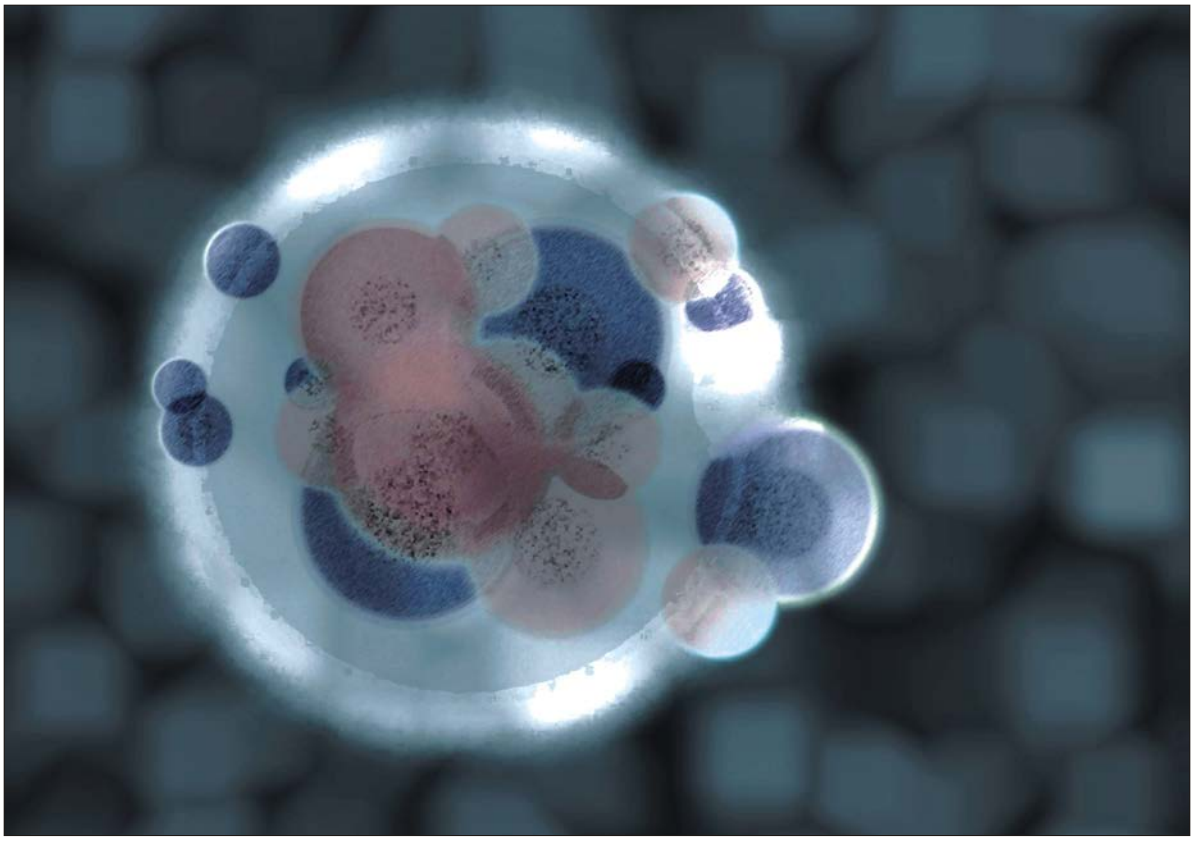

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Effects of Nitric Oxide and Arginine Vasopressin on Sodium Intake Induced by Central Angiotensin II. Part 2

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Abstract: We study the effects of angiotensin receptors antagonists, arginine vasopressin receptor antagonist, L-arginine and L-NAME, injected into supraoptic nucleus of the hypothalamus (SON) on sodium intake induced by the injection of angiotensin II (ANGII). Holtzman rats weighing 200-250 g with canulae implanted into the SON were used. The drugs were injected in 0.5 μL over 30-60 sec. Sodium intake after injection of saline SAL+SAL 0.15 M NaCl was 0.10±00.1 mL 2 h^{-1}; SAL+ANGII injected into SON increased sodium intake. Losartan injected prior to ANGII into SON decreased sodium intake induced by ANGII. PD123319 injected prior to ANGII produced no changes in sodium intake induced by ANGII. AVPA receptor V_1 antagonist injected prior to ANGII reduced sodium intake with a less intensity than losartan. L-arginine injected prior to ANGII decreases sodium intake at a same intensity than losartan. L-NAME injected prior to ANGII potentiated sodium intake induced by ANGII. Losartan injected simultaneously with L-arginine prior to ANGII blocked the natriorexigenic effect of ANGII. These results confirm the importance of SON in the control of sodium intake. Also suggest that both AT_1 and arginine vasopressin V_1 receptors interact with nitrergic pathways within the SON influencing the sodium metabolism by changing sodium appetite induced by ANGII.

Key words: Angiotensin antagonists, receptors, vasopressin antagonist receptor, nitric oxide, sodium metabolism, supraoptic nucleus

INTRODUCTION

Cardiovascular and hydroelectrolytic balance was influenced by vasopressin and nitric oxide in areas of the central nervous system mainly in the circumventricular structures (Saad *et al*., 2006a). Nitric oxide influences the angiotensin effect in many physiological mechanisms such as salivary secretion (Saad *et al*., 2002).

Application of angiotensin antagonist DuP753 blocks the ANGII-induced depolarization in the SON neurons. In contrast, application of the type AT_2 antagonist PD123177 was ineffective in blocking this response (Yang *et al*., 1982). The systemic application of ibersartan and losartan abolished the ANGII central physiologic responses (water intake, sodium intake and increase arterial pressure (Camargo and Saad, 1999).

NO synthase inhibitors such as L-NAME have been used widely to determine the role of endogenous NO. L-NAME reduced urinary sodium excretion (Mousseau *et al*., 1996). It has been demonstrated that NO may facilitate the release of excitatory transmitters, possibly through a presynaptic cyclic GMP-dependent mechanism (Wu *et al*., 1997). The influence of NO on angiotensin effects has been demonstrated (Saad *et al*., 2002). L-NAME increases blood pressure that is due to an increase in salt sensibility (Hodge *et al*., 2002). Endothelial and neuronal nitric oxide synthase inhibitors influence angiotensin II pressor effect in central nervous system (Saad *et al*., 2006b).

The role of renin-angiotensin system in the control of arterial blood pressure and salt appetite in rats has been demonstrated (Thunhorst and Johnson, 1994). The participation of SON in the regulation of water and sodium balance has been demonstrated (Antunes *et al*., 1998).

Since the SON are involved in the control of hydromineal and cardiovascular balance we investigated

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whether the natriorhexigenic effect, induced by ANGII injection into the SON could be mediated by angiotensin, vasopressin receptors and nitric oxide within the SON.

MATERIALS AND METHODS

Subjects: Holtzman rats weighing 200-250 g with canulae implanted unilaterally into SON were used. The animals were housed in individual metabolic cages. Food (Purina Rat Chow) and tap water is available, ad libitum, for the duration of the experiments. The room temperature was maintained at 22±2°C. The light cycle was held at 12:12 with lights on 06:00 h. All experiments were conducted during the light period, between 09:00 am and 03:00 pm. If it started 09:00 am it ended 11:00 pm.

Surgical procedures

Cerebral cannula: The animal were anesthetized with ketamine (80 mg kg^{-1} of body weight) plus xylazine (7 mg kg^{-1} of body weight) intraperitoneally (ip) and implanted with 10 and 12 mm long and 0.7 mm OD stainless steel cannulae into the SON, according to the coordinates of the Paxinos and Watson (1986) rat brain atlas. The coordinates were 1.4-1.8 mm caudal to bregma, 2.3 mm lateral to middle line and 9.2 mm bellow the duramater. The cannulae were fixed to the skull with the aid of jeweler screws and dental acrylic resin and protected with a stylet. Rats recovered from surgery for a minimum of 5 days beginning of testing.

Central drugs injections: Injections into SON were made using 10 μL Hamilton syringes connected by polyethylene tubing (PE-10) to 30-gauge injection cannulas. At the time of testing, the obturator was removed and the injection cannula introduced into the chronically implanted guide cannula. The injection cannula was 0.5 mm longer than the guide cannula. The injection volume was 0.5 μL delivered over 20 to 30 sec. After injection, the styles were replaced and the rats were placed back into the cage.

Drugs: ANGII purchased from Sigma (Chemical Co., St. Louis, MO) and dissolved in saline (0.15 M NaCl) at 10 nmol/0.5 μL.

PD123319 and losartan purchased from DuPont, Merck, Wilmington, DE USA and dissolved in saline (0.15 M NaCl), at 80 nmol/0.5 μL.

d(CH_2)$_5$-Tyr(Me)-AVP (AVPA) purchased from Bachem, Inc., Torrance, Ca, USA and dissolved in saline (0.15 M NaCl), at 80 nmol/0.5 μL.

L-arginine purchased from Sigma (Chemical Co., St. Louis, MO) dissolved in saline (0.15 M NaCl) at 20 μg/0.5 μL.

N^G-nitro-L-arginine methyl ester (L-NAME) purchased from Sigma (Chemical Co., St. Louis, MO), dissolved in saline (0.15 M NaCl) at 40 μg/0.5 μL.

Sodium intake: ANGII (10 pmol/0.5 μL) or vehicle was injected into the SON, water and was offered. Each antagonist was also injected into the SON at the dose of 80 nmol/0.5 μL, 15 min before sodium was offered. The antagonists were injected into the SON 10 min before ANGII was injected into the SON. L-arginine (20 μg/0.5 μL) nitric oxide donor and L-NAME (40 μg/0.5 μL) nitric oxide inhibitor were injected into SON 10 min prior to ANGII injection into the SON. Sodium intake was recorded each 30 min over a 2 h period using individual metabolic cages.

Histology: At the end of the experiments, the rats were anesthetized with ether and given at 0.5 μL injection of fast green dye via the intracranial cannula, followed by perfusion with saline and buffered formalin. The brains were removed, fixed in 10% formalin, frozen to -25°C and cut into 20-30 μm coronal sections and cut into 20-30 μm coronal sections and stained with hemathoxilin-eosin. Only animals in which the injection was placed in the lateral medial and caudal portion of the SON were use in this study (Fig. 1).

Data analysis: Results are reported as means±standard error of the mean (SEM) for the indicated experiments. Statistical analysis was subjected ANOVA followed by the Newman-Keels post-hoc test. Differences were considered significant at $p<0.05$.

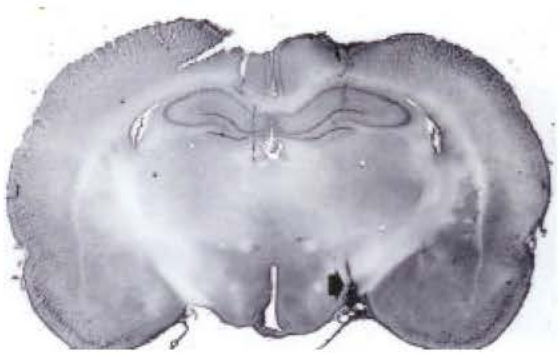

Fig. 1: Photomicrography of a hematoxylin-stained transverse section showing the site of injection into the SON (Arrow). CP cannulae pathways. The cannulae reach the lateral medial and caudal portion of the SON

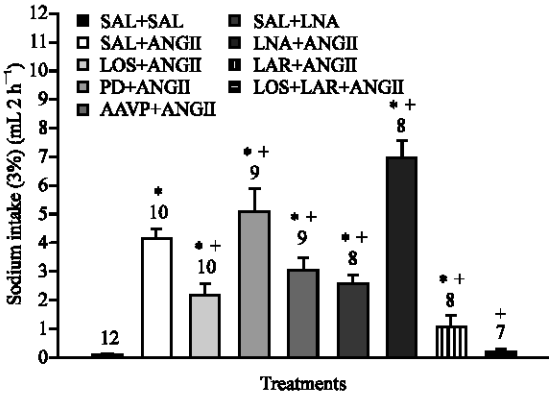

Fig. 2: Effect of pretreatment with losartan (LOS), PD123319 (PD), d(CH_2)$_5$-Tyr(Me)-AVP (Antagonist of AVP) (AVPA), L-arginine (LAR, L-NAME (LNA) and Losartan+L-arginine or vehicle (saline) into the SON on sodium intake evoked by injection of ANGII into the SON. The number of animals is represented at the top of each column. Data are reported as mean±SEM. *p<0.05 compared to the saline group; $^{+}$p<0.05 compared to the ANGII group (Newman-Keuls post-hoc test)

RESULTS

Sodium intake: Sodium intake after injection of 0.15 M NaCl into SON was 0.1±0.01 mL 2 h^{-1}. ANGII injected into SON increased sodium intake (4.2±0.3 mL 2 h^{-1}) (p<0.05). Losartan injected into SON prior to ANGII injection into the SON decreased the sodium intake (2.2±0.4 mL 2 h^{-1}) (p<0.05). AVPA (arginine vasopressin V_1 receptor antagonist) injected into the SON decreased the sodium ingestion induced by ANGII injection into the LSA (3.1±0.4 mL 2 h^{-1}) (p<0.05). Sodium intake induced by injection of ANGII into SON was reduced by previous injection of L-arginine into the SON (1.1±0.4 mL 2 h^{-1}) (p<0.05). L-NAME alone injected in this site produce an increased in 3% NaCl (2.6±0.3 mL 2 h^{-1}) (p<0.05). The natriorexigenic effect of ANGII was potentiated by previous injection of L-NAME into SON (7.0±0.60 mL 2 h^{-1}) (p<0.05). PD 123319 produced no effect in sodium intake induced by ANGII (5.1±0.8 mL 2 h^{-1}). Losartan in association with L-arginine abolished the sodium ingestion induced by ANGII (0.2±0.1 mL 2 h^{-1}) (p<0.05) Fig. 2.

DISCUSSION

Recently we demonstrated the interaction between nitrergic and vasopressinergic pathways of the SON on water intake induce by central application of ANGII. The relationship between nitric oxide and vasopressin in the regulation of body fluid also has been demonstrated (Mornagui *et al*., 2006).

These results demonstrate that injection of AT_1 antagonist losartan into the SON reduced sodium intake, induced by angiotensinergic activation. PD123319, a selective antagonist of AT_2 receptors, had no effect on sodium intake. McKinley *et al*. (2003) showed that angiotensin AT_1 and AT_2 receptors are also plentiful in the brain.

Previous injection of d (CH2)5-Tyr (Me)-AVP, an arginine vasopressin V_1 receptor antagonist, into the SON decreased the ANGII-induced increased sodium intake. These results indicated that NaCl 3% intake induced by ANGII involves the V_1 vasopressin receptors.

Studies utilizing autoradiography, selective to ANGII-receptor antagonists revealed that the SON of the rat contains AT_1 receptors (Tsutsumi and Saavedra, 1991). Furthermore it has been reported that salt appetite can be triggered by iontophoretically applied ANGII into the anterior median septum (Mousseau *et al*., 1996). An endogenous origin for ANGII is suggested by various reports of angiotensin-like immunoreactivity in the magnocellular neurons of the SON (Renaud and Bourque, 1991). Possible ANGII is released locally from axon collaterals or somato-dendritic sites as proposed for vasopressin (Richard *et al*., 1991).

Present studies also demonstrate that L-NAME increased sodium intake induced by ANGII stimuli. These results clearly demonstrated that ANGII implicated NO to produce the natriorexigenic effect and that the SON is an important area for this behavior. It has been demonstrated that NO attenuated the ANGII-induced sodium intake. Vasoconstriction and ANGII can evoke losartan-resistant tubular Na^+ reabsorption, but the tubular action are concealed by NO (Suo *et al*., 2002; Zhou *et al*., 2002). The chloroquine stimulates nitric-oxide synthase both centrally, stimulating vasopressin secretion and within the kidney, were it modulates glomerular hemodinamics and tubular function (Ahmed *et al*., 2003). Other studies demonstrated that alpha-1 and beta-adrenoceptors of the lateral hypothalamus are possibly involved with central mechanism dependent on ANGII and SFO that control water and sodium intake (Camargo *et al*., 2000).

Also these results show that ANGII-induced sodium appetite was dependent on AT_1 receptors. NO also play a role in this response. Treatment with AT_1 ANGII receptor blocker, losartan, reversed the increase in sodium intake. Treatment with L-NAME induces an increase in sodium intake. The action of L-NAME may be due a local

vasoconstriction. Further, the salt-sensitive component appears to be ANGII-dependent, as it was associated with increasing plasma ANGII levels and could be reversed by treatment with an ANGII receptor antagonist (Hodge *et al*., 2002).

Dehydration produced in the supra optic nucleus a significantly increase in c-Fos staining with a great percentage of the Fos cells and increase in vasopressin. Changes in Fos staining were also observed in the NTS, RVL, parabrachial nucleus and PVN. Rehydration with water or saline produces differential effects on plasma AVP, Fos staining and sodium concentration (Gottlieb *et al*., 2006).

Whereas the AT_1 receptors of the SON mediate NaCl ingestion induced by angiotensinergic activation of the SON, the arginine vasopressin V_1 AVPergic neurons inhibit sodium ingestion. The AT_2 receptor antagonist receptor produced no effect on sodium intake. These results clearly demonstrated that ANGII implicated NO mechanism to induce sodium intake.

SON is an important structure of the central nervous system that regulates the natriorexigenic response induced by activation of the renin-angiotensin system of the SON. NO contributes to the fine regulation of vasopressin synthesis and release (Vacher *et al*., 2003). Therefore these investigations confirm the importance of SON and AT_1, AT_2, V_1 receptors and NO in the control of sodium intake. These results were strongly supported by previous studies of our laboratory demonstrating that SON participated in cardiovascular and hydromineral regulation (Saad *et al*., 2004, 2006c).

CONCLUSION

Angiotensinergic neural pathways of SON implicated vasopressinergic and nitrergic mechanism in the control of sodium intake, important mechanism for the sodium metabolism.

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