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Role of angiotensin II and vasopressin receptors within the supraoptic nucleus in water and sodium intake induced by the injection of angiotensin II into the medial septal area

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Abstract

In this study we investigated the effects of the injection into the supraoptic nucleus (SON) of non-peptide AT1- and AT2-angiotensin II (ANG II) receptor antagonists, DuP753 and PD123319, as well as of the arginine-vasopressin (AVP) receptor antagonist d(CH_2)$_5$-Tyr(Me)-AVP, on water and 3% NaCl intake induced by the injection of ANG II into the medial septal area (MSA). The effects on water or 3% NaCl intake were assessed in 30-h water-deprived or in 20-h water-deprived furosemide-treated adult male rats, respectively. The drugs were injected in 0.5 µl over 30-60 s. Controls were injected with a similar volume of 0.15 M NaCl. Antagonists were injected at doses of 20, 80 and 180 nmol. Water and sodium intake was measured over a 2-h period. Previous administration of the AT1 receptor antagonist DuP753 into the SON decreased water (65%, N = 10, P<0.01) and sodium intake (81%, N = 8, P<0.01) induced by the injection of ANG II (10 nmol) into the MSA. Neither of these responses was significantly changed by injection of the AT2-receptor antagonist PD123319 into the SON. On the other hand, while there was a decrease in water intake (45%, N = 9, P<0.01), ANG II-induced sodium intake was significantly increased (70%, N = 8, P<0.01) following injection of the V1-type vasopressin antagonist d(CH_2)$_5$-Tyr(Me)-AVP into the SON. These results suggest that both AT1 and V1 receptors within the SON may be involved in water and sodium intake induced by the activation of ANG II receptors within the MSA. Furthermore, they do not support the involvement of MSA AT2 receptors in the mediation of these responses.

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Key words
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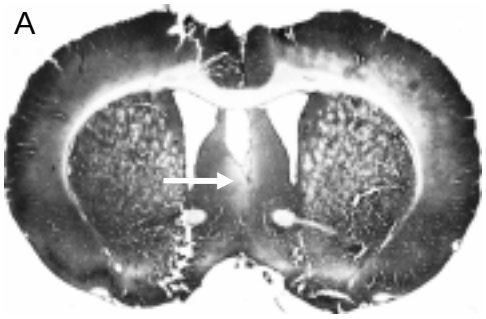
- V1 receptors
- Water intake
- Sodium intake
- Medial septal area
- Supraoptic nucleus
- Rats

Central injection of angiotensin II (ANG II) elicits prompt and pronounced responses such as increased blood pressure, thirst, sodium appetite, and the release of vasopressin (1). Electrical stimulation of the basal forebrain causes the release of arginine-vasopressin (AVP) and a prolonged thirst (2). Furthermore, exogenous application of ANG II increases the firing rate of the magnocellular neurosecretory neurons of the supraoptic nucleus (SON). This effect seems to be produced by the activation of functional ANG II receptors within the SON since it is reversibly blocked by the angiotensin peptide antagonist salarasin (3,4). Application of the nonpeptide type 1 angiotensin antagonist DuP753 blocks the ANG II-induced depolarization in the SON. In contrast, application of the type 2 antagonist PD123177 was ineffective in blocking this response (5). Neurons of the septal nuclei are also sensitive to angiotensin (6). Since the septum is known to send efferents to the SON (7), we investigated whether the dipsogenic and natriorhexigenic effects induced by the injection of ANG II into the medial septal area (MSA) could be mediated by angiotensin and vasopressin receptors within the SON.



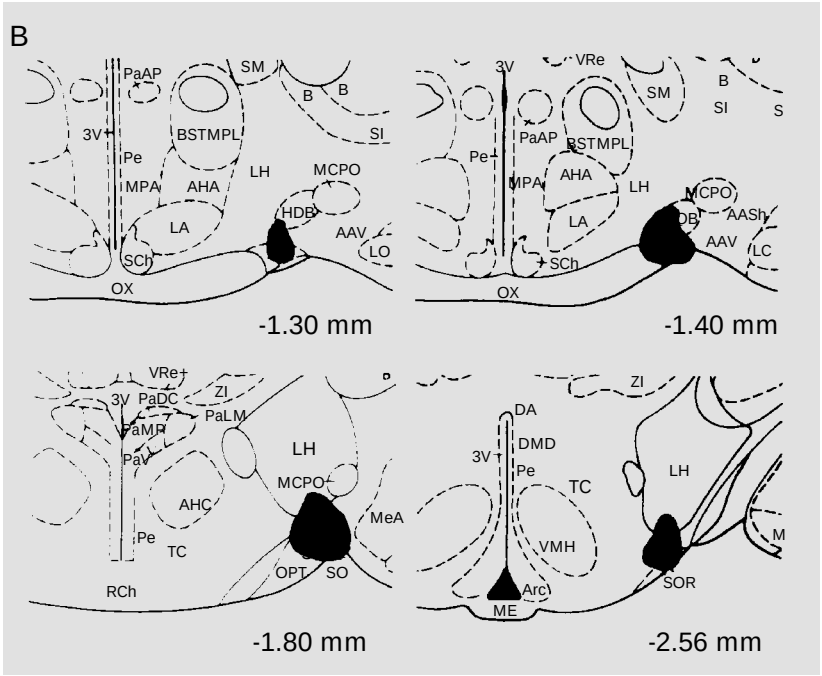


Figure 1 - *A*, Photomicrograph of a hematoxylin-stained transverse section of the rat brain showing the site of injection into the MSA. *B*, Schematic representation of the location of the injection sites in the SON region (black areas). The numbers in the upper right part of each section indicate the rostrocaudal distance of that section from the bregma. LH, Lateral hypothalamic area; OX, optic chiasm; OPT, optic tract; SO, supraoptic nucleus; SOR, retro-chiasmatic supraoptic nucleus.

Male Holtzman rats weighing 250-300 g were anesthetized with tribromoethanol (20 mg/100 g body weight, *ip*) and implanted with 10- and 12-mm long and 0.7-mm OD stainless steel cannulas into the MSA and SON, according to the coordinates of the Paxinos and Watson rat brain atlas (8). The cannulas were fixed to the skull with the aid of jeweler screws and dental acrylic resin and protected with a stiletto. Since water and sodium deprivation increases CNS synthesis of ANG II, further increasing water and sodium intake (9), water and 3% NaCl solution were removed 30 and 20 h before the intracranial injections, respectively. Sodium depletion was further accomplished by the administration of a single dose of furosemide (10 mg, *sc*) to sodium-deprived rats. Moreover, at the time of ANG II injection food pellets were replaced with sodium-deficient ones, and the rat's cage was washed thoroughly to remove enviromental sodium residues. On the subsequent day (20 h after furosemide injection) food was removed and 3% NaCl was restored to the rats. ANG II (10 nmol) or 0.5 ml vehicle was injected into the MSA 20 min before 3% NaCl was offered.

The screening test consisted of an injection of ANG II (10 nmol/0.5 µl) into the MSA followed by the measurement of water intake over 30 min. Each antagonist was also injected alone into the MSA at the dose of

180 nmol/0.5 µl, 20 min before water or 3% NaCl was offered. Water or sodium intake was recorded over a 2-h period using individual metabolic cages. The antagonists were injected into the SON 15 min before ANG II was injected into the MSA. ANG II (Sigma Chemical Co., St. Louis, MO), PD123319 and DuP753 (DuPont, Merck, Wilmington, DE) and $d(CH_2)_5$-Tyr(Me)-AVP (Bachem, Bubendorf, Switzerland) antagonists were injected at doses of 20, 80 and 180 nmol/0.5 µl.

At the end of the experiments, the rats were anesthetized with ether and perfused through the heart with saline followed by 10% formalin. The brains were then removed, stored in 10% formalin for 1 week and cut into 20-30-µm coronal sections, which were stained with hematoxylin-eosin for light microscopy to determine the position of the cannula inside the MSA and SON (Figure 1). Data from rats for which the injection sites were visibly outside the target nuclei were excluded from analysis. Results are reported as means ± SEM. Data were subjected to two-way ANOVA *vs* doses, followed by the Newman-Keuls *post-hoc* test.

The injection sites are shown in Figure 1. Water and 3% NaCl intake following the injection of ANG II into the MSA of controls and rats injected with antagonists are shown in Figure 2. Rats injected with ANG II into the MSA showed a significant increase in water and sodium intake compared with the 0.15 M NaCl-injected group. DuP753 injected into the SON decreased (two-way ANOVA) water and sodium intake induced by ANG II injection in a dose-dependent manner. In contrast, injection of the vasopressin V1 receptor antagonist, $d(CH_2)_5$-Tyr(Me)-AVP, into the SON caused a significant decrease in water intake and increased (two-way ANOVA) the sodium intake induced by ANG II injection into the MSA. Injection of PD123319 produced no effect on water or saline ingestion. The injection of DuP753, PD123319, and $d(CH_2)_5$-Tyr(Me)-AVP alone (180 nmol) into the SON did not alter water (11.5 ± 0.9, 10.3 ± 0.7, and 10.2 ± 0.8 ml/2 h, respectively) or 3% NaCl (6.4 ± 0.9, 4.9 ± 0.8, and 7.3 ± 1.6 ml/2 h, respectively) intake by water-deprived or sodium-depleted rats, respectively.

The present results show that whereas injection of the selective AT1 antagonist DuP753 into the SON reduced water and sodium intake induced by angiotensinergic activation of the MSA, the injection of PD123319, a selective antagonist for AT2 receptors, had no effect on these responses. In addition, they also show that the previous injection of $d(CH_2)_5$-Tyr(Me)-AVP, a V1

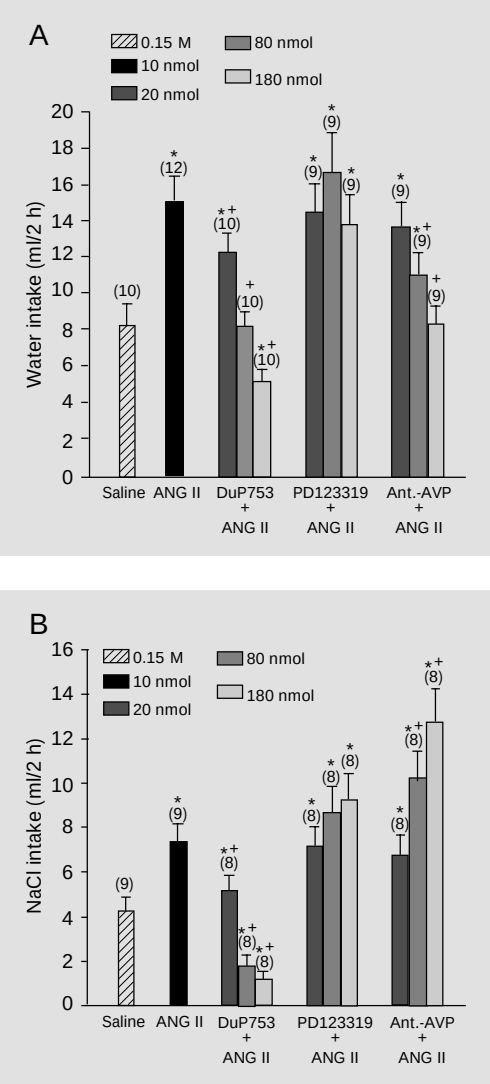


Figure 2 - Effect of pretreatment with DuP753, PD123319, $d(CH_2)_5$-Tyr(Me)-AVP (Ant.-AVP) or vehicle (saline) into the SON on water (A) and sodium (B) intake evoked by injection of ANG II into the MSA. Data are reported as mean ± SEM. *P<0.05 compared to the saline group; +P<0.05 compared to the ANG II group (Neuman-Keuls *post-hoc* test).

receptor antagonist, into the SON inhibited ANG II-induced water intake and increased the sodium intake.

Quantitative autoradiography with selective ANG II-receptor antagonists revealed that the SON of the rat contains AT1 receptors (10). Furthermore, it has been reported that water and salt appetite can be triggered by iontophoretically applied ANG II into the anteromedian septum (11). The vasopressin V1 receptor is also found in the MSA (12,13), and treatment with the V1 receptor antagonist caused a marked decrease in receptor affinity for AVP (14). An endogenous origin for ANG II is suggested by various reports of angiotensin-like immunoreactivity in the magnocellular neurons of the SON (15). 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 (16). The septal influence on the control of AVP and ANG II secretion is presumably mediated by cells projecting from this area to the vasopressin-containing magnocellular neurons of the SON (17).

In summary, the present results suggest that, whereas the AT1 receptors of the SON mediate water and NaCl ingestion induced by angiotensinergic activation of the MSA, the V1 receptors activate water intake but inhibit sodium ingestion.

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