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Adrenoceptors of the medial septal area modulate water intake and renal excretory function induced by central administration of angiotensin II

W.A. Saad[1,2], I.F.M.S. Guarda[3], L.A.A. Camargo[1], T.A.F.B. Santos[3], S. Simões[2] and Willian A. Saad[4]

[1]Departamento de Fisiologia e Patologia, Faculdade de Odontologia, Universidade Estadual Paulista, Araraquara, SP, Brasil
[2]Departamento de Odontologia, Universidade de Taubaté, Taubaté, SP, Brasil
[3]Departamento de Anestesiologia, Hospital Médico 9 de Julho, São Paulo, SP, Brasil
[4]Departamento de Cirurgia, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil

Abstract

We investigated the role of α-adrenergic antagonists and clonidine injected into the medial septal area (MSA) on water intake and the decrease in Na^+, K^+ and urine elicited by ANGII injection into the third ventricle (3rdV). Male Holtzman rats with stainless steel cannulas implanted into the 3rdV and MSA were used. ANGII (12 nmol/µl) increased water intake (12.5 ± 1.7 ml/120 min). Clonidine (20 nmol/µl) injected into the MSA reduced the ANGII-induced water intake (2.9 ± 0.5 ml/120 min). Pretreatment with 80 nmol/µl yohimbine or prazosin into the MSA also reduced the ANGII-induced water intake (3.0 ± 0.4 and 3.1 ± 0.2 ml/120 min, respectively). Yohimbine + prazosin + clonidine injected into the MSA abolished the ANGII-induced water intake (0.2 ± 0.1 and 0.2 ± 0.1 ml/120 min, respectively). ANGII reduced Na^+ (23 ± 7 µEq/120 min), K^+ (27 ± 3 µEq/120 min) and urine volume (4.3 ± 0.9 ml/120 min). Clonidine increased the parameters above. Clonidine injected into the MSA abolished the inhibitory effect of ANGII on urinary sodium. Yohimbine injected into the MSA also abolished the inhibitory effects of ANGII. Yohimbine + clonidine attenuated the inhibitory effects of ANGII. Prazosin injected into the MSA did not cause changes in ANGII responses. Prazosin + clonidine attenuated the inhibitory effects of ANGII. The results showed that MSA injections of α_1- and α_2-antagonists decreased ANGII-induced water intake, and abolished the Na^+, K^+ and urine decrease induced by ANGII into the 3rdV. These findings suggest the involvement of septal α_1- and α_2-adrenergic receptors in water intake and electrolyte and urine excretion induced by central ANGII.

Correspondence
W.A. Saad
Departamento de Fisiologia e Patologia
Faculdade de Odontologia, UNESP
Rua Humaitá, 1680
14801-903 Araraquara, SP
Brasil
Fax: +55-16-201-6488
E-mail: silvana@foar.unesp.br

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- Water excretion
- Medial septal area

Introduction

Central administration of angiotensin II (ANGII) induces thirst in satiated animals by interacting with neurotransmitters, especially catecholamines (1,2). Adrenergic neurotransmitters from several hypothalamic areas may participate in the effect of ANGII regulating hydromineral fluid intake and renal electrolyte excretion in a process that involves α_1- and α_2-adrenoceptors (3-5). Central injection of an α-adrenergic antagonist suppresses water intake induced by intracerebroventricular (*icv*) ANGII (6,7). Several areas of the limbic system participate in the regulation of sodium, potassium and water excretion (8). Previous studies have demonstrated the effects of α-antagonists and agonists injected into the lateral hypothalamus on the water and sodium intake induced by ANGII injection into the subfornical organ (3). The adrenergic pathways of the septal area play an important role in the regulation of electrolyte and water excretion. α-Adrenoceptors present an excitatory effect, whereas ß-adrenoceptors present an inhibitory effect (9,10). Rats with electrolytic lesions of the septal area drink more water than normal ones in response to thirst stimuli mediated by ANGII (11). Extensive neural pathways from circumventricular structures to the septal area are involved in the regulation of fluid intake and cardiovascular regulation (12,13). Clonidine, an α_2-adrenergic receptor agonist, has a potent and well-known antidipsogenic action (14-17). Peripheral or central injection of clonidine reduces water intake induced by peripheral or central administration of ANGII (18,19). Central treatment with yohimbine (an α_2-adrenergic receptor antagonist) reduces the antidipsogenic effect of clonidine, suggesting the participation of α_2-adrenergic receptors in this effect (18). Idazoxan (an α_2-adrenergic and imidazoline receptor antagonist) reduced the effect of clonidine on hypertonic NaCl and water intake (20). The natriuretic-kaliuretic response elicited by cholinergic stimulation of the lateral hypothalamic area depends in part on synapses located in the medial septal area (MSA), the response elicited by cholinergic stimulation of the MSA also utilizes synapses located in the lateral hypothalamic area (21). Increased renal sodium and potassium excretion has been obtained with noradrenaline and other adrenergic drugs injected into the septal area (9). There is some evidence that fibers from the subfornical organ converge to the nucleus medianus and also project to the supraoptic nucleus, paraventricular nucleus and throughout the lateral preoptic area-lateral hypothalamic area (22). The central part of the subfornical organ, which is linked to circulating ANGII (23), also contains ANGII-immunoreactive terminal fields which seem to come from cells in the MSA (24). In view of the importance of the circumventricular structures and MSA for the hydromineral balance in rats (9,25), as well as the important interactions between areas of the circumventricular structures and the MSA, we determined the effect of the injection of clonidine, yohimbine and prazosin into the MSA on water intake and on the antinatriuretic, antikaliuretic and antidiuretic effects induced by the administration of ANGII into the third ventricle (3rdV).

Material and Methods

Animals

Male Holtzman rats weighing 240-280 g at the beginning of the experiments were housed in individual metabolic cages. Standard Purina pellets (Na^+ content 5 nmol/100 g) and tap water were available *ad libitum*. The temperature in the animal colony was maintained at approximately 23ºC. The 12/12-h light-dark cycle began with lights on at 8:00 am. All animals used in the experiments received the same drugs but at different times. However, each animal was used

in four experiments at most.

Brain surgery

After an acclimatization period of 7 days, the animals were restrained in a stereotaxic apparatus (Kopf model) and maintained under intraperitoneal tribromoethanol (20 mg/100 g body weight; Aldrich Chemical Company Inc., Milwaukee, WI, USA) anesthesia throughout surgery. A longitudinal incision was made in the skin of the head of each animal, the subcutaneous tissue was pulled back and the skull was drilled with a spherical drill. A stainless steel cannula (14 x 0.7 mm OD) was introduced into the MSA and another cannula (10 x 0.7 mm OD) was introduced into the 3rdV. The skull was positioned by having the bregma and lambda at the same level. The coordinates for approaching the MSA and 3rdV were obtained from the Paxinos and Watson atlas (26). For the MSA the following coordinates were used: AP, 0.9 mm caudal to the bregma; L, 0.0 mm in the midline, and V, 4.6 mm below the dura mater. For the 3rdV, the following coordinates were used: AP, 0.2 mm caudal to bregma; L, 0.0 mm on the sagittal line, and V, 7.8 mm below the dura mater. The cannula was fixed to the skull with screws and acrylic resin. A prophylactic dose of penicillin (30,000 IU) (Pentabiótico, Fontoura Wyeth, São Paulo, SP, Brazil) was given intramuscularly and presurgically. The insertion of a close-fitting stylet kept the lumen free of debris and clots.

Intracerebral injection techniques

Bolus intracranial injections were made after gently removing the animal from its cage, replacing the stylet with an injector that protruded 1.0 mm beyond the tip of the guide cannula in order to reach the MSA and 3rdV. This injector was connected by a PE-10 tubing to a 10-µl microsyringe, and a total volume of 1.0 µl was injected over a period of 30-60 s. The stylet and injector were constantly wiped with cotton soaked in 70% alcohol. After the injection, the injector was removed, the stylet was replaced and the animals were returned to their cages so that we could observe the water intake, as well as renal sodium, potassium and water excretion.

Drugs

The drugs were dissolved in sterile 0.15 M NaCl and injected into the MSA or 3rdV with a Hamilton microsyringe (10 µl) connected by PE-10 polyethylene tubing (25 cm) to a needle (0.3 mm OD), and introduced into the brain through the cannula previously fixed to the animals' heads. The volume of injection was always 1 µl injected over a 30-60-s period.

The drugs used were clonidine hydrochloride (Boehringer-Ingelheim Ltd., London, UK), prazosin hydrochloride (Pfizer, Guarulhos, SP, Brazil), yohimbine hydrochloride (Sigma, St. Louis, MO, USA), and Ile^5-angiotensin II (Sigma). Saline (0.15 M NaCl) was used as control.

Histology

After the experiments, the animals were anesthetized with ether and perfused through the heart with saline and 10% formalin. The brain was removed and stored in 10% formalin for at least 1 week. It was then frozen and coronal sections (20-30 µm) were cut and stained with hematoxylin-eosin for examination by light microscopy. Only the results of rats whose MSA and 3rdV were reached by the injection were used. Figure 1A and B shows the site of injection into the MSA and 3rdV as indicated by the arrows.

Statistical analysis

Data are reported as means ± SEM and were analyzed by one-way analysis of vari-

ance. Values were considered to be statistically significant when $P<0.05$. The Newman-Keuls *post hoc* test was used to assess the difference between individual means.

Experimental protocol

The study of water ingestion, as well as renal sodium, potassium and water excretion produced by injecting ANGII into the 3rdV was started 5 days after brain surgery. Water ingestion and the renal parameters were determined during different experimental sessions and in several groups of animals after the injection of the following drugs into the MSA and 3rdV of satiated rats: saline into the MSA and 3rdV (control); saline into the MSA and ANGII (12 nmol) into the 3rdV; clonidine (20 nmol) or yohimbine or prazosin (80 nmol) into the MSA and saline into the 3rdV; clonidine (20 nmol) or yohimbine or prazosin (80 nmol) into the MSA and ANGII into the 3rdV; yohimbine or prazosin (80 nmol) + clonidine (20 nmol) into the MSA and ANGII (12 nmol) into the 3rdV.

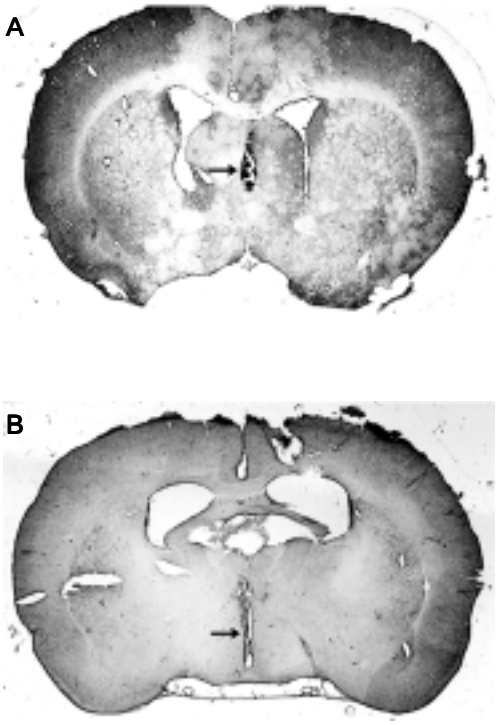

Figure 1. Photomicrographs of a hematoxylin-eosin-stained transverse section of the rat brain showing the sites (arrows) of injection into the medial septal area (A) and into the third ventricle (B).

Water intake was recorded for 2 h after the injection of ANGII into the 3rdV. Prazosin and yohimbine were also injected 20 min before ANGII. When associated with clonidine, prazosin and yohimbine were injected 20 min before clonidine. The volume of water was measured using graduated (0.1 ml markings) tubes fitted with metal drinking spouts. No solid food was made available to the animals during the experiments.

Urine was collected after a period of solid food deprivation, and the animals were weighed and received a 5% water overload by gavage, consisting of a volume of water at 37ºC equal to their body weight. The animals were returned to their cages, with no water or solid food available. Excreted urine was collected into graduated tubes through a funnel located at the bottom of the cage. After 60 min the rats were submitted to a second water overload (5% of body weight) by gavage. Twenty minutes later, a control urine sample was collected and the drug was diluted in 1 µl 0.15 M NaCl solution and injected into the MSA/3rdV. Urine samples were collected 2 h after this injection. Sodium and potassium concentrations in the urine samples were measured with an IL-143 flame spectrophotometer (Instrumental Laboratories, Lexington, MA, USA).

Results

Effect of pretreatment with intraseptal yohimbine and prazosin on the water intake induced by ANGII injected into the 3rdV

The injection of ANGII (12 nmol) into the 3rdV produced a progressive increase in water intake starting 30 min after injection. After injection of 0.15 M NaCl (control) into the MSA/3rdV, water intake was 0.4 ± 0.1 ml/120 min. The injection of ANGII into the 3rdV produced an increase in water intake to 12.5 ± 1.7 ml/120 min. Clonidine (20 nmol) injection into the MSA before ANGII injec-

tion into the 3rdV decreased water intake to 2.9 ± 0.5 ml/120 min. Yohimbine (80 nmol) and prazosin (80 nmol) injected into the MSA before injection of ANGII into the 3rdV decreased the dipsogenic effect of ANGII, with water intake of 3.0 ± 0.4 and 3.1 ± 0.2 ml/120 min, respectively. Yohimbine and prazosin injected before clonidine into the MSA, and before injection of ANGII into the 3rdV abolished the effect of ANGII, reducing water intake to control values, i.e., 0.2 ± 0.1 and 0.2 ± 0.1 ml/120 min, respectively (Figure 2).

Effect of the injection of yohimbine or prazosin before clonidine into the MSA on renal sodium excretion after the administration of ANGII into the 3rdV

Injection of 0.15 M NaCl into the MSA/3rdV induced a urinary sodium excretion of 129 ± 27 µEq/120 min. Intracerebroventricular ANGII reduced sodium excretion to 23 ± 7.0 µEq/120 min. Clonidine injected into the MSA increased sodium excretion to 246 ± 14 µEq/120 min. Treatment with clonidine into the MSA reduced the inhibitory effect of ANGII on sodium excretion (179 ± 27 µEq/120 min). Sodium excretion after yohimbine administration into the MSA was 123 ± 11 µEq/120 min. Treatment with yohimbine attenuated the inhibitory effect of ANGII on renal sodium excretion (103 ± 16 µEq/120 min). Sodium excretion after prazosin injection into the MSA was 125 ± 12 µEq/120 min. Prazosin produced no significant change in the inhibitory effect of ANGII on sodium excretion (14 ± 2 µEq/120 min). Treatment with yohimbine before clonidine and ANGII attenuated the decrease in sodium excretion induced by ANGII, but with less intensity compared to clonidine injected before ANGII, with values of 59 ± 8 µEq/120 min. Treatment with prazosin before clonidine and ANGII produced no significant change in sodium excretion (33 ± 8 µEq/120 min) (Figure 3).

Effect of the injection of yohimbine or prazosin before clonidine into the MSA on renal potassium excretion after the application of ANGII into the 3rdV

Potassium excretion after injection of 0.15 M NaCl into the MSA/3rdV was 101 ± 12 µEq/120 min. ANGII injected into the 3rdV caused a reduction in renal potassium excretion to 27 ± 3 µEq/120 min. Treatment with clonidine into the MSA increased potassium excretion (191 ± 15 µEq/120 min). Intraseptal clonidine reduced *icv* ANGII-inhibited potassium excretion (117 ± 5 µEq/120 min). Potassium excretion after yohim-

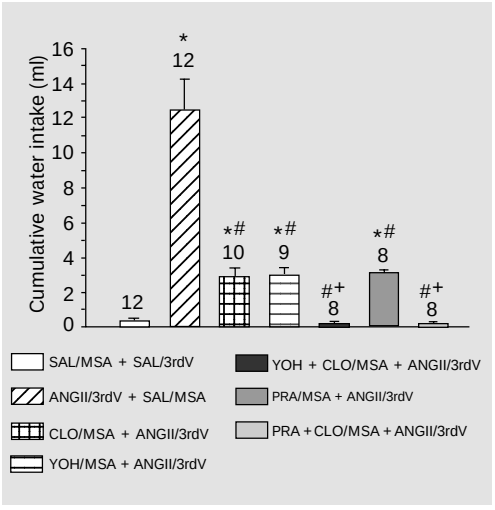

Figure 2. Cumulative water intake induced by intracerebroventricular injection of angiotensin II (ANGII, 12 nmol/µl) in rats treated with intraseptal injection of clonidine (CLO, 20 nmol/µl), yohimbine (YOH, 80 nmol/µl) or prazosin (PRA, 80 nmol/µl). The number of animals is indicated at the top of each column. The results are reported as means ± SEM. *P<0.05 compared to SAL/MSA + SAL/3rdV. #P<0.05 compared to SAL/MSA + ANGII/3rdV. +P<0.05 compared to CLO/MSA + ANGII/3rdV (Newman-Keuls *post hoc* test). SAL, saline; MSA, medial septal area; 3rdV, third ventricle.

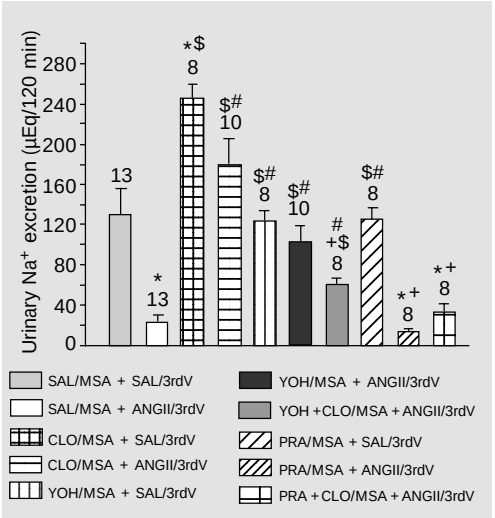

Figure 3. Urinary sodium excretion induced by intracerebroventricular injection of angiotensin II (ANGII, 12 nmol/µl) into the third ventricle (3rdV) of rats treated with intraseptal clonidine (CLO, 20 nmol/µl), yohimbine (YOH, 80 nmol/µl) or prazosin (PRA, 80 nmol/µl). The results are reported as means ± SEM. The number of animals is indicated at the top of each column. *P<0.05 compared to SAL/MSA + SAL/3rdV. #P<0.05 compared to CLO/MSA + SAL/3rdV. $P<0.05 compared to SAL/MSA + ANGII/3rdV. +P<0.05 compared to CLO/MSA + ANGII/3rdV (Newman-Keuls *post hoc* test). SAL, saline; MSA, medial septal area.

bine injection into the MSA was 106 ± 16 μEq/120 min. The decrease in renal potassium excretion after ANGII injection into the 3rdV was attenuated by yohimbine, with values of 91 ± 11 μEq/120 min. Yohimbine injected before clonidine and ANGII attenuated potassium excretion (52 ± 11 μEq/120 min). Potassium excretion after treatment with prazosin was 32 ± 7 μEq/120 min. Prazosin produced no change in the inhibitory effect of ANGII on renal potassium excretion (24 ± 6 μEq/120 min). Potassium excretion after treatment with prazosin injected before clonidine and ANGII was 45 ± 6 μEq/120 min (Figure 4).

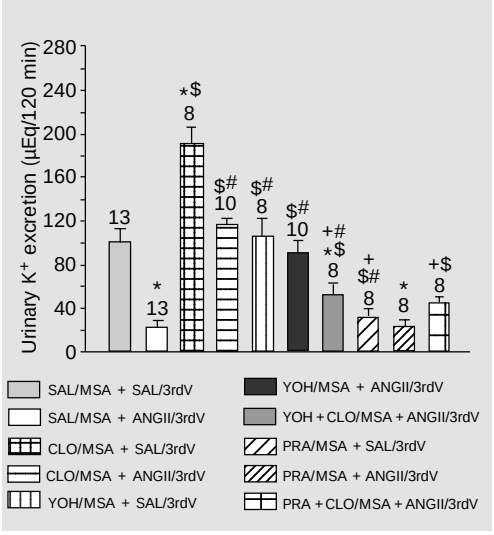

Figure 4. Urinary potassium excretion induced by intracerebroventricular injection of angiotensin II (ANGII, 12 nmol/μl) into the third ventricle (3rdV) of rats treated with intraseptal clonidine (CLO, 20 nmol/μl), yohimbine (YOH, 80 nmol/μl) or prazosin (PRA, 80 nmol/μl). The number of animals is indicated at the top of each column. The results are reported as means ± SEM. *P<0.05 compared to SAL/MSA + SAL/3rdV. #P<0.05 compared to CLO/MSA + SAL/3rdV. $P<0.05 compared to SAL/MSA + ANGII/3rdV. +P<0.05 compared to CLO/MSA + ANGII/3rdV (Newman-Keuls *post hoc* test). SAL, saline; MSA, medial septal area.

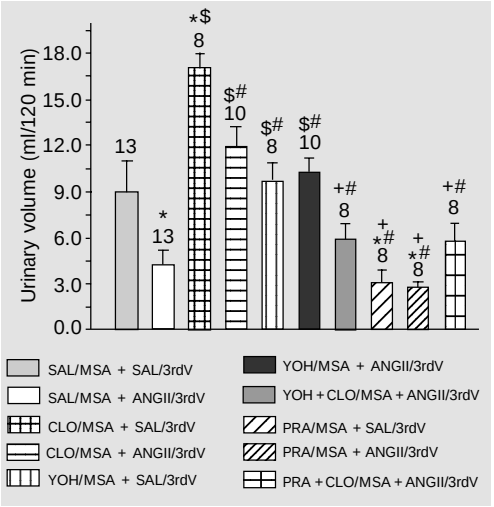

Figure 5. Urinary volume excretion induced by intracerebroventricular injection of angiotensin II (ANGII, 12 nmol/μl) into the third ventricle (3rdV) of rats treated with intraseptal clonidine (CLO, 20 nmol/μl), yohimbine (YOH, 80 nmol/μl) or prazosin (PRA, 80 nmol/μl). The results are reported as means ± SEM. The number of animals is indicated at the top of each column. *P<0.05 compared to SAL/MSA + SAL/3rdV. #P<0.05 compared to CLO/MSA + SAL/3rdV. $P<0.05 compared to SAL/MSA + ANGII/3rdV. +P<0.05 compared to CLO/MSA + ANGII/3rdV (Newman-Keuls *post hoc* test). SAL, saline; MSA, medial septal area.

Effect of the injection of yohimbine or prazosin before clonidine into the MSA on urine volume after injection of ANGII into the 3rdV

Urine volume after injection of 0.15 M NaCl into the MSA/3rdV was 9.0 ± 2.0 ml/120 min. ANGII injected into the 3rdV caused a reduction of urine volume to 4.3 ± 0.9 ml/120 min. Treatment with clonidine into the MSA increased urine volume to 17.1 ± 0.9 ml/120 min. Clonidine abolished the inhibitory effect of ANGII on urine volume (11.9 ± 1.3 ml/120 min). Urine volume after intraseptal treatment with yohimbine was 9.7 ± 1.2 ml/120 min. Yohimbine attenuated the reduction of urine volume caused by ANGII (10.3 ± 0.9 ml/120 min). Yohimbine injected before clonidine and ANGII abolished the effect of clonidine on urine volume (5.9 ± 1.0 ml/120 min). The urine volume after intraseptal prazosin was 3.1 ± 0.8 ml/120 min. Prazosin produced no change in the urine volume caused by ANGII (2.8 ± 0.3 ml/120 min). Prazosin injected before clonidine and ANGII abolished the effect of clonidine on urine volume (5.8 ± 1.1 ml/120 min) (Figure 5).

Discussion

The present results show that the α_2-adrenoceptor agonist clonidine injected into the MSA attenuated the dipsogenic response produced by ANGII injection into the 3rdV. Prior injection of the α_1- and α_2-adrenergic receptor antagonists, prazosin and yohimbine, into the MSA reduced the *icv* ANGII-induced water intake. However, it has been shown that central treatment with an adrenergic agent also increases the water intake induced by ANGII in rats (5). Prazosin and phentolamine injected into the rostral hypothalamus attenuate the drinking response induced by *icv* ANGII injection (6). Thus, the

inhibitory and facilitatory effects of noradrenaline injected into the central nervous system on ingestive behavior suggest a dual role for noradrenaline in the central control of water and salt intake induced by ANGII (27). The present results also show that ANGII injection into the 3rdV decreased sodium, potassium and water excretion in water-loaded rats. Injection of clonidine into the MSA abolished the antinatriuresis, antikaliuresis and antidiuresis induced by ANGII injected into the 3rdV. The decrease in sodium, potassium and water excretion induced by ANGII injection into the 3rdV in water-loaded rats (with volume expansion) was abolished by pretreatment with yohimbine injected into the MSA. Although several studies have shown the natriuretic effect of ANGII, the present results showed the antinatriuretic effect of ANGII injected into the 3rdV. Intracerebroventricular injection of ANGII produced a decrease in sodium excretion. The different effects of ANGII on natriuresis suggest that central control of natriuresis may involve more than one mechanism depending on the excitatory stimuli that activate different areas of the central nervous system. Urine volume was also reduced by *icv* administration of ANGII (28). Other studies have demonstrated several interactions between adrenergic, cholinergic and angiotensinergic pathways in the central nervous system (3). Adrenergic stimulation of the MSA influences sodium and potassium excretion (29). Natriuresis resulting from α_2-adrenoceptor activation has been demonstrated by the application of noradrenaline, clonidine, but not phenylephrine (30). The same occurs with injection of adrenergic agonists or antagonists into the MSA on the renal excretory function response to *icv* ANGII. Taken together, these results show that the central injection of an α-adrenergic agonist or antagonist can disrupt the renal effect of ANGII in rats and suggest that the adrenergic pathways of the MSA can produce a dual effect on electrolyte excretion. Clonidine induced an increase in sodium, potassium and water excretion which was reduced by treatment with yohimbine and prazosin by blocking the α_2-adrenoceptor subtype. The release of norepinephrine may be an excitatory step along the ANGII-induced sodium, potassium and water excretion pathway and when an adrenergic antagonist is used it can block these responses. In spite of this excitatory effect, the adrenergic pathways could also be involved in an inhibitory system for electrolyte and water excretion. The participation of the lateral hypothalamic area in these mechanisms of water intake was also demonstrated (21). The autoradiographic localization of [3]-clonidine binding to non-adrenergic sites was similar to, but distinct from, α_2-adrenergic receptors in human brain (31). Prazosin is effective in blocking the natriuretic and kaliuretic response to the α_1-adrenoceptor agonist phenylephrine (32). The present study shows that injection of prazosin into the MSA did not modify the inhibitory responses induced by ANGII injected into the 3rdV on sodium, potassium and water excretion. Clonidine induced natriuresis, kaliuresis and diuresis when injected into the MSA. Evidence has shown that clonidine, when centrally injected alone, produces diuresis, kaliuresis and natriuresis (33-35). The diuresis produced by the central administration of clonidine has been attributed to the inhibition of vasopressin release (35) and natriuresis has been attributed to renal sympathoinhibition (34,36). The present results suggest that the facilitatory effect of clonidine on natriuresis and kaliuresis is mediated by the activation of α_1-adrenoceptors and the inhibitory effects are mediated by α_{2A}-adrenoceptors (37). Circumventricular structures present excitatory and inhibitory mechanisms responsible for regulating the renal sodium, potassium and water excretion (29). The adrenergic neurotransmission in the median preoptic nucleus may actively participate in ANGII-induced dipsogenesis, di-

uresis, kaliuresis, and pressor responses in a process that involves α_1- and α_2-adrenoceptors (38). Thus, the α_2-receptors of the MSA play an inhibitory role and the α_1-receptors play an excitatory role in sodium, potassium and water excretion. Another important fact is that the effect of α_1- and α_2-adrenoceptors on ANGII, which affects water and electrolyte regulation, is due to the release or inhibition of vasopressin or atrial natriuretic factor. The administration of clonidine has been reported to increase the circulating levels of atrial natriuretic factor (29). Vasopressin or atrial natriuretic factor has some influence on sodium, potassium and water.

The participation of imidazoline receptors in the effect of α_2-adrenoceptors has been postulated. It has been demonstrated that rilmenidine, an imidazoline agonist, when injected into the paraventricular nucleus of the hypothalamus, decreases the hypertensive effect of ANGII injected into the 3rdV (39). It has also been observed that rilmenidine injection into the paraventricular nucleus of the hypothalamus, prior to ANGII injection into the subfornical organ blocks the dipsogenic effect of ANGII (33). These observations support the notion that yohimbine reversed the effect of ANGII when injected alone by blocking the imidazoline receptors. ANGII may produce antinatriuresis, antikaliuresis and antidiuresis by acting on imidazoline receptors. This response was confirmed by yohimbine blocking the clonidine effect. Yohimbine injected with clonidine failed to fully reverse the effect of ANGII, probably due to the action of clonidine on the imidazoline receptors. These findings agree with the results of other studies reporting that imidazoline drugs such as clonidine, rilmenidine and the catecholamine α_2-adrenoceptor agonist α-methylnorepinephrine have distinct mechanisms of action (40).

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