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PRELIMINARY REPORT

Arginine Vasopressin and Oxytocin Responses to Angiotensin II Are Mediated by AT1 Receptor Subtype in Normal Men

P. Chiodera, R. Volpi, A. Caiazza, N. Giuliani, M.G. Magotti, and V. Coiro

This study was performed to determine whether the stimulatory effect of plasma angiotensin II (ANG II) on arginine vasopressin (AVP) and oxytocin (OT) secretion in humans is mediated by AT1 subtype receptors. For this purpose, the effects of the AT1 receptor antagonist losartan (50 mg orally) or a placebo on the AVP and OT responses to ANG II (intravenous infusion for 60 minutes of successively increasing doses of 4, 8, and 16 ng/kg min; each dose for 20 minutes) administration were evaluated in seven normal men. In additional experiments, the same subjects were tested with losartan (50 mg orally) alone or placebo alone. Neither losartan nor placebo given alone modified the basal levels of AVP and OT. ANG II infusion induced significant increments in both serum AVP and OT levels (mean peaks were 1.55 and 1.41 times higher than baseline, respectively). Both hormonal responses to ANG II were completely abolished by pretreatment with losartan. These data provide evidence of AT1 receptor involvement in mediation of the ANG II–stimulating effect on AVP and OT secretion.

CIRCULATING ANGIOTENSIN II (ANG II) is known to play a role in regulation of the homeostasis of blood pressure, cardiovascular function, and fluid and electrolyte balance.[1,2] These effects are exerted through activation of at least two specific receptors (ie, AT1 and AT2 subtypes) in peripheral tissues and in the CNS. Particularly, blood-borne ANG II actions in the CNS include not only a centrally mediated pressor response, but also the stimulation of water drinking and hormonal secretions from both the anterior and posterior pituitary.[3]

In the present study, we focused on the receptor mechanism mediating the plasma ANG II–stimulating action on posterior pituitary hormone secretion. This study was made possible by the recent availability for administration in humans of the specific AT1 receptor antagonist losartan.[4] Therefore, the arginine vasopressin (AVP) and oxytocin (OT) responses to an intravenous infusion of ANG II were tested in normal men either in the presence or in the absence of a concomitant administration of losartan.

MATERIALS AND METHODS

Seven healthy male subjects, aged 29 to 35 years, volunteered for this study. All subjects were within 10% of their ideal body weight (IBW) (body weight, 68.5 ± 6.7; mean body-mass index, 23) and were without clinical and laboratory evidence of hepatic or renal disease. Each man was tested with ANG II alone and on a different occasion with ANG II after treatment with losartan. The two tests were performed in random order, with an interval of at least 1 week. After the end of the study, two additional tests with the administration of losartan or placebo alone were performed in random order at weekly intervals. All tests followed the same procedure. At 8:30 AM on the morning of the test, a 19-gauge cannula was inserted into the left antecubital vein of subjects lying in the recumbent position, after an overnight fast and rest in bed. The cannula served for blood sampling. At the same time, in the ANG II and ANG II plus losartan tests, a double-lumen indwelling catheter was inserted into the right antecubital vein and was used for infusion of ANG II in 5% glucose.

ANG II Test

Two basal blood samples were taken at the time of the insertion of the cannulae (time −30) and 30 minutes later (time 0). After withdrawal of the second basal sample (time 0), subjects received a 60-minute infusion of ANG II (Asp 1, Ile 5 ANG II dissolved in 5% glucose) in successively increasing doses of 4, 8, and 16 ng/kg min; each dose was given for 20 minutes. Further blood samples were collected 20, 40, 60, 90, 120, 150, and 180 minutes after the beginning of the ANG II infusion.

From the Department of Internal Medicine, School of Medicine, University of Parma, Parma, Italy; and the Division of Internal Medicine, Hospital of Fidenza, Fidenza, Italy.
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Address reprint requests to P. Chiodera, MD, Cattedra di Endocrinologia, Università di Parma, via Gramsci 14, 43100 Parma, Italy.

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ANG II Plus Losartan Test

This test was performed as the previously described ANG II test, except for the oral administration of 50 mg losartan (NeoLotan; Neopharmed, Rome, Italy) at time −30. In the ANG II test, a placebo was given instead of losartan.

Losartan Test

This test was performed as the previously described ANG II plus losartan test, except for the infusion of a 5% glucose solution instead of ANG II.

Placebo Test

This test was performed as the previously described losartan test, except for the administration of a placebo instead of losartan.

Assays

All blood specimens were collected in prechilled plastic tubes containing Na_2EDTA (1 mg/mL) and aprotinin (100 KIU/mL). Twenty-five milliliters of blood was drawn at each sampling time. Tubes were collected and promptly centrifuged at 4°C. Plasma was separated and frozen at −20°C until assayed. All samples from each subject were analyzed in duplicate in the same assay. OT and AVP were measured with specific radioimmunoassay methods.[5] The intraassay coefficient of variation was 8% for AVP and 7.6% for OT; the interassay coefficient of variation was 12.8% for AVP and 11.8% for OT. The sensitivity of the RIA was 1.1 pmol/L for AVP and 2.0 pmol/L for OT.

Osmolality, hematocrit, and sodium concentrations were also measured in all specimens. Osmolality was measured with an advanced osmometer (Osmette S; Sedas s.r.l., Milan, Italy); hematocrit was determined with a Drummond microhematocrit (Drummond Scientific, Broomal, CA). Samples were placed into plastic tubes containing K_3EDTA for the measurement of hematocrit and into plastic tubes containing lithium-eparine for the measurement of osmolality. Blood was collected into additional tubes for serum sodium evaluation by flame photometry. During the study, mean blood pressure was monitored with a mercury sphygmomanometer at each sampling time during ANG II or ANG II plus losartan. Statistical analysis was performed with the Wilcoxon's matched-paired rank-sum test and two-way ANOVA for repeated measures followed by specific means comparisons test, as appropriate. Results are reported as mean ± SE.

RESULTS

Figure 1 and Table 1 show the results of the ANG II test and of the ANG II test performed after losartan administration. In the ANG II test, plasma AVP and OT levels increased sharply and in a similar fashion in response to ANG II. The mean peak levels of both hormones were observed at 60 minutes after the beginning of ANG II infusion ($P < .01$ *v* baseline for both AVP and OT; Wilcoxon matched-paired rank-sum test). Treatment with losartan abolished both OT (from 3.4 ± 0.5 pmol/L to 2.3 ± 0.3 at 60 minutes; $P < .05$) and AVP (from 3.75 ± 0.6 pmol/L to 2.4 ± 0.5 at 60 minutes; $P < .05$) responses to ANG II (two-way ANOVA for repeated measures followed by specific means comparisons test).

The administration of losartan alone or placebo alone did not change the basal AVP and OT concentrations (Fig 2).

During the ANG II test, blood osmolality (time 0, 284.0 ± 2.0 mOsmol/kg), hematocrit (time 0, 43.8% ± 1.4%), and serum sodium concentrations (time 0, 140.3 ± 0.4 mEq/L) did not change in any subject. Similar values were observed during the

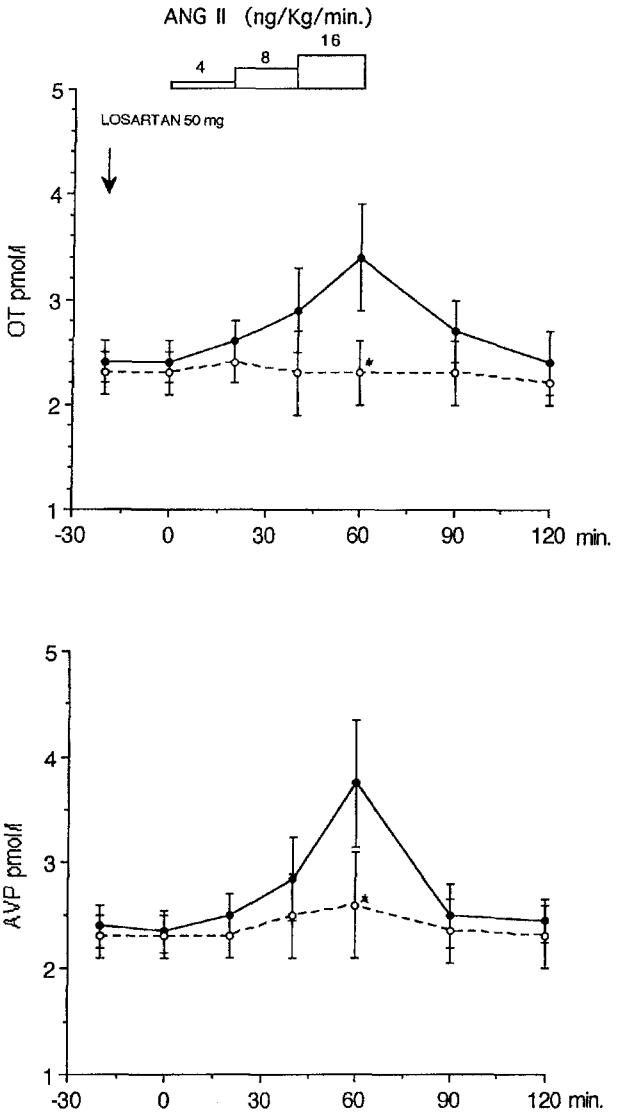

Fig 1. Plasma AVP and OT levels (mean ± SE) during ANG II (●—●) and ANG II + losartan (●- - -●) treatment. *$P < .05$ between control and experimental tests (ANOVA followed by specific mean comparison test).

ANG II plus losartan test, the losartan test, and the placebo test. Mean blood pressure increased significantly during ANG II infusion from a basal value of 94.2 ± 1.5 to 104.6 ± 1.4 mm Hg at 60 minutes ($P < .01$). Losartan did not change blood pressure observed at time 0 (93.6 ± 1.5 mm Hg), but significantly decreased the blood pressure increment induced by ANG II (96.5 ± 1.5; $P < .01$) at 60 minutes. When losartan was given alone, the mean blood pressure decreased significantly from 93.5 ± 1.6 (basal value) to 89.7 ± 1.7 (60 minutes) ($P < .05$; Wilcoxon matched-paired rank-sum test).

None of the subjects experienced side effects after ANG II and losartan treatment.

DISCUSSION

The results of this study show for the first time in humans that AT1 receptors mediate ANG II–stimulating action on AVP and

OT secretion. AT1 receptor subtypes have been described both inside and outside the blood-brain barrier (BBB) not only in hypothalamic-pituitary structures related to posterior pituitary hormone secretion, such as the supraoptic (SON) and paraventricular nuclei (PVN), the median eminence, and the posterior pituitary itself, but also in various cerebral structures known as areas involved in cardiovascular regulation by the brain.[6] Even though ANG II is unable to cross the BBB, circulating ANG II is thought to play an important role in the maintenance of blood pressure by acting on receptors located in the circumventricular organs, which are located outside the BBB, but project to cerebral structures controlling cardiovascular function.[7-12] This pathway is also connected with the SON and PVN and thus may be supposed to mediate ANG II stimulation of posterior pituitary hormone secretions. Like ANG II, losartan is unable to cross the BBB[13] and, thus, inhibition of ANG II–induced AVP and OT responses might be attributed to blockade of AT1 receptors in the circumventricular organs. However, we cannot exclude direct stimulatory effects of circulating ANG II on the posterior pituitary[14] and/or hypothalamic AVP- and OT-secreting structures located outside the BBB, such as the median eminence. In fact, evidence has been provided that ANG II stimulates AVP release from median eminence terminals.[15] Losartan might have produced its inhibitory effect on ANG II action by blocking AT1 receptors at these sites.

Both ANG II and AVP are potent vasoconstrictors and influence heart function through different mechanisms.[16] On the other hand, there is no evidence of a role for OT in hemodynamic homeostasis in normal human subjects, even though the effects of OT in regulation of blood pressure and volume have been shown in rats and dogs.[17-20] Further studies are needed to establish whether in humans ANG II might influence AVP and OT secretion in physiological situations and/or in pathological conditions.

Table 1. Individual Basal and Peak Values of AVP and OT During ANG II and ANG II Plus Losartan Tests

	ANG II Test		ANG II + LOS Test	
Subject No.	Basal	Peak	Basal	Peak
OT (pmol/L)				
1	2.6	3.2	2.5	2.3
2	2.6	3.3	2.4	2.1
3	2.0	2.6	2.0	2.0
4	2.0	3.3	2.0	2.4
5	2.3	3.1	2.1	2.0
6	3.1	5.8	3.3	3.3
7	2.0	2.8	2.1	2.0
Mean ± SE	2.3 ± 0.2	3.4 ± 0.4	2.3 ± 0.2	2.3 ± 0.2
AVP (pmol/L)				
1	2.5	3.2	2.5	2.0
2	2.6	3.5	2.5	2.3
3	2.6	3.6	2.6	2.5
4	1.4	2.4	1.5	1.2
5	2.3	3.5	2.4	2.1
6	3.2	6.7	2.9	5.0
7	1.9	3.1	2.0	1.7
Mean ± SE	2.4 ± 0.2	3.7 ± 0.5	2.3 ± 0.2	2.4 ± 0.5

Abbreviation: LOS, losartan.

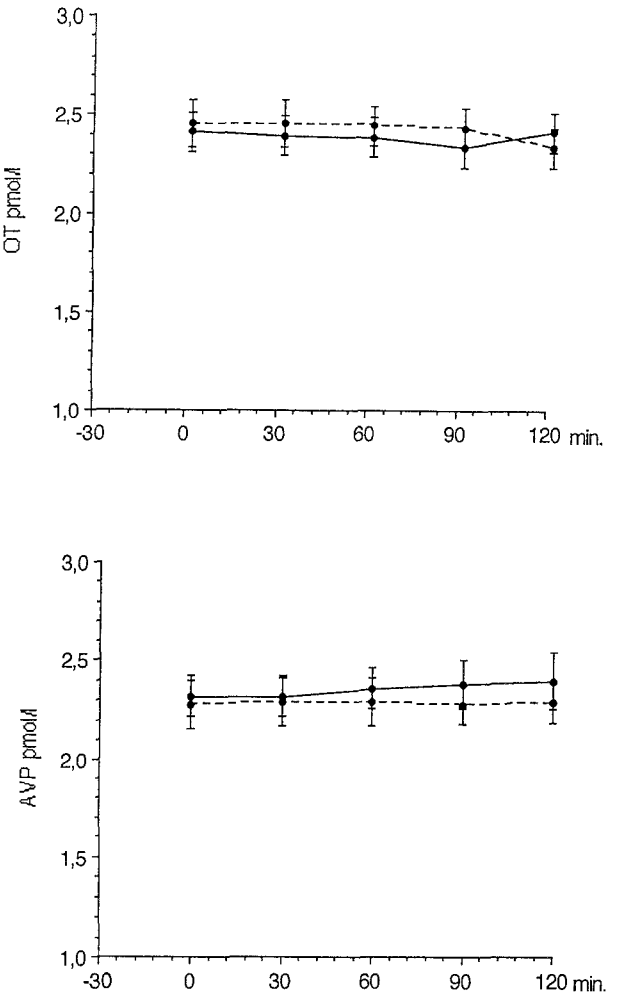

Fig 2. Plasma OT and AVP levels (mean ± SE) during losartan (●—●) and saline (●- - -●) administration. Each point represents the mean ± SE.

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REFERENCES

1. Phillips MI: Function of angiotensin in the central nervous system. Ann Rev Physiol 49:413-435, 1987

2. Wright JW, Harding JW: Brain angiotensin receptor subtypes AT1, AT2 and AT4 and their functions. Regul Peptides 59:269-295, 1995

3. De Gasparo M, Bottari S, Levens NR: Characteristics of angiotensin II receptors and their role in cell and organ physiology, in Laragh JH, Brenner BM (eds): Hypertension: Pathophysiology, Diagnosis and Management (ed 2). New York, NY, Raven, 1995, pp 1695-1720

4. Wong PC, Timmermans PBMWM: Physiological effects of a new class of highly specific angiotensin II receptor antagonists, in Laragh JH, Brenner BM (eds): Hypertension: Pathophysiology, Diagnosis and Management (ed 2). New York, NY, Raven, 1995, pp 3079-3098

5. Chiodera P, Louis F, Legros JJ: Simultaneous radioimmunoassay for plasma arginine-vasopressin and oxytocin using DEAE sephadex A25 extraction. J Endocrinol Invest 7:287-293, 1984

6. Phillips MI, Shen L, Richards EM, et al: Immunohistochemical mapping of angiotensin AT1 receptors in the brain. Regul Peptides 44:95-107, 1993

7. Peach MJ: Renin-angiotensin system: biochemistry and mechanisms of actions. Physiol Rev 57:313-370, 1977

8. Ramsay DJ: Effects of circulating angiotensin II in the brain, in Ganong WF, Martini L (eds): Frontiers in Neuroendocrinology, vol 7. New York, NY, Raven, 1982, pp 263-285

9. Veljkovic S, Jovanovic MD, Japundzic N, et al: The area postrema and the hypertensive effects of angiotensin. Metab Brain Dis 4:61-65, 1989

10. Papas S, Smith P, Ferguson AV: Electrophysiological evidence that systemic angiotensin enfluences rat area postrema neurons. Am J Physiol 258:R70-R76, 1990

11. Wong PC, Price WA, Chiu AT, et al: Nonapeptide angiotensin II receptor antagonists. VIII. Characterization of functional antagonism displayed by DUP 753, an orally antihypertensive agent. J Pharmacol Exp Ther 252:719-725, 1990

12. Gehlert DR, Gackenheimer SL, Schober DA: Autoradiographic localization of subtypes of angiotensin II antagonist binding in the rat brain? Neuroscience 44:501-514, 1981

13. Barbella Y, Cieco M, Israel A: Effect of losartan, a nonapeptide angiotensin II receptor antagonist, on drinking behavior and renal actions of centrally administered renin. Proc Soc Exp Biol Med 202:401-406, 1993

14. Lutz-Bucher B, Koch B, Alzein M: Direct stimulatory effect of angiotensin II on neurohormone release from posterior pituitary and modulation by opiates. Neuroendocrinol Lett 4:103-107, 1982

15. Negro-Vilar A, Steele MK: Evidence that angiotensin II (A II) stimulates vasopressin (AVP) release from median eminence terminals. Fed Proc 41:1354, 1992 (abstr)

16. Hegarty AA, Hayward LF, Felder RB: Influence of circulating angiotensin II and vasopressin on neurons of the nucleus of the solitary tract. Am J Physiol 270:R675-R681, 1996

17. Weitzman RE, Glatz TH, Fisher DA: The effect of hemorrhage and hypertonic saline upon plasma oxytocin and arginine vasopressin in conscious dogs. Endocrinology 103:2154-2160, 1978

18. Morris M, Barnard RR Jr, Sain LE: Osmotic mechanisms regulating cerebrospinal fluid vasopressin and oxytocin in the conscious rat. Neuroendocrinology 39:377-383, 1984

19. Cheng SWT, North WG: Responsiveness of oxytocin-producing neurons to acute salt loading in rat: Comparison with vasopressin-producing neurons. Neuroendocrinology 42:174-180, 1986

20. Morris M, Alexander N: Baroreceptor influences on oxytocin and vasopressin secretion. Hypertension 13:110-114, 1988