vehicle. The drug solution was prepared on the day of the experiment.

Microdialysis

Microdialysis in the MnPO area and analysis of NA were performed by means of procedures described in our previous studies (20–22). Briefly, the dialysis probe (AI–1–12–1, Eicom Co.) whose tip had a 1.0–mm–long semipermeable membrane (in vivo recovery at 2 μl/min is almost 25%) was inserted into the implanted guide cannula. The probe was continuously perfused at a rate of 2 μl/min using a perfusion pump with a solution (NaCl 147mM, $CaCl_2$ 2.3mM, KCl 4mM). All dialysates were thus collected at 20 min intervals.

Intracranial injection

The third day after the implantation, the effects of microinjection of ANG II or vehicle into the SFO on extracellular content of NA in the MnPO area were tested. The dialysis in the MnPO area was achieved 6–7 h before the ANG II or vehicle injection into the SFO. The water bottle and the food box were removed 1 h before the injection. Each rat was removed from its home cage, and the obturator was removed. The injector, filled with injectate and connected to a 10 μl Hamilton gas chromatography syringe via approximately 80 cm of polyethylene tubing, was inserted into the implanted guide cannulae. The injectate within the tip of the injector was separated from the tip of the cannula, and therefore from the rat, by a 20 nl air bubble. The rat was then placed in the metabolism cage. In the previous study, we have demonstrated that microinjection of ANG II into the SFO in a dose of 10^{-6} M elicits a robust drinking response (19, 22). In this study, injections of ANG II were thus administered in a dose of 10^{-6} M. Because it is crucial to minimize diffusion of injectate in neuroanatomic localization experiments, all injections of the drug solution or vehicle were given in a volume of 50 nl. The injections were achieved at a rate of 5 nl/s. After the dialysis experiment, the animal was placed in its home cage and water and food were available ad libitum before the start of the experiment of the second day.

On the next day, the effects of water intake on the NA release in the MnPO area caused by the ANG II injection in the same dose used in the first injection into the SFO were examined. Tap water was available ad libitum immediately before the ANG II injection into the SFO. The latency to the onset of drinking was recorded, and water intake was then monitored for 120 min following the injection. To determine whether the second injection of ANG II into the SFO causes similar alterations in the NA levels observed in the first injection of the drug, the effects of the second injection of ANG II on the release of NA in the MnPO area

were examined in a part of the animals (n=6) under the condition without drinking.

Measurement of blood pressure and metaraminol adminstration

On the third day after the implantation, 8 rats were anesthetized with urethane (1.4–1.5 g/kg b.w.t., i.p.). A polyethylene cannula was inserted into the left femoral artery. The mean arterial pressure (M.A.P.) was directly measured with a blood pressure transducer (P–50, Gould Inc.) and a blood pressure meter (AP–6111G, Nihon Koden), and recorded on a recorder (Ws–681G, Nihon Koden). The left femoral vein was catheterized to inject the peripheral vasoconstrictor metaraminol. A tracheal cannula was inserted. The animal was then paralyzed with tubocurarine (2 mg/kg b.w.t, i.p.) and artificially ventilated to prevent respiratory influence on blood pressure. The rectal temperature was monitored and maintained at 37±0.5 ℃. Metaraminol was administered in a dose of 5 μg. Microinjections of ANG II (10^{-6} M, 50 nl) into the SFO were achieved at 100 min after the metaraminol administration.

Measurement of NA

The dialysates were analyzed for concentrations of NA, using HPLC (Eicom EP–10) with electrochemical detection (Eicom ECD–100). A mobile phase consisting of 0.1M sodium acetate, 0.1M citric acid, 0.75mM sodium1–octanesulfonate, 0.3 mM EDTA and 21% methanol (pH 3.9) was used to elute the monoamine from a reverse phase column (3.0 x 100 mm SC–3ODS column, Eicom Co.). The graphite working electrode was set at +750mV versus a Ag/AgCl reference electrode and the flow rate was 0.5 ml/min.

Histology

At the termination of each experiment, the animal was then sacrificed with an overdose of sodium pentobarbital and perfused through the heart with isotonic saline to clear blood, which was followed by 10% formalin for fixation. The brain was removed and stored in the formalin saline before being cut on a freezing microtome at 50 μm in transverse sections. Sections were mounted on glass slides and stained with Neutral red for microscope examination. The stereotaxic coordinates for the sites of dialysis probe and injection cannula were determined according to the atlas of Paxinos and Watson (14).

Statistics

All values are expressed as mean ± S.E.M. Data were analyzed by means of one–way or two–way repeated measures analysis of variance (ANOVAs) and subsequent Tukey's protected t–test. A probability of less than 0.05 was required for significance.

Results

Histological verification of the injection and dialysis sites

Histological analysis from the rat brains demonstrated that 29 out of 32 rats tested had the probe placement in the MnPO (Fig. 1D–F). The data from the remaining 3 rats having the probe placement outside the MnPO were not included in the analysis. Of the 29 animals having the probe placement in the MnPO, 16 and 6 received either the ANG II or vehicle injection into the SFO under the condition that accompanied water intake on the second experimental day (Fig. 1D) and the ANG II injection into the SFO under the condition without water ingestion in either the first or second day (Fig. 1E), respectively. The remaining 6 rats were utilized for the examination of the effects of changes in arterial pressure on NA release in the MnPO area (Fig. 1F).

Of the16animals that received the ANG II or vehicle injection, 15rats had correct SFO injector placement (Fig. 1A) such that ANG II administration produced a drinking response (more than 2.7 ml in 20 min). The remaining one rat with histology showing the tip of the injector through the ventral ependyma of the SFO exhibited little drinking (0.2 ml in 20 min) with the dose of the peptide used in this study and thus was not

included in further analysis. In all cases in which the injector tip for ANG II administration was within 0.2 mm of the dorsal border of the SFO, the injection of the peptide would invariably result in a drinking response (5.8±1.2 ml in 20 min ; 39±10 s ; n=9). In all animals that received the second ANG II injection into the SFO under the condition without water ingestion, the tip of the injection cannula was located within or just above the SFO (Fig. 1B). All animals tested for the effects of changes in arterial pressure on the NA release had good SFO injector placements (Fig. 1C).

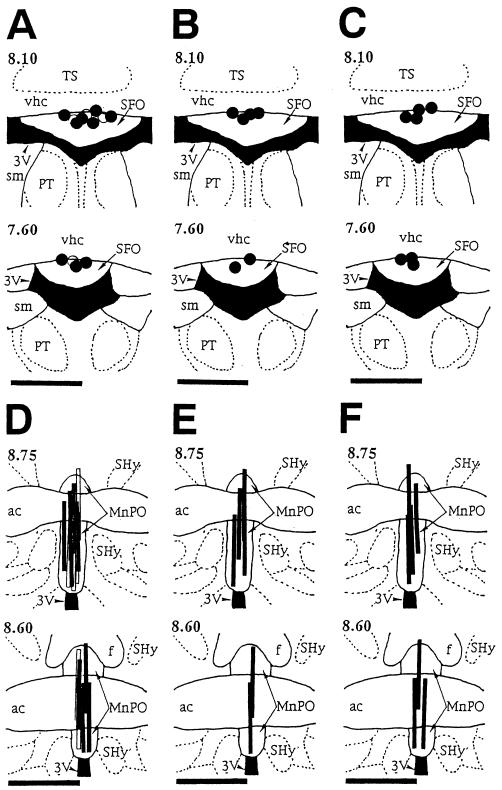

Fig. 1. A-C : The location of the cannula tips. A : Closed and open circles on schematic transverse sections indicate the loci of the cannulas that were utilized for microinjection of ANG II and saline vehicle into the SFO, respectively. B : Closed circles depict the loci of the cannulas in the rats that received the ANG II injections under the condition without drinking in both the first and second days. C : Closed circles indicate the loci of the cannulas in the rats that received the ANG II injection under urethane anesthesia. D-F : Vertical bars on schematic illustrations indicate the locations of the 1 -mm-long tip of the dialysis probe in the MnPO. D : Closed and open bars depict the locations of the probes in the rats that received the ANG II and saline vehicle injections into the SFO, respectively. E : Closed bars depict the probe placements in the rats that received the ANG II injections under the condition without drinking in both the first and second days. F : Closed bars indicate the probe placements in the rats that received the ANG II injection under urethane anesthesia. ac, anterior commissure ; f, fornix ; MnPO, median preoptic nucleus ; PT, paratenial thalamic nucleus ; SFO, subfornical organ ; SHy, septo- hypothalamic nucleus ; sm, stria medullaris of the thalamus ; TS, triangular septal nucleus ; vhc, ventral hippocampal commissure ; 3V, third ventricle. Scale bar=1 mm.

Effects of water intake on the ANG II-induced NA release in the MnPO area

In the 15 rats having both good injector and probe placements, the effects of ANG II (n=9) or saline vehicle (n=6) injections into the SFO on the release of NA in the MnPO area were examined under the condition without water ingestion. The basal levels of NA in the MnPO area immediately before the ANG II and saline vehicle injections into the SFO were 14.5±3.1 and 15.2±3.9 pg/40μl dialysate, respectively. No significant differences in the basal levels of NA were observed between the ANG II and vehicle treatment groups. Injections of ANG II into the SFO significantly enhanced the NA release in the MnPO area compared to the injection of saline ($F(1,13)=37.965$, $P<0.001$, Fig. 2A).

On the next day, the effects of water intake on the NA release in the MnPO area induced by ANG II injected into the SFO were examined. Injections of ANG II into the SFO elicited a robust drinking response within 60 min after the injection ($P<0,001$ for 20 min, $P<0.01$ for 40 min, $P<0.05$ for 60 min ; n=9 ; Fig. 2 B). The total water intake in 120 min was 7.3±1.5 ml (ranging 2.9–10.1 ml). The latency of drinking response was 37±8 s (ranging 7–91 s). The saline injection into the SFO, on the other hand, did not cause any significant water intake (total water volume in 120 min, 0.3±0.1 ml ; n=6 ; Fig. 2B). The baseline control levels of NA immediately before the ANG II (10.6±2.6 pg/40μl dialysate ; $F(1,16)=12.530$, $P<0.05$) or saline (11.0±2.8 pg/40μl dialysate ; $F(1,10)=12.076$, $P<0.05$) injection in the MnPO area were significantly lower compared to those of the first experimental day. The ANG II injection into the SFO significantly increased the NA release in the MnPO area compared to the saline injection ($F(1,13)=28.637$, $P<0.001$, Fig. 2A).

The amount of the increase in the NA release induced by ANG II injected into the SFO was much lower

in the condition that accompanied the water intake. To determine the statistical significance of this effect, a two–way ANOVA was calculated for the data of Fig. 2A, with water treatment as the between–group factor (two levels) and with time as the within–animal factor (eight levels). There were significant main effects of treatment ($F(1,16)=27.469$, $P<0.01$) and time ($F(7,112)=21.736$, $P<0.01$) and a significant interaction ($F(7,112)=19.995$, $P<0.001$). In planned comparisons, the group without water intake differed from the group that accompanied drinking at 20 min after the ANG II injection ($P<0.001$).

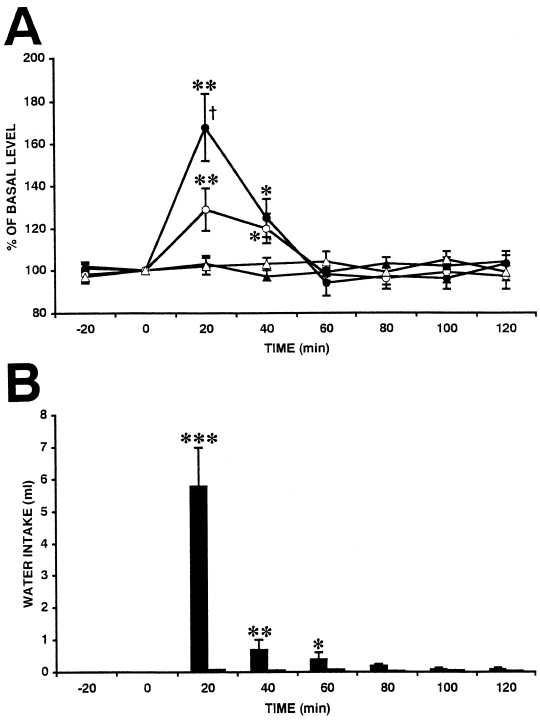

Fig. 2 Effects of water intake on the release of NA caused by microinjection of ANG II into the SFO. A: Changes in the release of NA in the MnPO area after the injection of ANG II (filled and unfilled circles, n=9) and saline vehicle (filled and unfilled triangles, n=6) into the SFO. Values are expressed as percentage of the sample taken immediately before the injection. Results in this and subsequent figures except for Fig.4 A are shown as mean ± S.E.M. The filled circles and triangles indicate the alterations in the NA release under the condition without drinking. The unfilled circles and triangles indicate the changes in the NA release under the condition that accompanied water intake. *P <0.01, **P<0.001compared with the basal control level (0 min). †P<0.001compared with those of the ANG II injection under the condition that accompanied water intake (filled circle) at 20min. B: The water intake (in each 20min) in response to the ANG II injection (closed histogram bars, n=8) or the vehicle injection (open histogram bars, n=6) into the SFO. *P<0.05, **P<0.01, ***P<0.001 compared with those of the saline injection.

Effects of the repeat ANG II injection into the SFO on NA release in the MnPO area

To determine whether the repeat injection of the ANG II into the SFO influences on the release of NA in the MnPO area, the effects of the second injection of the drug into the SFO on the NA release were examined under the condition without drinking. The basal concentrations of NA immediately before the ANG II injection into the SFO were significantly lower in the second day (10.4 ± 4.2 pg/40μl dialysate; n=6) than in the first day (14.3 ± 3.9 pg/40μl dialysate; n=6) ($F(1,10)=10.772$, $P<0.05$). In both the experimental days, injections of ANG II into the SFO significantly enhanced the NA release in the MnPO area (Fig. 3). The amount of the ANG II–induced increase in the NA release compared to the basal levels immediately before the drug injection was similar on both days.

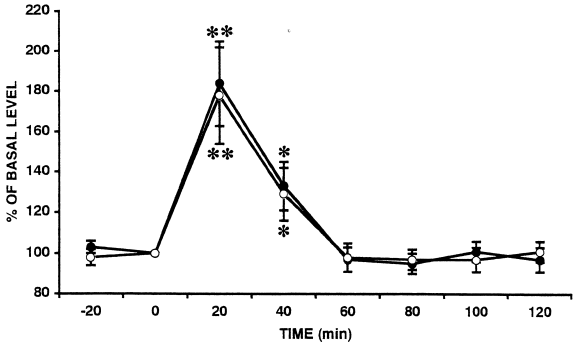

Fig. 3. Effects of repeat injection of ANG II on the release of NA in the MnPO area under the condition without drinking. Changes in the NA release are expressed as percentage of the sample taken immediately before the microinjection (n=6). Circles and open circles indicate the alterations in the NA release in response to the first and second injection of ANG II into the SFO, respectively. *P<0.05, **P< 0.001 compared with the basal control level (0 min).

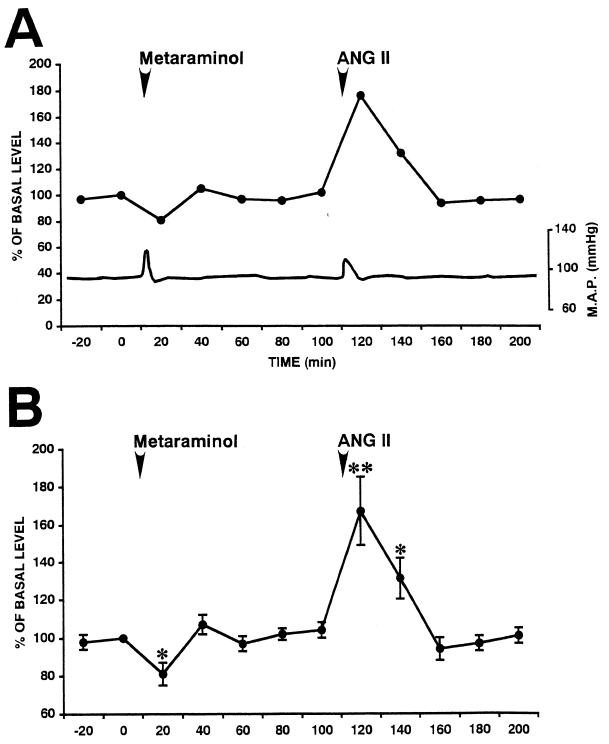

Fig. 4. Effects of increases in blood pressure following intravenous administration of metaraminol and microinjection of ANG II into the SFO on the release of NA in the MnPO area under urethane anesthesia. Values are expressed as percentage of the sample taken immediately before the intravenous injection of metaraminol. Arrows indicate the time of injection of the drugs. A: An example of the change in the NA release (upper) and mean arterial pressure (M.A.P., lower) to the intravenous injection of metaraminol and injection of ANG II into the SFO. B: Results in the 7 rats tested are summarized. *P<0.01, **P<0.001 compared with the basal control level (0 min).

NA release in the MnPO area to an elevation in blood pressure

The basal concentrations of NA in the MnPO area were 13.8±2.9 pg/40μl dialysate (n=7) under urethane anesthesia. Intravenous infusions of metaraminol significantly decreased the NA release in the MnPO area that accompanied a 18–41 mmHg (23±5 mmHg) elevation in M.A.P. ($F(1,10)=14.990$, $P<0.01$) (Fig. 4). Injections of ANG II into the SFO, on the other hand, significantly increased the release of NA in the MnPO area ($F(1, 10)=43.957$, $P<0.001$) that accompanied a 18–38 mmHg (22±5 mmHg) increase in M.A.P. (Fig. 4).

Discussion

The present data show that ANG II injected into the SFO enhances the release of NA in the MnPO area, and are consistent with the previous report (20). It has been suggested that ANG II acts as a neuromodulator in the central nervous system because of its interactions with neurotransmitters, especially the catecholamines (4, 15). Neuroanatomical studies have revealed that neurons in the SFO send angiotensinergic fibers to the MnPO (11, 12) and that stimulation of the SFO enhances the activity of MnPO neurons through ANG II receptors (24, 25). It has been shown that ANGII can increase the release of NA in the hypothalamus, perhaps via a presynaptic mechanism (6, 21). Thus, it is suggested the hypothesis that, in the MnPO area, ANG II released from angiotensinergic nerve terminals following activation of the SFO acts onto NA terminals, as a presynaptic mechanism, which result in increased NA release. However, there is no previous literature that the angiotensinergic fibers arising from the SFO terminate on NA terminals in the MnPO. Anatomical studies have revealed that the MnPO is richly innervated by noradrenergic nerve terminals derived from 3 cell groups, the locus coeruleus, the A2 cell group in the dorsal vagus complex, and the A 1 cell group in the ventrolateral medulla (16, 17). On the other hand, the existence of efferent projections from the SFO which are able to activate these noradrenergic cells in the brain stem is not known. It has been shown that the SFO is also innervated by noradrenergic projections from the A 1 and A2 cell groups (3, 9). If a single NA neuron projects simultaneously to the MnPO and the SFO, it would be possible that stimulation of the SFO per se causes NA release in the MnPO area via an axon reflex. The precise mechanisms underlying the increased NA release elicited by the ANG II injection into the SFO remains to be explained.

In the present study, the elevation in the NA release in the MnPO area elicited by ANG II injected into the SFO was significantly reduced by water intake. The results provide the first demonstration that water ingestion attenuates the release of NA in the MnPO area induced by ANG II activation of the SFO, and suggest that the noradrenergic system in the MnPO plays an important role in the mediation of dipsogenic response induced by activation of SFO neurons in response to an increase in the circulating level of ANG II. It has been demonstrated that NA depletions in the AV3V region lead to attenuated drinking (1, 2, 8). Reduction of systemic fluid volume has been shown to enhance NA turnover in the MnPO area (21, 26). In addition, a recent report has indicated that inactivation of either angiotensinergic or α–adrenergic systems in the MnPO following local administration of the antagonists diminishes the water intake induced by ANG II injected into the SFO (19). These observations and our data suggest the proposition that the release of NA in the MnPO may be an excitatory step along the ANG II–eliciting thirst pathway, and imply that signals from peripheral organs such as the stomach in response to water intake may decrease neural inputs of noradrenergic projections to the MnPO and/or the activity of noradrenergic system in the MnPO.

Previous observations have suggested that activation of SFO neurons produces an increase in blood pressure through their efferent fibers to the MnPO (8, 24). Although either ANG II injected into the SFO or metaraminol administered intravenously elicited an elevation in arterial pressure, the opposite effects of ANG II and metaraminol on the release of NA were found in this study, suggesting the possibility that an elevation in arterial pressure may be not attribute to the increased NA release caused by ANG injected into the SFO. However, it has been demonstrated that the noradrenergic system in the MnPO is implicated in the regulation of the blood pressure response to ANG II (1, 2). Additionally, it remains to verify the possibility that metaraminol may penetrate the blood–brain barrier or influence on SFO neurons via fenestrated capillaries. Thus, we cannot answer the question of whether an increase in arterial pressure may be involved in the enhanced NA release in the MnPO area.

In conclusion, the present data show that ANG II injected into the SFO enhances the release of NA in the MnPO and the NA release is reduced by water ingestion, suggesting that the noradrenergic system in the MnPO may participate in the modulation of the dipsogenic response induced by ANG II acting at the SFO.

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Abstract

The present study was carried out to investigate the role of noradrenergic systems in the median preoptic nucleus (MnPO) in the drinking response caused by microinjection of angiotensin II (ANG II) into the subfornical organ (SFO) in the awake rat. Extracellular levels of noradrenaline (NA) in the region of the MnPO were monitored with in vivo microdialysis methods. Injections of ANG II (10^{-6} M, 50 nl) into the SFO significantly increased NA release in the MnPO area. The increase in the NA release induced by the ANG II injection was significantly reduced by water ingestion. In urethane–anesthetized rats, injections of ANG II into the SFO produced an elevation in mean arterial pressure (M.A.P.). Intravenous infusions of metaraminol (5 μg), on the other hand, slightly suppressed the release of NA in the MnPO area that accompanied an elevation in M.A.P. These results suggest that the noradrenergic system in the MnPO may be involved in the dipsogenic response induced by ANG II acting at the SFO.

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