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GABAergic modulation of the ANG II-induced drinking response in the rat medial preoptic nucleus

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Abstract

The present study was designed to examine the participation of γ-aminobutyric acid (GABA) receptor mechanisms in the medial preoptic nucleus (MPO) in the drinking response caused by angiotensin II (ANG II) activation of the subfornical organ (SFO) in the awake rat. Local administration of ANG II (5 pmol, 50 nl) into the SFO elicited drinking. The water intake induced was significantly attenuated by previous injections (50 nl) into the MPO of the $GABA_A$ agonist muscimol (0.5, 5 and 50 pmol), but not by the $GABA_B$ agonist baclofen (0.5, 5 and 50 pmol) or vehicle, into the MPO. On the other hand, the ANG II-induced water intake was significantly enhanced by previous injections (50 nl) into the MPO of the $GABA_A$ antagonist bicuculline (0.5 and 5 pmol), but not the $GABA_B$ antagonist phaclofen (0.05, 0.5 and 5 pmol). Muscimol (50 nmol) injected into the MPO significantly reduced the water intake elicited by intracellular fluid depletion (i.e., hypertonic saline: 2 M, 2 ml/kg bw ip), whereas bicuculline (5 pmol) was without effect. These results show the involvement of the GABAergic system within the MPO in the dipsogenic responses induced by ANG II acting at the SFO and intracellular fluid depletion, and suggest that the system may serve to attenuate the ANG II-induced dipsogenic response through $GABA_A$ receptors.

Keywords: Medial preoptic nucleus; Subfornical organ; Drinking; Angiotensin II; Intracellular fluid depletion; GABA agonist; GABA antagonist

1. Introduction

The subfornical organ (SFO), a circumventricular structure that lacks a functional blood–brain barrier (VanHouten et al., 1983), is implicated in angiotensin II (ANG II)-induced drinking (Simpson and Routtenberg, 1978; Mangiapane and Simpson, 1980; Eng and Miselis, 1981; Miselis, 1981; Lind and Johnson, 1982; Gutman et al., 1988; Tanaka and Nomura, 1993; Tanaka, 2002; Kariya et al., submitted for publication) and pressor responses (Mangiapane and Simpson, 1980; Lind and Johnson, 1982; Gutman et al., 1988). The SFO has multiple efferent and afferent connections with the medial preoptic nucleus (MPO) (Camacho and Phillips, 1981; Miselis, 1981; Lind and Johnson, 1982; Lind et al., 1984, 1985; Chiba and Murata, 1985; Tanaka, 1989; Tanaka et al., 1995). Several studies have shown that the connections between these nuclei are considered to be important for regulating ANG II-induced SFO actions. Destruction of the MPO (Lind and Johnson, 1982) or transection of neural pathways between the nuclei (Eng and Miselis, 1981; Miselis, 1981) prevents the dipsogenic and pressor responses to activation of SFO neurons caused by ANG II. Immunohistochemical tracing studies have revealed that SFO neurons send angiotensinergic efferent fibers to the MPO (Lind et al., 1984, 1985). Stimulation of the SFO projections causes an increase in the release of noradrenaline in the MPO area (Tanaka et al., 1997), and water ingestion diminishes the noradrenaline release in the MPO area caused by angiotensinergic activation of the SFO (Miyakubo et al., accepted for publication). Local administration of either the ANG II antagonist saralasin (Tanaka and Nomura, 1993; Tanaka, 2002) or the α-adrenoceptor antagonist phentolamine (Tanaka, 2002) into the MPO attenuates the drinking response elicited by ANG II injected into the SFO. In addition, previous reports have

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suggested that the angiotensinergic SFO projections to the MPO are involved in the modulation of blood pressure (Tanaka et al., 1995, 2002a,b).

It has been demonstrated that the MPO area contains γ-aminobutyric acid (GABA) neurons and terminals (Mugnaini and Oertel, 1985). With respect to the action of the GABAergic system, experimental findings have shown that GABA and its analogs influence the ANG II-induced drinking and pressor responses through the lamina terminalis along the anterior wall of the third ventricle (Jones and Mogenson, 1982; Unger et al., 1983; Abe et al., 1988). An electrophysiological investigation has indicated that GABAergic projections from the MPO inhibit the excitability of both vasopressin- and oxytocin-secreting cells in the supraoptic nucleus (Nissen and Renaud, 1994). In addition, we have recently found using microdialysis methods that an enhancement of noradrenaline release caused by body fluid volume depletion is attenuated by perfusion with the $GABA_A$ agonist muscimol through a dialysis probe (Sakamaki et al., 2003), and that GABAergic inputs to the MPO are derived from the organum vasculosum lamina terminalis (OVLT) (Ushigome et al., in press). These observations offer the proposition that the GABAergic system in the MPO may be involved in the control of body fluid homeostasis and cardiovascular function.

The purpose of the present study was to elucidate the participation of the GABAergic system in the MPO's regulation of the drinking response induced by angiotensinergic activation of the SFO, and to clarify the role of GABA receptor subtypes in the regulatory mechanism of this drinking response. In this study we examined the effects of local administration of the GABA agonists as well as antagonists into the MPO on the water intake elicited by ANG II injected into the SFO. In an attempt to verify whether the GABAergic system in the SFO is involved in the regulatory mechanism of drinking behavior elicited by other dipsogenic stimuli, we also investigated the effects of similar administration of the GABA agents on the drinking response caused by intracellular fluid depletion (i.e., hypertonic saline).

2. Materials and methods

The experiment was performed according to the guiding principles of the Physiological Society of Japan.

2.1. Animals

A total of 78 male Wistar rats weighing 260–380 g was used for the experiment. The animals were obtained from Nihon Charles River (Atsugi, Kanagawa, Japan). They were housed individually in hanging wire cages for at least 2 weeks before testing. Food and water were available ad libitum except where noted. Lights were on in the animal rooms for 12 h per day, and temperature was maintained at 23–25 °C.

2.2. Surgery

The animals were anaesthetized with sodium pentobarbital (60 mg/kg ip), and were placed in a stereotaxic frame. The dorsal surface of the skull was exposed by midline incision. In 41 rats, a 26-gauge stainless steel cannula was stereotaxically lowered into the SFO, angled at 15° to vertical meridian. The same gauge cannula was positioned in to the MPO. The remaining 33 animals received only the implantation of such cannulae in the MPO. The intracranial cannulae were secured with dental acrylic anchored by small jeweller's screws fixed in the skull. The 26-gauge cannulae served as a guide for a 33-gauge stainless steel injector cannula, which was inserted just before injections. The tips of injectors and guide cannulae were flush during insertion. Each guide cannula was filled by an obturator of the same gauge as the injector cannula when the animals were not being tested.

2.3. Drug

ANG II (Asp^1-Ile^5-ANG II) salt, muscimol, baclofen, bicuculline methiodide, and phaclofen were obtained from Sigma (St. Louis, MO). For microinjection, ANG II salt was dissolved in isotonic saline. The ANG II solution was frozen in aliquots. Aliquots were thawed immediately before each experiment. Muscimol, a $GABA_A$ agonist, baclofen, a $GABA_B$ agonist, bicuculline methiodide, a $GABA_A$ antagonist, and phaclofen, a $GABA_B$ antagonist, were dissolved in artificial cerebrospinal fluid (126 mM NaCl, 3 mM KCl, 1.25 mM KH_2PO_4, 1.3 mM $MgSO_4$, 3 mM $CaCl_2$, $NaHCO_3$, 10 mM glucose; pH 3.5–6.0). These drug solutions were prepared on the day of the experiment and refrigerated until used.

2.4. Protocol for microinjection

Four days after each surgery each animal was put into a metabolism cage and baseline drinking behavior was observed. All testing was done at least 3 h into the light part of each rat's light/dark cycle. On the next day each rat was removed from its home cage, and the obturator was removed. The injectors, filled with injectate and connected to two 5-μl Hamilton gas chromatography syringes via approximately 1.0 m of polyethylene tubing, were inserted into the implanted guide cannulae. The rat was then placed in the metabolism cage.

Immediately following placement of the rat in the cage, the ANG II solution was injected into the SFO. Since it has been demonstrated that injections of ANG II in a dose of 5 pmol into the SFO elicit a robust drinking response (Tanaka and Nomura, 1993; Tanaka et al., 1997; Tanaka, 2002; Kariya et al., submitted for publication), ANG II was administered in this dose. The latency to the onset of drinking was recorded, and water intake was then monitored for 20 min following the injection. Each rat was given only

one intracranial injection per day. On the next day and the subsequent 2 days, the effects of pretreatment with the drug in several doses or vehicle in the MnPO on the drinking response to ANG II injected into the SFO was tested. Injections of muscimol or baclofen were applied in doses of 0.5, 5 and 50 pmol. Injections of bicuculline or phaclofen were administered in doses of 0.05, 0.5 and 5 pmol. The drugs or vehicle treatment was achieved 30 s before the ANG II injection into the SFO. Because it is crucial to minimize diffusion of injectate in neuroanatomic localization experiments, all injections of the drug solutions or vehicle were given in a volume of 50 nl. Injections were achieved at a rate of 5 nl/s using a microinjection pump (EP-60, Eicom).

In 19 animals having the placement of the cannula tip in the MPO only, the effects of pretreatment with muscimol (50 nmol), bicuculline (5 nmol) or vehicle in the MPO on the drinking response elicited by intracellular fluid depletion. Four intact rats were served as a control. Hypertonic saline (2 M, 2 ml/kg bw) was administered intraperitoneally immediately after the muscimol or bicuculline treatment, and water intake was then measured for 120 min following the injection.

2.5. Histology

At the termination of the experiment, injections of isotonic saline (50 nl) or artificial cerebrospinal fluid (50 nl) containing 2% Pontamine sky blue dye were made in order to confirm more precisely the location of cannula tips and to measure the spread of the injected solution. Twenty minutes after the injections, each animal was sacrificed with an overdose of sodium pentobarbital and perfused through the heart with isotonic saline to clear blood, which was followed by 10% formalin saline for fixation. The brain was then removed and stored in formalin saline for 24 h. Transverse sections of 50 μm were cut on a freezing

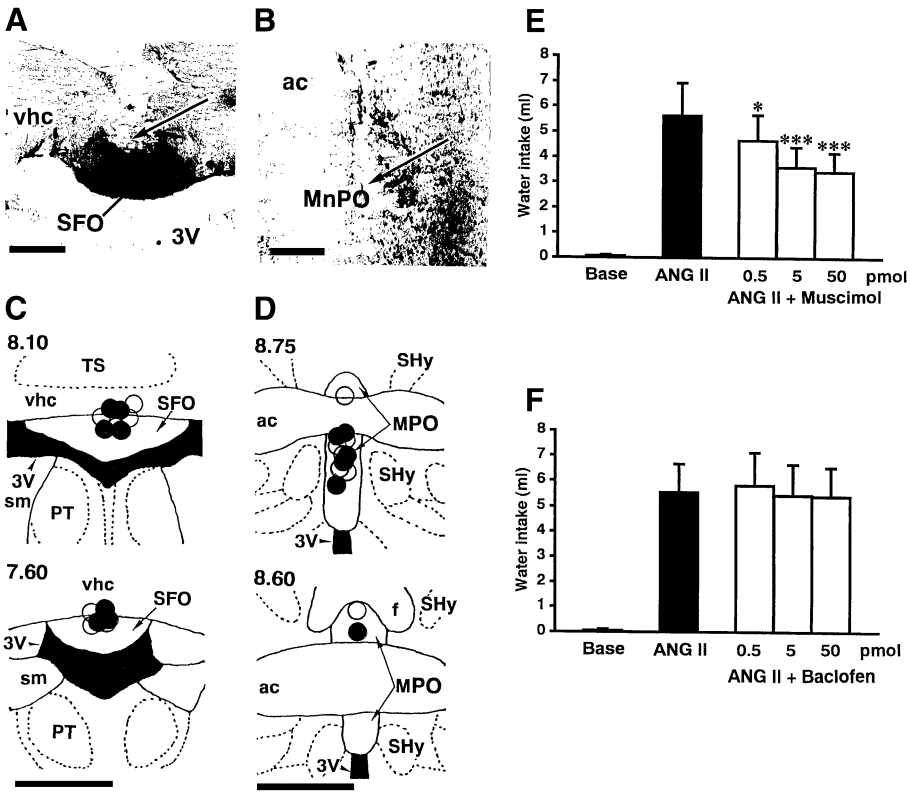

Fig. 1. Effects of previous injection of muscimol or baclofen into the medial preoptic nucleus (MPO) on the water intake caused by angiotensin II (ANG II) injected into the subfornical organ (SFO). (A and B) Photographs from Neutral red-stained coronal sections illustrate the location of the injector cannula (arrows) in the SFO (A) and the MPO (B). (C and D) The location of the cannula tips. (C) Closed and open circles on schematic transverse sections (8.10 and 7.60 mm anterior to the interaural line) depict the loci of the tip of the cannulae used for injections of ANG II in the muscimol-treated and the baclofen-treated rats, respectively. (D) Closed and open circles on two representative transverse sections (8.75 and 8.60 mm anterior to the interaural line) indicate the loci of the tip of cannulae utilized for injections of muscimol and baclofen, respectively. (E and F) Total water intake (ml/20 min) in the baseline drinking behavior (Base) and in response to the single injection of ANG II into the SFO or the ANG II injection combined with previous injection of muscimol [$n=7$, (E)] or baclofen [$n=7$, (F)] into the MPO. Results in this and subsequent figures are expressed as means ± S.E.M. Total water intake to ANG II injected into the SFO was significantly decreased by pretreatment with muscimol (0.5, 5 and 50 pmol), but not by baclofen, in the MPO. $*P<.05$, $***P<.001$ compared with ANG II. ac, anterior commissure; f, fornix; MPO, medial preoptic nucleus; PT, paratenial thalamic nucleus; SFO, subfornical organ; SHy, septohypothalamic nucleus; sm, stria medullaris of the thalamus; TS, triangular septal nucleus; vhc, ventral hippocampal commissure; 3 V, third ventricle. Scale bar = 0.5 mm.

microtome. Sections were then mounted on glass slides and stained with Neutral red for microscope examination. The stereotaxic coordinates for the cannula tips were determined according to the atlas of Paxinos and Watson (1986).

2.6. Statistical analysis

All values reported are means ± S.E.M. Data were analyzed with a one-way repeated measures analysis of variance (ANOVA) and subsequent protected Tukey's *t* test for the effects of the treatment. The criterion for significance was $P < .05$ in all cases.

3. Results

3.1. Drinking elicited by ANG II injected into the SFO

A total of 37 rats had correct SFO injector placement (Figs. 1A, C, 2A, 3A) such that ANG II administration caused a drinking response (more than 2.4 ml in 20 min). Other rats ($n = 4$) with histology showing the tip of the injector in the surrounding region of the SFO exhibited little or no drinking with the dose of ANG II used in this study (data not shown) and thus were 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 (Figs. 1C, 2A), the injection of the peptide would invariably result in a drinking response (volume, 5.6 ± 0.4 ml in 20 min vs. baseline water intake less than 0.2 ml in 20 min; latency, 38 ± 6 s; $n = 37$).

In 34 out of 37 rats having good cannula placement in the region of the SFO, the tip of the injection cannulae was located within or adjacent the MPO (Figs. 1B, D, 2B, 3B), and the remaining 3 rats had the tip of the cannulae greater than 0.3 mm away from the main body of the MPO (data not shown). The data from these 3 animals were not included in the analysis.

3.2. Effects of pretreatment with the GABA agonists on the ANG II-induced drinking response

Previous injections of muscimol into the MPO significantly attenuated the water intake caused by ANG II injected into the SFO [$F(1,12) = 17.986$, $P < .05$ for 0.5 pmol; $F(1,12) = 46.064$, $P < .001$ for 5 pmol; $F(1,12) = 51.008$, $P < .001$ for 50 pmol; $n = 7$; Fig. 1E]. Similar injections of baclofen in any of the doses used in this study into the MPO were without effect on the water intake induced by the ANG II injection ($n = 7$; Fig. 1F). Previous

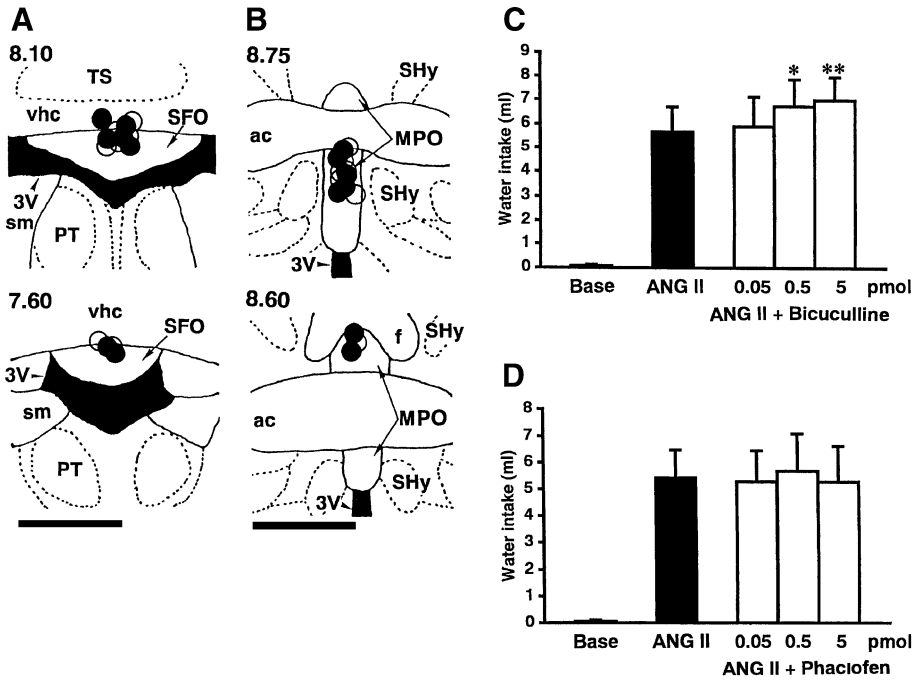

Fig. 2. Effects of previous injection of bicuculline or phaclofen into the MPO on the water intake caused by ANG II injected into the SFO. (A and B) The location of the cannula tips. (A) Closed and open circles on schematic transverse sections depict the loci of the tip of the cannulae used for injections of ANG II in the bicuculline-treated and the phaclofen-treated rats, respectively. (B) Closed and open circles on two transverse sections indicate the loci of the tip of cannulae utilized for injections of bicuculline and phaclofen, respectively. (C and D) Total water intake (ml/20 min) in the baseline drinking behavior (Base) and in response to the single injection of ANG II into the SFO or the ANG II injection combined with previous injection of bicuculline ($n = 7$, C) or phaclofen ($n = 7$, D) into the MPO. The water intake to the ANG II injection into the SFO was significantly enhanced by pretreatment with bicuculline (0.5 and 5 mol), but not by phaclofen, in the MnPO. $*P < .05$, $**P < .01$ compared with ANG II. For abbreviations, see Fig. 1.

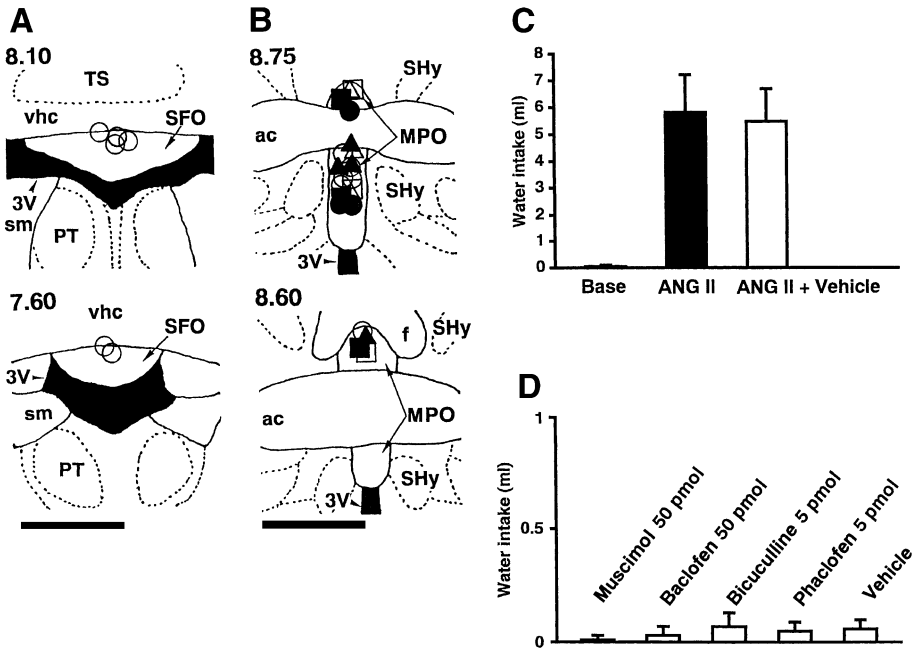

Fig. 3. Effects of previous injection of vehicle into the MPO on the water intake elicited by ANG II injected into the SFO, and total water intake (ml/20 min) in response to the single injection of muscimol, baclofen, bicuculline, phaclofen, or vehicle into the MnPO. (A and B) The location of the cannula tips. (A) Open circles on schematic transverse sections depict the loci of the tip of the cannulae used for injections of ANG II. (B) Open circles on two transverse sections indicate the loci of the tip of cannulae utilized for previous injections of vehicle into the MPO. Closed and open squares show the loci of the tip of the cannulae used for the single injection of muscimol and baclofen into the MPO, respectively. Closed and open triangles indicate the loci of the tip of the cannulae utilized for the single injection of bicuculline and phaclofen into the MPO, respectively. Closed circles depict the loci of the tip of the cannulae used for the single injection of vehicle ($n=3$) into the MPO. (C and D) Total water intake in the baseline drinking behavior (Base) and in response to the single injection of ANG II into the SFO or the ANG II injection combined with previous injection of vehicle into the MPO [$n=6$; (C)], and in response to the single injection of muscimol (50 pmol; $n=3$), baclofen (50 pmol; $n=3$), bicuculline (5 pmol; $n=4$), phaclofen (5 pmol; $n=3$), or vehicle ($n=3$) into the MPO (D). Pretreatment with vehicle in the MPO did not affect the ANG II-induced water intake (C). For abbreviations, see Fig. 1.

injections of vehicle in the MPO did not cause any significant changes in the water ingestion ($n=6$; Fig. 3C).

Previous injections of any of the agonists or vehicle into the MPO had no significant effect on the latencies to the onset to drinking response induced by ANG II injected directly into the SFO (Table 1).

Table 1
Latencies to the onset of drinking before and after treatment with the GABA agonists, the GABA antagonists, or vehicle in the MnPO in response to the ANG II injection into the SFO

Drug	Dose (pmol)	No. of animals	Pretreatment (s)	Treatment (s)
Muscimol	0.5	7	35 ± 9	39 ± 7
	5	7	35 ± 9	38 ± 9
	50	7	35 ± 9	34 ± 8
Baclofen	0.5	7	41 ± 7	39 ± 8
	5	7	41 ± 7	41 ± 10
	50	7	41 ± 7	37 ± 9
Bicuculline	0.05	7	37 ± 9	38 ± 9
	0.5	7	37 ± 9	35 ± 10
	5	7	37 ± 9	40 ± 8
Phaclofen	0.05	7	35 ± 9	37 ± 8
	0.5	7	35 ± 9	40 ± 11
	5	7	35 ± 9	37 ± 9
Vehicle		6	38 ± 8	40 ± 9

Values are expressed as means ± S.E.M.

3.3. Effects of pretreatment with the GABA antagonists on the ANG II-induced drinking response

Previous injections of bicuculline in the doses of 0.5 pmol ($F(1,12)=18.956$, $P<.05$; $n=7$) and 5 pmol ($F(1,12)=27.369$, $P<.01$), but not 0.05 pmol, in the MPO significantly increased the water intake caused by ANG II injected into the SFO ($n=7$; Fig. 2C). Pretreatment with phaclofen in any of the doses in the MPO did not affect the ANG II-induced water intake ($n=7$; Fig. 2D).

Previous injection of any of the drugs the MPO had no significant effect on the latencies to the onset to drinking response induced by the ANG II injection into the SFO (Table 1).

3.4. Microinjection of the GABA agents or vehicle alone into the MPO

In 13 out of 14 rats having the cannula placement within or adjacent the MPO (Fig. 3B), whether local injections of the GABA agents or vehicle alone into the MPO cause drinking were examined. The tip of the cannula of the remaining one animal was located in the site approximately 0.4 mm dorsal from the MPO (data not shown), and the result of the animal was not included in the analysis.

Injections of any of muscimol (50 pmol; $n=3$), baclofen (50 pmol; $n=3$), bicuculline (5 pmol; $n=4$), phaclofen (5 pmol; $n=3$), or vehicle ($n=3$) did not produce a remarkable change in the water intake compared with the baseline water intake (less than 0.2 ml in 20 min; Fig. 3D).

3.5. Effects of microinjection of muscimol or bicuculline into the MPO on the drinking response caused by injections of hypertonic saline

To determine whether the GABAergic system in the MPO participate in the regulatory mechanism of dipsogenic responses elicited by intracellular fluid depletion, the effects of microinjection of muscimol or bicuculline at the highest dose into the MPO on the drinking response induced by treatment with 2 M NaCl solution (2 ml/kg bw ip) were examined in 17 out of 19 rats having the cannula placement within or adjacent the MPO (Fig. 4A). The tip of the cannula of the remaining two animals was located in the site more than 0.3 mm dorsal from the MPO (data not shown), and the results of the animals were not included in the analysis.

In four intact rats, intraperitoneal injections of hypertonic saline produced drinking (more than 1.7 ml in 120 min; $n=4$: Fig. 4B). Previous injections of muscimol (50 nmol; $n=6$) into the MPO significantly decreased the water intake produced by the treatment with hypertonic saline ($F(1,10)=25.927$, $P<.01$; $n=6$; Fig. 4B). Neither bicuculline (5 nmol; $n=6$) nor vehicle ($n=5$) administration into the MPO affected the drinking response to hypertonic saline (Fig. 4B).

4. Discussion

Our experiment demonstrated that previous injections of muscimol or bicuculline into the MPO cause changes in the drinking response induced by ANG II injected into the SFO. It has been demonstrated that neurons in the SFO contain GABA receptors (Weindl et al., 1992) and application of GABA and its analogs alters the activity of SFO neurons (Inenaga et al., 1995; Xu et al., 2001). Thus, it may be argued that the effects of local administration of these drugs in the MPO resulted from leaking into the ventricular system causing inhibitory or antagonistic effects in the sites. Since steeper concentration gradients facilitate faster diffusion (Selleck and Simpson, 1980), it is possible that the agents injected into the MPO could have affected the SFO by reflux up the cannula path and diffusion through tissue to the SFO. When injections of muscimol into the third ventricle were achieved in a dose of 50 pmol (50 nl), a marked alteration of either discharge rate or the responsiveness of SFO neurons to ANG II was not observed (Tanaka et

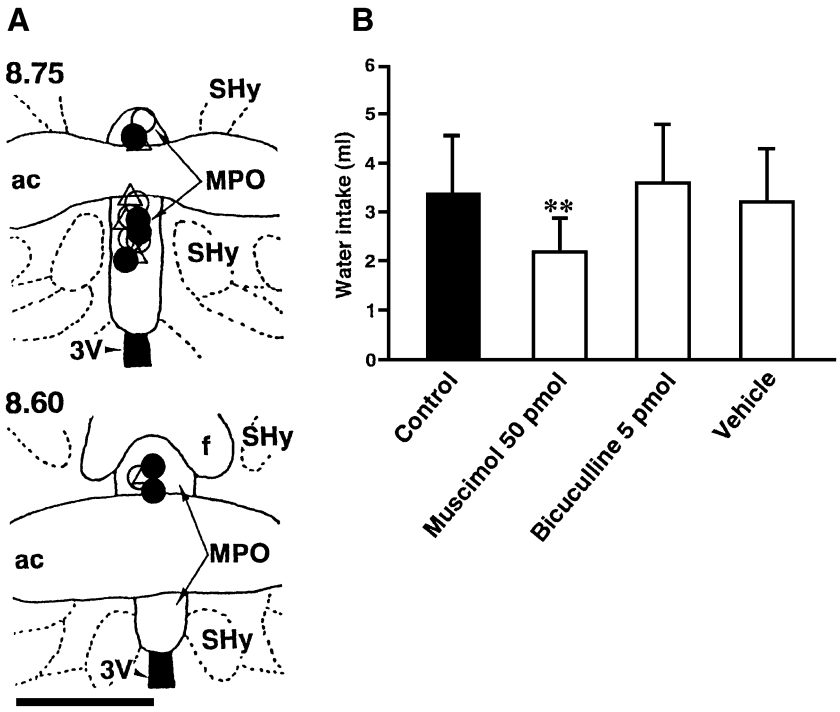

Fig. 4. Effects of previous injection of muscimol and bicuculline into the MPO on the water intake caused by intraperitoneal injection of hypertonic saline (2 M, 2 ml/kg bw). (A) The location of the cannula tips. Closed and open circles and closed triangles on two transverse sections indicate the loci of the tip of cannulae utilized for injections of bicuculline, phaclofen and vehicle, respectively. (B) Total water intake (ml/20 min) in response to the single injection of hypertonic saline (Control; $n=4$) or the hypertonic saline combined with previous injection of muscimol (50 pmol; $n=6$), bicuculline (5 pmol; $n=6$) or vehicle [$n=5$, (D)] into the MPO. The water intake to the hypertonic saline was significantly decreased by pretreatment with muscimol in the MnPO whereas bicuculline and vehicle treatments were without effect. $**P<.01$ compared with Vehicle. Abbreviations see Fig. 1.

al., 2002a,b). Additionally, in the preliminary study, injections of muscimol in a dose of 50 pmol (50 nl) into the third ventricle did not cause any significant changes in the water intake induced by ANG II into the SFO (volume in 20 min: 5.8 ± 1.0 ml in the pretreatment vs. 5.5 ± 0.9 ml in the treatment, $n = 6$). It may be thus considered that the attenuation or enhancement of water intake is mediated by direct action of the drug in the MPO. It may be also argued that the $GABA_A$ agonist and antagonist might be producing effects on behavior through secondary effects of the agents. Although no attempt was made in this study to provide control experiments to demonstrate whether similar doses of the GABA agents cause taste aversions or disrupt hedonistic drinking, the findings in which injections of the agents at the highest dose did not affect the basal water ingestion imply that the injection of the agents do not induce illness, malaise, or disorientation that can disrupt a drinking response. In addition, previous observations have shown that, in urethane-anesthetized rats, local application of the $GABA_A$ agents in into the MPO area does not produce a marked change in blood pressure (Ushigome et al., in press). Therefore, it seems likely that the effects of the $GABA_A$ agents observed in this study might be a direct action on the ANG II-induced drinking response.

The present data provide the first evidence that the GABAergic system in the MPO serve to attenuate the dipsogenic response caused by angiotensinergic activation of the SFO. The findings in which the water intake induced by ANG II injected into the SFO is reduced by pretreatment with muscimol, but not by baclofen, in the MPO suggest that the inhibitory action of the GABAergic system in the MPO may be mediated through $GABA_A$ receptor mechanisms. In this study, we observed that previous injections of bicuculline, but not phaclofen, enhance the water intake elicited by the ANG II injection. The observations raise the proposition that the GABAergic system in the MPO may tonically inhibit the neural inputs from the SFO through $GABA_A$ receptors or there is a neural pathway that activates the GABAergic inhibitory system in response to ANG II acting at the SFO.

On the other hand, our results indicate that the responses elicited by pretreatment with either muscimol or bicuculline are not large and the injection of bicuculline alone into the MPO does not cause a significant drinking response. In addition, no significant changes in the latencies of the onset of drinking in response to the injection of the GABA agents were found. These results suggest that at least there are other non-$GABA_A$ synapses that are sufficient for the drinking. It is tempting to speculate that the GABAergic system in the MPO may participate in carrying signals from other components that are involved in the modulation of dipsogenic response rather than controlling the inputs from the SFO. In an attempt to verify this speculation, the effects of local administration of muscimol and bicuculline into the MPO on the drinking response caused by intracellular fluid depletion were examined. Muscimol injected into the MPO significantly reduced the water intake in response to hypertonic saline treatment, whereas bicuculline injections did not affect the drinking response. The data show that the $GABA_A$ receptor mechanism within the MPO may be also implicated in the elicitation of osmotic thirst, and imply that the GABAergic system mentioned above, which causes an increase in the ANG II-induced water intake in response to the bicuculline administration, may be inactivated by intracellular fluid depletion.

Previous studies have suggested that GABA and its analogs influence the ANG II-induced drinking and pressor responses through the lamina terminalis along the anterior wall of the third ventricle (Jones and Mogenson, 1982; Unger et al., 1983; Abe et al., 1988). Our data clearly showed that the MPO is an important site for the GABAergic control of the dipsogenic action produced by ANG II. The presence of GABAergic neurons and terminals in the MPO area has been demonstrated (Mugnaini and Oertel, 1985). However, the precise location of the GABA interneurons acting in the inhibitory pathway is still unknown. Because of neural inputs to the MPO come from the OVLT, SFO, medial hypothalamus, and several brainstem regions (Camacho and Phillips, 1981; Miselis, 1981; Lind and Johnson, 1982; Saper and Levisohn, 1983; Saper et al., 1983; Lind et al., 1984, 1985; Kawano and Masuko, 1993), it is possible to speculate that one of the projections from these sites is the GABAergic inhibitory pathway. Indeed, recent microdialysis observations have revealed that perfusion with the phaclofen, but not bicuculline, through a dialysis probe enhances the release of noradrenaline in the MPO area caused by electrical stimulation of the OVLT, suggesting that the OVLT may exert GABAergic inhibitory influence on the NA release in the MPO area through $GABA_B$ receptor mechanisms (Ushigome et al., in press). The OVLT contains neurons that participate in osmoregulation (McKinley et al., 1988). Since our data show that the inhibitory effect of the GABAergic system in the MPO area on the drinking response caused by ANG II activation of the SFO may be mediated via $GABA_A$ receptors, it is possible that $GABA_A$ and $GABA_B$ receptors in the MPO may play distinctly different roles in the regulation of body fluid balance.

It has been postulated that the interaction between the angiotensinergic and catecholaminergic systems in the MPO may be important for the elicitation of drinking behavior (Bellin et al., 1987, 1988; Johnson et al., 1992; Silva et al., 1996; Tanaka, 2002). Ablation of the MnPO (Lind and Johnson, 1982) or pretreatment with the ANG II antagonist saralasin (Tanaka and Nomura, 1993; Tanaka, 2002) or the α-adrenoceptor antagonist (Tanaka, 2002) reduces water intake induced by ANG II injected into the SFO. The MPO is richly innervated by angiotensinergic nerve terminals derived from the SFO (Lind et al., 1984, 1985). Electrical or chemical (ANG II) stimulation of the SFO causes an increase in the excitability of MPO neurons via ANG II receptors (Tanaka, 1989; Tanaka et al., 1995) and the release of noradrenaline in the MPO area (Tanaka et al.,

1997). The ANG II-induced elevation in the noradrenaline release in the MPO area is attenuated by water ingestion (Kariya et al., submitted for publication). Inactivation of the angiotensinergic system in the periventricular structures including the MPO area following injections of the ANG II antagonist saralasin into the third ventricle decreases the extracellular catecholamine concentration in the MPO area (Tanaka et al., 2002a,b). These findings and our data raise the possibility that the GABAergic system in the MPO may act to inhibit the activity of the angiotensinergic and cathecholaminergic systems that modulate the dipsogenic response elicited by ANG II through $GABA_A$ receptors. It has been reported that lesions of either the ventromedial hypothalamus (Bastos et al., 1997) or the lateral hypothalamus (Silva et al., 1995) reduce the dipsogenic effects of ANG II and noradrenaline injected into the MPO, indicating that the MPO projections to the hypothalamic regions are involved in the elicitation of drinking behavior. It might be thus expected that the GABAergic system may regulate the activity of the MPO projections. Further studies are in progress.

In conclusion, the present study demonstrates that the GABAergic system in the MPO helps modulate the dipsogenic responses elicited by ANG II in the SFO and intracellular fluid depletion. Our data further suggest that this GABAergic system can attenuate the ANG II-induced dipsogenic response through $GABA_A$ receptors.

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