Water deprivation upregulates angiotensin II receptors in rat anterior pituitary

ANITA ISRAEL, JUAN M. SAAVEDRA, LAURA PLUNKETT
National Institute of Mental Health, Laboratory of Clinical Science, Section on Clinical Psychopharmacology, Washington, DC 20205-1000

ISRAEL, ANITA, JUAN M. SAAVEDRA, AND LAURA PLUNKETT. *Water deprivation upregulates angiotensin II receptors in rat anterior pituitary.* Am. J. Physiol. 248 (Endocrinol. Metab. 11): E264–E267, 1985.—Angiotensin II (ANG II) receptors were quantitated in pituitary glands of individual male Long Evans rats by autoradiography after incubation of 8-μm thick pituitary sectious with ^{125}I-[Sar^1]ANG II. Rat anterior pituitary had a single class of high-affinity saturable ANG II receptors with a B_{max} of 1,360 ± 109 fmol/mg protein and a K_a of 0.510 ± 0.03 × 10^9 M^{-1}. Five days of water deprivation produced a marked increase in the number of anterior pituitary ANG II receptors (B_{max}: 2,428 ± 233 fmol/mg protein, a 79% iucrease, $P < 0.001$) and a decrease in affinity for the ligand (K_a: 0.337 ± 0.01 10^9 M^{-1}, a 34% decrease, $P < 0.05$). Our results suggest a role for anterior pituitary ANG II receptors in the regulation of fluid and electrolyte metabolism in the rat.

dehydration; angiotensin II binding sites; augiotensin II receptors autoradiography; neuropeptide receptors autoradiography

ANGIOTENSIN II (ANG II) has marked effects on the release of hormones from the anterior pituitary gland (3, 9, 19, 20, 24). Some of these actions may be mediated, at least in part, by specific receptors located in anterior pituitary cells (17). ANG II receptors have been characterized in rat anterior pituitary membrane preparations (4, 12, 14, 22) and in dispersed anterior pituitary cells (4).

We studied the regulation of anterior pituitary ANG II receptors after water deprivation, a state resulting in a substantial elevation of circulating ANG II levels (11) and in alterations of ANG II receptors in brain and peripheral tissues (12). We used a newly developed quantitative autoradiographic method that allows the complete kinetic analysis and characterization of receptors for neuropeptides in single rat pituitary glands (6, 7).

MATERIALS AND METHODS

Animals. Male 24-wk-old Long Evans rats were purchased from Blue Spruce Farms, Altamont, NY, and were housed at a constant temperature of 24°C, with lights on from 06:00 to 18:00 h. Rats were randomly divided into two groups, one given free access to tap water and rat chow and another water deprived for 5 days.

Determination of ANG II receptor binding by autoradiography. Rats were killed by decapitation between 09:00 and 11:00 h, and pituitary glands were immediately removed and frozen by immersion in isopentane at −30°C. Frozen 8-μm thick sections were cut in a cryostat at −14°C, less than 24 h after killing, thaw mounted onto subbed glass slides, desicated under vacuum at 4°C for 2 h, and kept at −20°C until incubation. Within 48 h after section preparation, binding sites for ANG II were labeled in vitro by incubation with ^{125}I-[Sar^1]ANG II (a gift from Dr. M. Khosla, Cleveland Clinic, Cleveland, OH; iodinated by a modified cloramine-T method at Melloy Laboratories, Springfield, VA; specific activity, 1,666 Ci/mmol). Tissue sections were preincubated for 15 min at 20°C in 5 ml of 10 mM sodium phosphate buffer, pH 7.4, containing NaCl (120 mM), Na_2 EDTA (5 mM), bacitracin (0.1 mM), and bovine serum albumin (0.2%) and then incubated for 60 min in fresh buffer with concentrations of ^{125}I-[Sar^1]ANG II from 25 pM to 10 nM. Nonspecific binding was determined in the presence of unlabeled ANG II (Sigma Chemical, St. Louis, MO) in concentration ranging from 0.125 to 50 μM. After incubation, the slides were washed four times (60 s each) with ice-cold 50 mM Tris-HCl buffer, pH 7.56, and dried under a cold stream of air. Sets of ^{125}I standards were prepared as described (6, 7).

Incubated tissue sections and ^{125}I standards were placed in cassettes (CGR Med. Baltimore, MD) and opposed against [^{3}H]ultrofilm (LKB Instruments, Rockville, MD) at room temperature for 1 day (concentrations from 0.625 to 10 nM) or 2 days (concentrations from 30 to 300 pM). The films were developed at 20°C for 4 min with undiluted D19 Kodak developer, and optical densities were quantitated by computerized densitometry (13, 25). The optical densities observed in the pituitary gland were related to the concentration of radioactivity present by comparison with standard curves generated by processing sets of standards with each of the autoradiograms (6, 7) and correcting by the decay factor of ^{125}I.

Statistical analysis. Analysis of binding data and Scatchard plots were performed via the computer program LIGAND (15). Significance between groups was determined through use of the Student's *t* test.

RESULTS

High concentrations of ANG II receptors were found in the anterior pituitary (Figs. 1 and 2). Rat anterior pituitary presented a single class of specific, saturable,

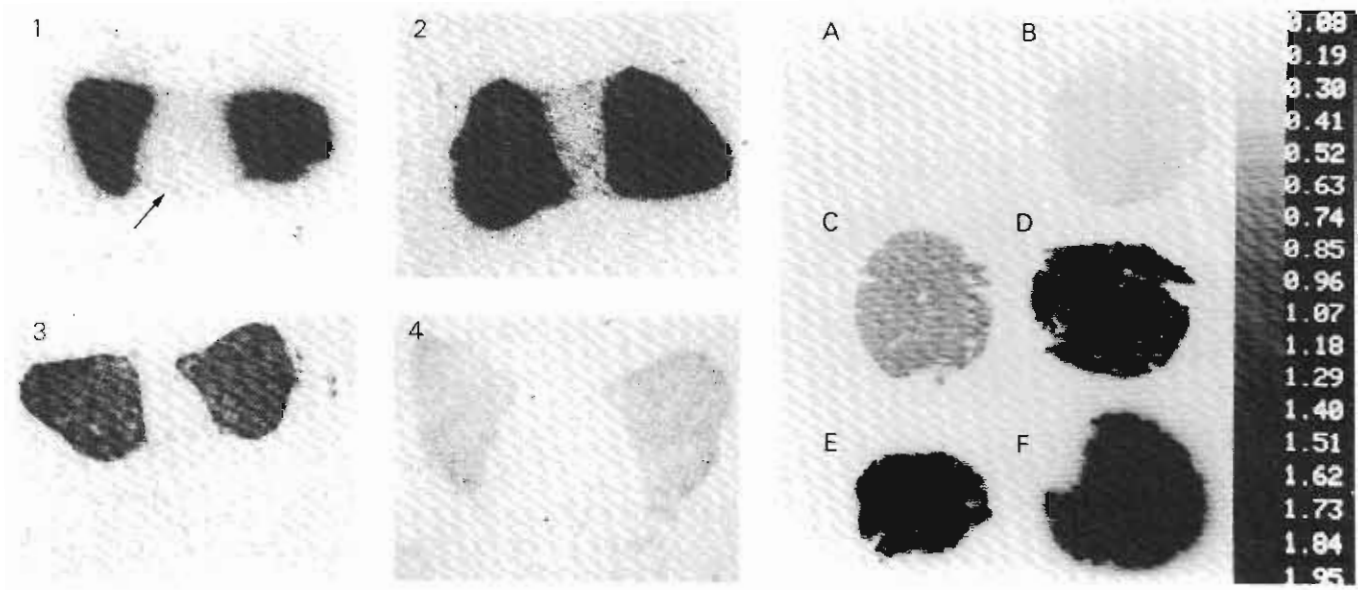

FIG. 1. Autoradiographic image with computerized densitometry of angiotensin II (ANG II) receptors in pituitary gland. *Left panel*: *1–3*, rat pituitary gland incubated with 5.0, 1.25, and 0.32 nM ^{125}I-[Sar^1]-ANG II. *Arrow* points to posterior pituitary lobe. *4*, Adjacent section incnbated with 5 nM ^{125}I-[Sar^1]ANG II and 25 μM unlabeled ANG II. *Right panel*: ^{125}I standards with computerized densitometry. Each section contains a different amount of radioactivity, dpm/mg of protein: 9.7 (*A*), 20.8 (*B*), 72.8 (*C*), 200.5 (*D*), 536.1 (*E*), 1,008.0 (*F*).

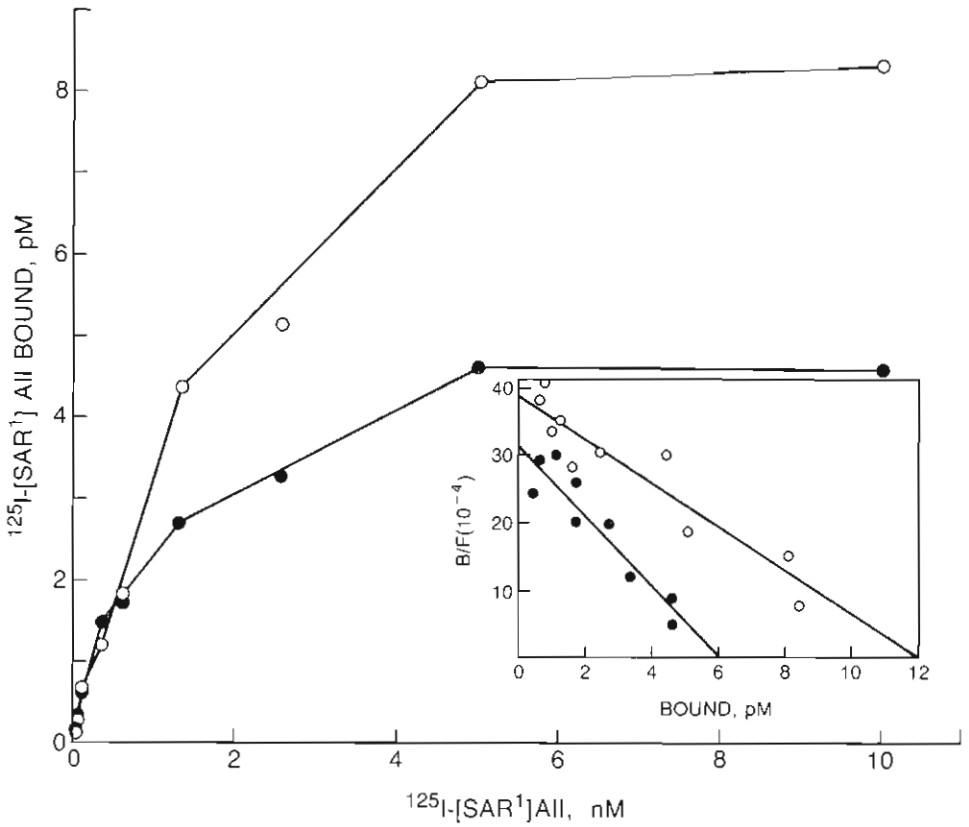

FIG. 2. Saturation curves and Scatchard analysis of specific ^{125}I-[Sar^1]ANG II binding to rat anterior pituitary gland. Tissue sections were incubated for 60 min with ^{125}I-[Sar^1]ANG II (concentrations from 25 pM to 10 nM). Nonspecific binding was determined in the presence of unlabeled ANG II (from 0.125 to 50 μM). Data represent a typical experiment that was replicated 9 times. *Closed circles* (●——●), control rats; *open circles* (○——○), water-deprived rats. *Inset*: Scatchard analysis of same data.

high-affinity ANG II receptors, with a B_{max} of 1,360 ± 109 fmol/mg protein and a K_a of 0.510 ± 0.03 × 10^9 M^{-1} (n = 9) (Fig. 2). Nonspecific binding in anterior pituitary was <5% of total binding (results not shown). In contrast, the posterior pituitary presented a low density of ANG II receptors, 100 ± 12 fmol/mg protein, when measured with a ^{125}I-[Sar^1]ANG II concentration of 10 nM.

Five days of water deprivation resulted in a large increase in the number of ANG II receptors in the anterior pituitary (Fig. 2) (+79%, B_{max}: 2,428 ± 233 fmol/mg protein, $P < 0.001$). In addition, the affinity constant of ANG II receptors in the anterior pituitary was significantly decreased after water deprivation (K_a: 0.337 ± 0.01 × 10^9 M^{-1}, −34%, $P < 0.05$). No changes occurred in posterior pituitary ANG II receptors after water dep-

rivation; their density, when incubated with 10 nM ^{125}I-[Sar^1]ANG II, was 80 ± 14 fmol/mg protein.

DISCUSSION

Our results demonstrate that ANG II receptors can be characterized by autoradiography in single-rat pituitary glands. Rat anterior pituitary showed one class of saturable high-affinity ANG II binding sites, in agreement with prior observations (4, 12, 24). The concentration of ANG II receptors in the anterior pituitary, however, was several times higher than previously reported for rat anterior pituitary membrane preparations (3–293 fmol/mg protein) (4, 12, 24), and it is one of the highest ANG II receptor concentrations in rat tissues (6, 7). In contrast, a very low number of ANG II receptors occurred in posterior pituitary, which represented less than 10% of the total ANG II specific binding of the anterior pituitary.

In the anterior pituitary, ANG II receptor number is increased after prolonged water deprivation, a state associated with a severalfold increase in ANG II blood levels (11). The increased binding capacity of anterior pituitary ANG II receptors might serve to sensitize the anterior pituitary to circulating ANG II and could represent an amplification mechanism of physiological importance. A similar upregulation of ANG II receptors occurs in the subfornical organ after water deprivation and in the rat adrenal zona glomerulosa after sodium depletion (12). In addition, anterior pituitary ANG II receptors could be modulated by ANG II released from the median eminence to the portal circulation (24) or by ANG II formed in situ by the anterior pituitary angiotensin system (1, 2, 18, 23).

The consequences of increased stimulation of the anterior pituitary by ANG II during water deprivation have not been clarified, but this stimulation is likely to result in increased secretion of anterior pituitary hormones. In mammals, ANG II has been found to stimulate the release of prolactin (3), β-endorphin (9), and ACTH (19), effects probably related to ANG II binding to specific anterior pituitary cell types (17). ACTH can act synergistically with ANG II in the zona glomerulosa to increase aldosterone secretion (25). Prolactin has been shown to contribute to the regulation of fluid and electrolyte metabolism in mammals (5, 10, 16) and to act synergistically with ANG II to cause drinking and fluid retention (8). In addition, ANG II could be involved in the secretion of other not fully identified factors from the anterior pituitary, such as the recently reported aldosterone-stimulating factor (21).

Present address of Anita Israel: Central University of Venezuela, Caracas, Venezuela.

Received 30 August 1984; accepted in final form 8 November 1984.

REFERENCES

1. CHEVILLARD, C., AND J. M. SAAVEDRA. High angiotensin-converting enzyme activity in the neurohypophysis of Brattleboro rats. *Science* 216: 646–647, 1982.
2. CHEVILLARD, C., AND J. M. SAAVEDRA. Angiotensin-converting enzyme (kininase II) in pituitary gland of spontaneously hypertensive rats. *Regul. Pept.* 5: 333–341, 1983.
3. DUFY-BARBE, L., F. RODRIGUEZ, J. ARSAUT, D. VERRIER, AND J. D. VINCENT. Angiotensin II stimulates prolactin release in the rhesus monkey. *Neuroendocrinology* 35: 242–247, 1982.
4. HAUGER, R. L., G. AGUILERA, A. J. BAUKAL, AND K. J. CATT. Characterization of angiotensin II receptors in the anterior pituitary gland. *Mol. Cell Endocrinol* 25: 203–212, 1982.
5. HORROBIN, D. F. Prolactin as a regulator of fluid and electrolyte metabolism in mammals. *Federation Proc.* 39: 2567–2570, 1980.
6. ISRAEL, A., F. M. A. CORREA, M. NIWA, AND J. M. SAAVEDRA. Quantitative determination of angiotensin II binding sites in rat brain and pituitary gland by autoradiography. *Brain Res.* In press.
7. ISRAEL, A., F. M. A. CORREA, M. NIWA, AND J. M. SAAVEDRA. Quantitative measurement of angiotensin II (AII) receptors in discrete regions of rat brain, pituitary and adrenal gland hy autoradiography. *Clin. Exp. Hypertens.* In press.
8. KAUFMAN, S. The dipsogenic activity of prolactin in male and female rats. *J. Physiol.* 310: 435–444, 1981.
9. KRAFT, K., R. R. LANG, W. GAIDA, T. UNGER, AND D. GANTEN. Angiotensin stimulates β-endorphin release from anterior pituitary gland cell cultures of rats. *Neurosci. Lett.* 46: 25–29, 1984.
10. LORETZ, C., AND H. A. BERN. Prolactin and osmoregulation in vertebrates. *Neuroendocrinology* 35: 292–304, 1982.
11. MANN, J. F. E., A. K. JOHNSON, AND D. GANTEN. Plasma angiotensin II: dipsogenic levels and angiotensin-generating capacity of renin. *Am. J. Physiol.* 238 (*Regulatory Integrative Comp. Physiol.* 7): R372–R377, 1980.
12. MENDELSOHN, F. A. O., G. AGUILERA, J. M. SAAVEDRA, R. QUIRION, AND K. J. CATT. Characteristics and regulation of angiotensin II receptors in pituitary, circumventricular organs and kidney. *Clin. Exp. Hypertens.* A5 (7 and 8): 1081–1097, 1983.
13. MENDELSOHN, F. A. O., R. QUIRION, J. M. SAAVEDRA, G. AGUILERA, AND K. J. CATT. Autoradiographic localization of angiotensin II receptors in rat brain. *Proc. Natl. Acad. Sci. USA* 81: 1575–1579, 1984.
14. MUKHERJEE, A., P. KULKARNI, S. M. MCCANN, AND A. NEGRO-VILAR. Evidence for the presence and characterization of angiotensin II receptors in rat anterior pituitary membranes. *Endocrinology* 110, 665–667, 1982.
15. MUNSON, P. J. Ligand: a computerized analysis of ligand binding data. In: *Methods in Enzymology. Immunochemical Techniques*, edited by John J. Langone and Helen Van Vunakis. New York: Academic Press, 1983, part E, vol. 92, p. 543.
16. NICOLL, C. S. Physiological actions of prolactin. In: *Handbook of Physiology. Endocrinology.* Washington DC: Am. Physiol. Soc., 1974, sect. 7, vol. IV, chapt. 3, p. 253.
17. PAGLIN, S., H. STUKENBROK, AND J. D. JAMIESON. Interaction of angiotensin II with dispersed cells from the anterior pituitary of the male rat. *Endocrinology* 114: 2284–2292, 1984.
18. SAAVEDRA, J. M., J. FERNANDEZ-PARDAL, AND C. CHEVILLARD. Angiotensin-converting enzyme in discrete areas of the rat forebrain and pituitary gland. *Brain Res.* 245: 317–325, 1982.
19. SCHOLKENS, B. A., W. JUNG, W. RASCHER, A. SCHOMIG, AND D. GANTEN. Brain angiotensin II stimulates release of pituitary hormones, plasma catecholamine and increases blood pressure in dogs. *Clin. Sci.* 59: 53S–56S, 1980.
20. SCHRAMME, C., AND C. DENEF. Stimulation of prolactin release by angiotensin II in superfused rat anterior pituitary cell aggregates. *Neuroendocrinology* 36: 483–485, 1983.
21. SEN, S., F. M. BUMPUS, S. OBERFIELD, AND M. I. NEW. Development and preliminary application of a new assay for aldosterone-stimulating factor. *Hypertension* 5: I27–I31, 1983.
22. SIRETT, N. E., A. S. MCLEAN, J. J. BRAY, AND J. I. HUBBARD. Distribution of angiotensin II receptors in rat brain. *Brain Res.* 122: 299–312, 1977.
23. STEELE, M. K., M. S. BROWNFIELD, AND W. F. GANONG. Immunocytochemical localization of angiotensin immunoreactivity in

gonadotrops and lactotrops of the rat anterior pituitary gland. *Neuroendocrinology* 35: 155–158, 1982.
24. STEELE, M. K., A. NEGRO-VILAR, AND S. M. MCCANN. Effect of angiotensin II on in vivo and in vitro release of anterior pituitary hormones in the female rat. *Endocrinology* 109: 893–899, 1981.
25. UNNERSTALL, J. R., D. L. NIEHOFF, M. J. KUHAR, AND J. M. PALACIOS. Quantitative receptors autoradiography using [^{3}H]ultrofilm: application to multiple benzodiazepine receptors. *J. Neurosci. Methods* 6: 59–73, 1982.
26. WEITZMAN, E. D. Circadian rhythms and episodic hormone secretion in man. *Annu. Rev. Med.* 27: 225–243, 1976.