Journal of
Biological Sciences

Influence of Arginine Vasopressin Receptor and Nitric Oxide on the Water, Sodium Intake and Arterial Blood Pressure Induce by Angiotensin Injected into Third Ventricle of the Brain

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Abstract: As several structures of the central nervous system are involved in the control of hydromineral and cardiovascular balance we investigated whether the natriorhexigenic and pressor response induced by the injection of ANG II into the 3rd V could be mediated by vasopressinergic and nitrergic system. Male Holtzman rats weighing 200-250 g with cannulae implanted into the 3rd V were used. The drugs were injected in 0.5 µL over 30-60 sec. Controls were injected with a similar volume of 0.15 M NaCl. ANGII increased the water intake vs control. AVPA injected into 3rd V prior to ANGII decreased the dipsogenic effect of ANGII. L-arginine also decreased the water intake induced by ANGII. AVPA plus L-arginine inhibit the water intake induced by ANGII. 7NIT injected prior to ANGII potentiated the dipsogenic effect of ANGII. Pre-treatment with ANGII increased the sodium ingestion vs control. AVPA decreased the ANGII effect in sodium intake. L-arginine also decreased the natriorhexigenic effect of ANGII. The combination of L-arginine and AVPA inhibit the sodium intake induced by ANGII. 7NIT injected prior to ANGII potentiated the sodium intake induced by ANGII. ANGII induced an increase in Mean Arterial Pressure (MAP) vs control. AVPA and L-arginine induced a decreased in the pressor effect of ANGII. The combination of L-arginine and AVPA inhibit the pressor effect of ANGII. 7NIT injected prior to ANGII into 3rd V potentiated the pressor effect of ANGII. These data suggest that arginine vasopressin V_1 receptors and Nitric Oxide (NO) within the circumventricular structures may be involved in sodium intake and pressor response induced by the activation of ANGII receptors within the circumventricular neurons. These studies revealed the involvement of sodium appetite by utilizing the angiotensinergic, vasopressinergic and nitrergic system in the central regulation of blood pressure.

Key words: ANGII AVP V_1 receptors, NO, CNS, sodium appetite an blood pressure

INTRODUCTION

It is well established that the brain angiotensinergic, vasopressinergic and nitrergic system are involved in regulation of blood pressure[1]. Alterations in function of these systems play important role in determining the neurogenic component of hypertension[2]. It has recently reported that an inhibition of the brain-renin-angiotensin system by an intraventricular antisense oligodeoxynucleotides to angiotensinogen mRNA or to AT1aR mRNA significantly reduces blood pressure in rats with two kidney, 1 clip renovascular hypertension[3]. Electrical stimulation of the basal forebrain causes the release of Arginine-vasopressin (AVP)[4]. N^w-nitro-L-arginine methyl ester (L-NAME) reduces renal blood flow, urine flow rate and urinary sodium excretion[5]. Several studies have shown that NO may function as a neurotransmitter or a neuromodulator. Recognition of the role of nitric oxide in cell-to-cell communication has changed the concept of traditional neurotransmission. It has been demonstrated that NO may facilitate the release of excitatory transmitters, possibly through a presynaptic cyclic GMP-dependent mechanism[6]. The influence of NO on angiotensin effects has been demonstrated[7]. Treatment with L-NAME increases blood pressure that is at least in part salt sensitive[8]. NO is involved in the regulation of drinking behavior induced by central administration of ANGII and cellular

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dehydration and NO of the Subfornical Organ (SFO), a circumventricular structures, play an important role in this regulation[9]. As several structures of the central nervous system are involved in the control of hydromineral and cardiovascular balance it was investigated whether the natriorhexigenic and pressor response induced by the injection of ANG II into the 3rd V could be mediated by vasopressinergic and nitrergic system.

MATERIALS AND METHODS

Animals: The animals were housed in individual metabolic cages. All experiments were conducted during the light period, between 09:00 am and 03:00 pm. Food (Purina Rat Chow) and tap water is available ad libitum, for the duration of the experiments. The temperature was maintained at $22\pm2°C$. The light cycle was held at 12:12 with lights on 06:00 h. Male Holtzman rats weighing 250-300 g were anesthetized with tribromoethanol (20 mg/100 g body weight), intraperitoneally (ip) and implanted with 10 and 12 mm long and 0.7 mm OD stainless steel cannulae into the 3rd V according to the coordinates of the Paxinos and Watson[10] rat brain atlas. The cannulae were fixed to the skull with the aid of jeweler screws and dental acrylic resin and protected with a stiletto. Rats recovered from surgery for a minimum of 5 days beginning of testing. After the animals recovery from brain surgery (5 days) PE-10 polyethylene tubing connected to PE-50 tubing was inserted into the abdominal aorta through the femoral artery under 2,2,2-tribromoethanol anesthesia (20 mg/100 g body weight). The polyethylene tube was tunneled subcutaneously to the back of the rat and externalized at the dorsal cervical region. Catheters were filled with heparinized saline and plugged with 23-G obturators. Rats recovered from surgery (vascular catheter) for a minimum of 24 h before beginning of testing. ANG II purchased from Sigma (Chemical Co., St. Louis, MO) and dissolved in saline (0.15 M NaCl) at 10 nM/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 nM/0.5 μL. L-arginine and 7-nitroindazol purchased from Sigma (Chemical Co., St. Louis, MO), dissolved in saline (0.15 M NaCl) at 40 and 80 μg/0.5 μL, respectively. ANG II (10 pM/0.5 μL) or vehicle was injected into the 3rd V, 3% NaCl was offered. AVPA V$_1$ antagonist was also injected into 3rd V 15 min before ANGII. L-arginine nitric oxide donor and 7-nitroindazol nitric oxide inhibitor were injected into 3rd V 10 min prior to ANGII injection into the 3rd V. Water and sodium intake was recorded each 15 min over a 1 h period using

individual metabolic cages. Direct Mean Arterial Blood Pressure (MAP) was recorded in unanesthetized and unrestrained rats. The animal was removed from the home cage and placed in a test cage, without access to food or water. The previously implanted catheter was connected to a Statham (P23 Db) pressure transducer (Statham-Gould, Valley View, OH) coupled to a multi channel recorded (Dataq multirecord USA). This program permits the acquisition of cardiovascular data by computer.

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. Only animals in which the injection was placed in the 3rd V were use in this study. Results are reported as means±Standard Error of the Mean for the indicated experiments. Statistical analysis was subjected to two-way Analysis of Variance (ANOVA) followed by the Newman-Keuls post-hoc test. Differences were considered significant at $p<0.05$.

RESULTS

Water intake: ANGII increased the water intake vs control (10.1 ± 0.7 vs 0.3 ± 0.1 mL h^{-1}) (n=9), respectively $p<0.05$. AVPA injected into 3rd V prior to ANGII decreased the dipsogenic effect of ANGII (3.9 ± 0.4 mL h^{-1}) (n=8) $p<0.01$. L-arginine also decreased the water intake induced by ANGII (2.8 ± 0.4 mL h^{-1}) (n=8) $p<0.05$. AVPA plus L-arginine inhibit the water intake induced by ANGII (0.5 ± 0.1 mL h^{-1}) (n=7) $p<0.01$. 7NIT injected prior to ANGII potentiated the dipsogenic effect of ANGII (14.1 ± 0.9 mL h^{-1}) (n=7) $p<0.05$ (Fig. 1).

NaCl 3% intake: Pre-treatment with ANGII increased the sodium ingestion (2.2 ± 0.1 mL h^{-1}) (n=10) vs control (0.2 ± 0.02 mL h^{-1}) (n=8) $p<0.001$. AVPA decreased the ANGII effect (1.3 ± 0.1 mL h^{-1}) (n=8) $p<0.05$. L-arginine also decreased the natriorhexigenic effect of ANGII (1.1 ± 0.01 mL h^{-1}) (n=7) $p<0.05$. The combination of L-arginine and AVPA inhibit the sodium intake induced by ANGII (0.1 ± 0.02 mL h^{-1}) (n=7) $p<0.05$. 7NIT potentiated the sodium intake induced by ANGII (3.8 ± 0.4 mL h^{-1}) (n=7) $p<0.01$ (Fig. 2).

Mean arterial pressure: ANGII induced an increase in MAP (18 ± 1 vs control 3 ± 1 mm Hg) (n=10; n=9), respectively $p<0.01$. AVPA and L-arginine induced a
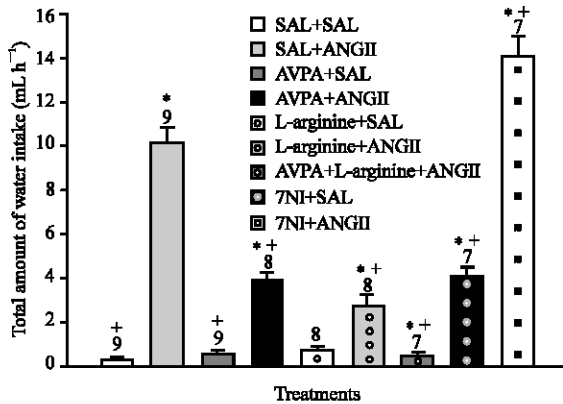
Fig. 1: Effect of pretreatment with d(CH$_2$)$_5$-Tyr(Me)-AVP (Ant.-AVP) (AVPA), L-arginine, 7 nitroindazol (7NIT) or vehicle saline (SAL) into the 3rd V on water intake evoked by injection of angiotensin II (ANGII) into the 3rd V. The number of animals is represented at the top of each column. Data are reported as mean±SEM. *p<0.05 compared to the saline group; $^+$p<0.05 compared to the ANG II group (Newman-Keuls post-hoc test)
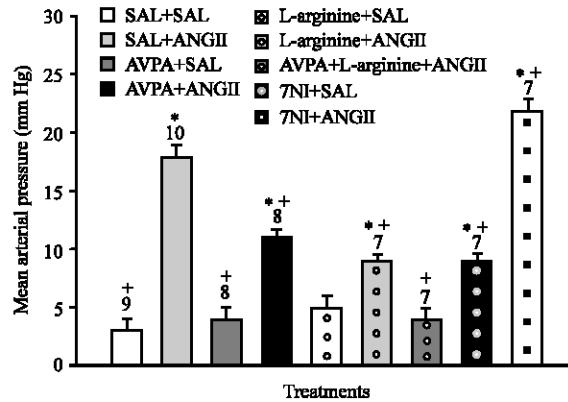

Fig. 2: Effect of pretreatment with d(CH$_2$)$_5$-Tyr(Me)-AVP (Ant.-AVP) (AVPA), L-arginine, 7 nitroindazol (7NIT) or vehicle saline (SAL) into the 3rd V on NaCl 3% intake evoked by injection of angiotensin II (ANGII) into the 3rd V. The number of animals is represented at the top of each column. Data 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)
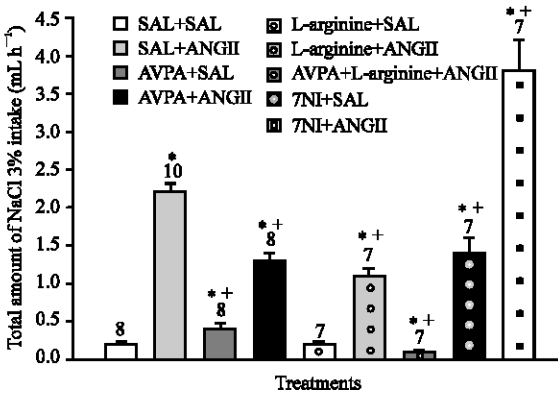
decreased in the pressor effect of ANGII (11±0.7 and 9±0.5 mm Hg) (n=8; n=7), respectively p<0.05. The combination of L-arginine and AVPA inhibit the pressor effect of ANGII (4±0.2 mm Hg) (n=7) p<0.05. 7NIT potentiated the pressor effect of ANGII (22±1 mmHg) (n=7) p<0.05 (Fig. 3).

Fig. 3: Effect of pretreatment with d(CH$_2$)$_5$-Tyr(Me)-AVP (Ant.-AVP) (AVPA), L-arginine, 7 nitroindazol (7NIT) or vehicle saline (SAL) into the 3rd V on the increase in mean arterial pressure evoked by injection of angiotensin II (ANGII) into the 3rd V. 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)

DISCUSSION

The present study demonstrated that the increased in water intake, sodium intake and increase in mean blood pressure induced by ANGII injected into 3rd V are influenced by vasopressinergic and nitrergic system. These data are confirmed by data of Rodrigues *et al.*[11]. In addition, they also show that the previous injection of d (CH$_2$)$_5$-Tyr (Me)-AVP, an arginine vasopressin V$_1$ receptor antagonist, into the 3rd V decreased the ANG II-induced an increased in water and sodium intake and decreased the MAP. These results indicated that the water, sodium intake and blood pressure induced by ANGII, involve the V$_1$ vasopressin receptors. Furthermore it has been reported that salt appetite can be triggered by iontophoretically applied ANGII into the anterior median septum[12]. The vasopressin V$_1$ receptor is also found in the septal area[13] and treatment with the arginine vasopressin V$_1$ receptor antagonist caused a marked decrease in receptor affinity for AVP[14]. It is thus possible that ANG II is released locally from axon collaterals or somato-dendritic sites in a manner similar to that proposed for oxytocin or vasopressin[15]. Acting in the brain vasopressin exerts both pressor and depressor effects[16]. Therefore, one can speculate that up regulation of V1aR mRNA could act to enhance this effect. We also demonstrated that 7NIT increased the sodium intake induced by ANGII stimuli. Intracerebroventricular (icv)

injection of N^G-monomethyl-L-arginine (NMMA), a blocker of nitric oxide synthase (NOS), preferentially increases plasma concentration of oxytocin and vasopressin in rats deprived of water for 24 h[17]. NO also play a role in these ANGII effects, such as increase in sodium intake and arterial pressure. Treatment with 7NIT induces an increase in blood pressure. 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[8]. These data were confirmed by the data of the cardiovascular responses to an acute increase in CSF sodium as well as the chronic pressor responses to high sodium intake in SHR[18]. ANGII influence nitrergic system to induce sodium intake and increase in arterial blood pressure as has been demonstrated in the experiments with L-arginine that decreased the water intake and sodium intake and mean arterial pressure. 7NIT potentiated the effects of ANGII. NO contributes to the fine regulation of vasopressin synthesis and release[19]. Vasopressin has been reported to mediate ANGII-induced pressor response[20]. Also heterogeneous actions of vasopressin on ANG II-sensitive neurons in the subfornical organ of rats have been demonstrated[21]. These investigations demonstrated that the role of the renin-angiotensin system in the control of electrolyte and cardiovascular balances is influenced by V_1 receptors and nitrergic system. We can also suggest that changes in arterial blood pressure induced by various hypertensive states[2], are due to an altered induction of the brain mRNA levels of AT_{1a} and V_{1a} receptors and nitric oxide synthase.

ACKNOWLEDGMENTS

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