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RAPID COMMUNICATIONS

INCREASED ATRIAL NATRIURETIC PEPTIDE (6-33) BINDING SITES IN THE SUBFORNICAL ORGAN OF WATER DEPRIVED AND BRATTLEBORO RATS

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Abstract. Binding sites for rat atrial natriuretic peptide (6-33) (ANP) were quantitated in the subfornical organ of chronically dehydrated homozygous Brattleboro rats unable to synthesize vasopressin; heterozygous Brattleboro rats, their controls, Long Evans rats and Long Evans rats after 4 days of water deprivation. Brain sections were incubated in the presence of ^{125}I-ANP and the results analyzed by autoradiography coupled to computerized microdensitometry and comparison to ^{125}I-standards. Brattleboro rats and water deprived Long Evans rats presented a higher number of ANP binding sites than their normally hydrated controls. Our results suggest a role of ANP binding sites in the subfornical organ in the central regulation of fluid balance and vasopressin secretion.

Introduction. The atrial natriuretic factor (ANF) and related peptides, first identified in the mammalian cardiac atrium are released into the circulation in response to volume expansion, and may play active roles in cardiovascular regulation and fluid homeostasis through receptor-mediated actions in the kidneys, adrenal glands and vasculature (1,2,3).

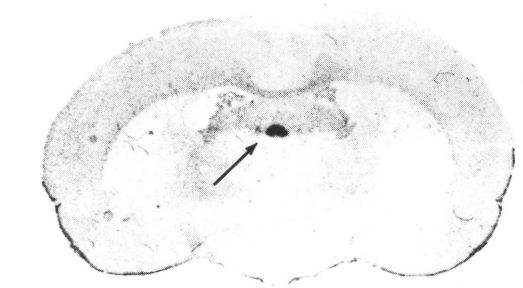
Certain effects of ANF, however, could be centrally mediated. ANF modulates vasopressin secretion acting at the hypothalamic and pituitary levels (4,5). Binding sites for rat ANF (8-33) are localized in the subfornical organ, a circumventricular structure devoid of blood-brain barrier and involved in blood pressure regulation and the secretion of vasopressin (6).

We have used quantitative autoradiographic methods coupled to computerized microdensitometry (8,9) to characterize and quantitate binding sites for ^{125}I-rat atrial natriuretic peptide (6-33) (ANP) in the subfornical organ of homozygous Brattleboro rats, unable to synthesize vasopressin and suffering from chronic dehydration (diabetes insipidus)(10), heterozygous Brattleboro rats and their normally hydrated controls, Long Evans rats. To assess the role of ANP in acute dehydration we studied ANP binding sites in water deprived Long Evans rats. ANP closely resembles rat ANF (8-33) containing at the N-terminus only two amino acids more (Ser-Leu) than this peptide (11).

Materials and Methods. Male 9-week old homozygous Brattleboro rats (DI), heterozygous Brattleboro rats (HZ), normally hydrated Long Evans controls (LE), and LE rats water deprived for 4 days were housed individually in metabolic cages under normal laboratory conditions for one week after being purchased from Blue Spruce Farms, Altamont, NY. Mean daily water intake was 8±2, 9±5 and 75±5 ml/100g weight for LE, HZ, and DI rats, respectively.

The rats were sacrificed by decapitation between 09.00 hr and 11.00 hr, their brains immediately removed and frozen by immersion in isopentane (-30°C). Within 24 hrs of sacrifice,

0037-9727/86 $1.50



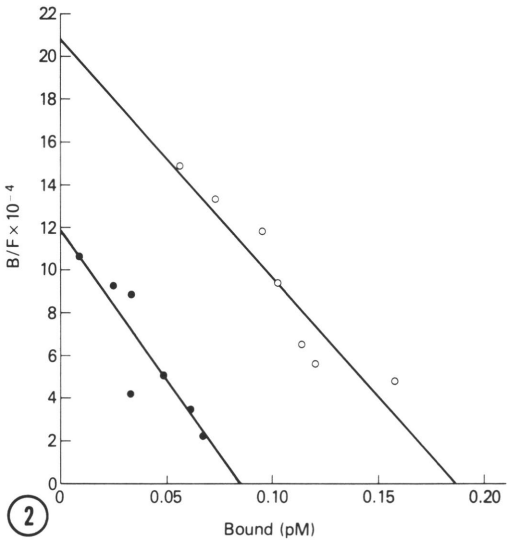


Figure 1: Autoradiographic localization of [^{125}I]-ANP binding sites in a coronal section at the level of the subfornical organ in the DI Brattleboro rat. The section was incubated in the presence of 0.3 nM [^{125}I]-ANP and exposed for 2.5 days to [^{3}H]-Ultrofilm. The arrow points to the subfornical organ.

Figure 2: Scatchard analysis of ^{125}I-ANP binding to the subfornical organ. Solid circles: LE. Open circles: DI. Data represent a typical experiment which was replicated 5 times (DI) or 8 times (LE) per group. Correlation coefficients (r) are: DI: -0.9188; LE: -0.8532.

tissue sections (16 µm) were cut in a cryostat at -14°C, thaw-mounted onto gelatin-coated glass slides, and placed under vacuum at 4°C until incubation.

Rat atrial natriuretic peptide (6-33) (ANP) binding sites were labeled in vitro by incubation with (3-[^{125}I]-iodotyrosyl ^{28}) ANP (specific activity 1750 Ci/mmol, Amersham Corp., Arlington Hts., IL). Scatchard analysis was performed using consecutive sections from individual brains. Tissue sections were preincubated at 20°C for 15 min in 50 mM Tris-HCl buffer, pH 7.4 and were incubated for 60 min at room temperature in 50 mM Tris-HCl buffer, pH 7.4, containing 100 mM NaCl, 5 mM $MgCl_2$, 0.5% bovine serum albumin, 40 µg/ml bacitracin, 4 µg/ml leupeptin, 2 µg/ml chymostatin, 0.5 µg/ml phenylmethylsulfonyl-fluoride (PMSF)(6), and ^{125}I-ANP in concentrations ranging from 10 to 400 pM. Non-specific binding was determined in consecutive sections in the presence of unlabeled ANP (atrial peptide, rat, 28 aminoacids, Peninsula Laboratories, Inc., Belmont, CA), in concentrations ranging from 0.025 to 1 µM. After incubation, the slides were washed three times (2 min each) in Tris-HCl buffer at 4°C and dried under a cold stream of air.

Quantitation of ANP binding sites was performed by autoradiography with [^{3}H]-Ultrofilm (LKB Industries, Rockville, MD) computerized microdensitometry and comparison to ^{125}I-standards (8,9).

Binding data were calculated and Scatchard plots were generated by linear regression. All data were presented as the mean ± S.E.M. Statistical differences between groups were analyzed using the Student's t-test.

Results. Saturable, single class binding sites for ANP were concentrated in the subfornical organ (Fig. 1, 2) of both DI and LE rats. The addi-

TABLE I. BRAIN ANP BINDING SITES IN LE, BRATTLEBORO AND WATER DEPRIVED RATS

Strain	Binding Affinity (K_a) ($x10^9 M^{-1}$)	Maximum Binding Capacity (B_{max}) (fmol/mg protein)
LE Control (8)	16.3 ± 2.8	29.0 ± 2.0
LE Water Deprived (8)	14.1 ± 3.0	74.4 ± 5.3 [a] [b]
HZ (5)	15.3 ± 1.5	43.7 ± 1.0 [a]
DI (5)	12.4 ± 2.9	67.4 ± 6.9 [a] [b]

Values are means ± S.E.M.
[a]: $p<0.005$, vs. control LE.
[b]: $p<0.005$, vs. HZ.

Numbers between parenthesis are number of animals per group, assayed individually.

tion of unlabeled ANP or ANF (8-33) resulted in a displacement of more than 80% of the ^{125}I-ANP binding (results not shown). DI rats had a much higher maximum binding capacity (B_{max}) than the LE controls (Fig. 2 and Table I). HZ rats had a B_{max} of intermediate value between those of LE controls and DI rats. LE rats, when water deprived for 4 days, showed an increase in the number of ANF binding sites in the subfornical organ, of a magnitude similar to that observed in DI rats (Table 1). In contrast there were no differences in the binding affinity constant (K_a) between the groups (Fig. 2 and Table I).

Discussion. ANF and related peptides have been proposed to be involved in the regulation of extracellular fluid (1). Evidence has been accumulating which suggests that these peptides may act as hormones, being released by left atrial cardiomyocites to the circulation to act at distant receptor sites (1,2,3). The peptides produce natriuresis and diuresis, effects which are physiologically opposite to those of the antidiuretic hormone, arginine vasopressin (1). Modulation of vasopressin release by ANF and related peptides could be considered an example of their hormonal effects. ANF can release vasopressin from isolated posterior pituitary lobes (5). However, distension of the left atrium induces a decrease in plasma levels of vasopressin (12) and synthetic ANF can inhibit dehydration-induced vasopressin release in vivo (4). Thus, the overall effect of ANF in vasopressin release by the intact hypothalamic-hypophyseal system may be one of decreased hormone release.

Vasopressin release from the posterior pituitary is modulated by stimulation of higher brain structures, such as those the subfornical organ, a circumventricular organ devoid of blood-brain barrier (7). During dehydration, blood borne factors such as angiotensin II stimulate subfornical organ receptors, resulting in increased drinking behavior, metabolic activity in this area, and vasopressin release (7, 13,14).

The discrete localization of ANP binding sites in the subfornical organ reported here is in agreement with previous observations of a high con-

centration of binding sites for the related peptide synthetic ANF (8-33) in this structure (6), and suggest that the subfornical organ could also be the target site in the central regulation of vasopressin secretion by blood-borne ANF.

Our observations of a high number of ANP binding sites in the subfornical organ of DI rats and of an increased number of ANP binding sites in water deprived LE rats supports a role of ANF peptides in the central control of fluid balance. Lack of vasopressin in homozygous Brattleboro rats results in excessive drinking, high circulating angiotensin II levels and a metabolically hyperactive subfornical organ-posterior pituitary axis (15). In normal animals acute dehydration produces a similar constellation of symptoms (13,16) as well as increased angiotensin II receptors in the subfornical organ (17). Dehydration also results in increased ANF content in the heart (18) probably a result of reduced release into the circulation.

The present findings of increased ANP binding sites in the subfornical organ of DI rats could represent up-regulation of ANP receptors due to decreased stimulation from blood borne ANP, or be directly related to alterations in angiotensin receptors in this area. Our findings suggest that the subfornical organ might be the target site for central interactions between ANF and angiotensin and provide further evidence for a role of ANF and related peptides in vasopressin secretion and in the regulation of fluid homeostasis.

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