Correction. In the article "Rearrangement of chicken immunoglobulin genes is not an ongoing process in the embryonic bursa of Fabricius" by J. C. Weill, C. A. Reynaud, O. Lassila, and J. R. L. Pink, which appeared in number 10, May 1986, of Proc. Natl. Acad. Sci. USA (83, 3336–3340), the authors request that the following correction be noted. An incomplete citation was given for ref. 24. The complete citation for ref. 24 is as follows: Ratcliffe, M. J. H., Lassila, O., Pink, J. R. L. & Vainio, O. (1986) Eur. J. Immunol. 16, 129–133.

Correction. In the article "Atrial natriuretic factor receptors in rat kidney, adrenal gland, and brain: Autoradiographic localization and fluid balance dependent changes" by David R. Lynch, Karen M. Braas, and Solomon H. Snyder, which appeared in number 10, May 1986, of Proc. Natl. Acad. Sci. USA (83, 3557–3561), the following correction should be noted. The page numbers (3357–3361) appearing on the actual pages should read 3557–3561.

Correction. In the article "Isolation of molecular probes associated with the chromosome 15 instability in the Prader-Willi syndrome" by T. A. Donlon, M. Lalande, A. Wyman, G. Bruns, and S. A. Latt, which appeared in number 12, June 1986, of Proc. Natl. Acad. Sci. USA (83, 4408–4412), the authors request that the following be noted: It has just come to our attention that some of the chromosomes in the paste-up in Fig. 1 are incorrect. Though the major conclusions of this paper appear to be correct, the above error casts doubt on the precise localizations of certain DNA probes tabulated but not found in the Prader-Willi deletion. Further study is required and is underway.
Atrial natriuretic factor receptors in rat kidney, adrenal gland, and brain: Autoradiographic localization and fluid balance dependent changes

(atriopeptin/hypertension/neuropeptide/electrolyte balance/vasorelaxant)

DAVID R. LYNCH, KAREN M. BRAAS, AND SOLOMON H. SYNDER*

Departments of Neuroscience, Pharmacology and Experimental Therapeutics, and Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Contributed by Solomon H. Snyder, December 31, 1985

ABSTRACT Mammalian atria contain natriuretic peptides designated atrial natriuretic factors (ANF). Using in vitro autoradiography with 125I-labeled ANF, we have localized high-affinity (Kd = 150 pM) ANF binding sites to the glomeruli of the kidney, zona glomerulosa of the adrenal gland, and choroid plexus of the brain. The numbers of sites in both kidney and adrenal are increased in rats deprived of water; increases are detectable within 72 hr of deprivation in the kidney and within 24 hr in the adrenal gland. Receptor numbers decline in rats given 2.0% NaCl as drinking water and in diabetic rats. The discrete localizations and dynamic alterations of these receptors suggest that ANF regulates fluid balance through diverse but coordinated actions on receptors in numerous organs including the kidney, adrenal, and brain.

The cardiac atria of mammals contain peptides, designated atrial natriuretic factors, or atriopeptins, that cause vasodilation and natriuresis (1). These factors have common sequence elements (2-6), and cloning of the cDNAs for these peptides suggests that they share a common precursor (7-11). ANF is found in specific atrial granules (12, 13) and is thought to be released, in response to atrial stretch, to act on target organs including the adrenal gland, brain, and kidney (14). The numbers of ANF-containing granules (15) and plasma ANF levels change with altered body fluid balance and blood pressure (16, 17).

Receptor binding sites for ANF have been characterized in homogenates of aorta (18, 19), kidney (18), and adrenal gland zona glomerulosa (20, 21). In preliminary studies, ANF receptors have been localized by autoradiography in brain (22, 23), adrenal gland zona glomerulosa (24), and glomeruli of the kidney (24). In the present study, we use in vitro autoradiography to localize ANF receptors discretely within the kidney, adrenal gland, and choroid plexus of the brain. These selective localizations may explain the diverse, coordinated actions of ANF. We also demonstrate changes in numbers of ANF receptors in response to altered fluid balance, revealing a reciprocal interaction between ANF levels and ANF receptors.

MATERIALS AND METHODS

125I-labeled rat ANF-(1-28) [125I-ANF-(1-28); 2200 Ci/mmol], labeled at the carbamyl-terminal tyrosine, was obtained from Russell Garlick at New England Nuclear Dupont (Boston, MA). Unlabeled rat ANF-(1-28) was obtained from Bachem Fine Chemicals (Torrance, CA).

Tissue Preparation. Male Sprague-Dawley, Wistar-Kyoto (WKY), Charles River CD, spontaneously hypertensive (SHR), Long–Evans, or Brattleboro rats (7–10 weeks old) were placed under sodium pentobarbital anesthesia and perfused through the left cardiac ventricle with phosphate-buffered saline (0.05 M sodium phosphate/0.150 M NaCl, pH 7.4), followed by 0.32 M sucrose in the same buffer. After perfusion, tissues were rapidly removed, embedded in brain paste, and frozen in a dry ice/ethanol slurry. Cryostat tissue sections (8 µm) were cut with a microtome, thaw-mounted onto chrome alum/gelatin-coated slides, and stored at −20°C until the autoradiographic procedure was performed.

Receptor Labeling. Tissue sections were brought to room temperature and incubated in 50 mM Hepes, pH 7.5/5.0 mM MgCl2/1.0% bovine serum albumin/0.1% bacitracin for 5 min, followed by incubation with 5–10 pM 125I-ANF in the same buffer for 60 min at 4°C. Blanks were incubated in the same solution in the presence of 100 nM unlabeled rat ANF-(1-28). The sections were washed twice for 15 min each in the same buffer, dipped in buffer without bovine serum albumin, dipped in distilled water, and dried rapidly under a stream of cool dry air.

Autoradiography. The dried labeled tissue sections were apposed either to Ultrofilm (LKB) or to Kodak NTB-3 emulsion-coated coverslips (25) for 10–14 days at 4°C. After the autoradiograms were developed, the tissue sections were stained with toluidine blue and mounted with Permount (Fisher Scientific, Philadelphia, PA). Autoradiograms prepared with Ultrofilm were quantitated by a computer-assisted image analysis system (Loats Associates, Westminster, MD). Optical density readings were converted to final of 125I-ANF bound per mg of protein by using standards prepared from brain paste containing known amounts of 125I (26, 27).

Stability of 125I-ANF. To ensure that the ligand was unchanged by the incubation conditions, aliquots of the 125I-ANF solutions, before and after incubation with tissue sections, were analyzed on a Brownlee RP300 HPLC column. Solution A was 0.1% trifluoroacetic acid and solution B was 0.1% trifluoroacetic acid/95% acetonitrile. The flow rate was 2 ml/min. A linear gradient was followed to 50% solution B in 60 min, and 2-ml fractions were collected. The ligand was eluted as a single peak both before and after incubation with tissue sections (data not shown), demonstrating the stability of the ligand in our incubation conditions.

Animal Models. ANF receptors were examined in tissues from a variety of experimental paradigms. For all injections, control animals received an equal amount of vehicle.

Water deprivation and salt-loading. Sprague–Dawley rats were dehydrated by withholding water for 1, 3, or 5 days. Additional rats were salt-loaded by substituting 2.0% NaCl

Abbreviations: ANF, atrial natriuretic factor; SHR, spontaneously hypertensive rats.

*To whom reprint requests should be addressed.
solution for drinking water for 7 days. Control animals had access to normal drinking water ad libitum.

Desoxycorticosterone acetate treatment. Sprague-Dawley rats were injected with desoxycorticosterone acetate (2.5 mg/kg body weight) dissolved in sesame oil as described (19).

Hypophysectomy. Hypophysectomized and sham-operated Sprague-Dawley rats were obtained from Charles River Breeding Laboratories. Hypophysectomized rats were maintained on 0.9% NaCl, whereas sham-operated rats were given normal drinking water.

Dexamethasone treatment. Dexamethasone (0.25 mg/kg of body weight) in 0.9% NaCl was injected subcutaneously into Sprague-Dawley rats for 10 days.

Lithium treatment. Sprague-Dawley rats were injected subcutaneously with LiCl (20 mg/kg body weight; equivalent to approximately 3 meq/kg of body weight) daily for 12 days. Additional animals were injected with NaCl (3 meq/kg). The average serum Li⁺ level for LiCl-treated rats was 1.2 mM 7 hr after injection on day 12. Serum Li⁺ levels for NaCl-treated and control rats were undetectable.

Diabetic rats. Sprague-Dawley rats were injected through the tail vein with streptozocin (Zanosar) (Upjohn) (65 mg/kg of body weight) in 0.9% NaCl. Blood glucose levels rose from control levels of approximately 100 mg/dl to 470 mg/dl after streptozocin treatment. Animals were sacrificed 28 days after injection.

Adrenalectomy. Sprague-Dawley rats, adrenalectomized or sham-operated as described (28) were obtained from Errol deSouza. Animals were maintained on 0.9% NaCl as drinking water and sacrificed 3 weeks later.

Brattleboro rats. Homozygous and heterozygous Brattleboro and control Long-Evans rats were obtained from Blue Spruce Farms (Altamont, NY).

SHR. Control Wistar–Kyoto rats and SHR were obtained from Charles River Breeding Laboratories.

RESULTS

Localization of ANF Receptors. Autoradiographic grains reflecting [125I]-ANF binding sites displayed a punctate pattern over the renal cortex with little labeling over the renal medulla (Fig. 1). Under higher magnification, silver grains were localized directly over glomeruli (Fig. 2). The labeling was confined to the glomeruli and was not found over the adjacent renal tubules or juxtaglomerular apparatus. We detected similar localizations in rabbit and guinea pig kidneys (data not shown).

In the adrenal gland, highest concentrations of [125I]-ANF binding sites were localized in the zona glomerulosa of the cortex. Lower levels were found in the zona fasciculata and zona reticularis, whereas no binding was found in the adrenal medulla (Fig. 3).

In the brain, others have observed binding of [125I]-ANF to several regions, including the subfornical organ and the area postrema (22, 23, 29). We detected weak binding of [125I]-ANF to these areas but did observe binding to the choroid plexus (Fig. 4). Our failure to observe substantial binding to the brain parenchyma may result from our relatively long wash times and low ligand concentrations.

Binding Characteristics. The physiological relevance of the labeled binding sites was indicated by their extremely high affinity for [125I]-ANF. Displacement of [125I]-ANF binding with unlabeled rat ANF-(1–28) yielded an IC₅₀ value of about 200 pM for binding to kidney glomeruli (Figs. 5 and 6). Scatchard analysis of the data indicates a Bₘₐₓ of 175 fmoi/mg of protein and a Kᵰ of 168 pM. Similar affinities were observed for [125I]-ANF binding to guinea pig and rabbit kidney glomeruli (data not shown). The Bₘₐₓ and Kᵰ values for [125I]-ANF

![Fig. 1. Localization of ANF receptors to renal cortex. Rat kidney tissue sections were incubated as described. Autoradiograms were generated by apposition to tritiated Ultrofilm, and dark-field photomicrographs were printed directly from the film. (A) Tissue section that was incubated in 5 pM [125I]-ANF. (B) Tissue section that was incubated in 5 pM [125I]-ANF with 100 nM unlabeled ANF. Specific binding is punctate and localized in the renal cortex over regions that correspond to glomeruli.](image-url)

![Fig. 2. Localization of ANF receptors to renal glomeruli. Rat kidney tissue sections were labeled and apposed to emulsion-coated coverslips as described. Localization of autoradiographic grains to glomeruli is shown in B and D; that the labeling is over discrete glomeruli in the renal cortex is shown in A and C. (A and B, ×80; C and D, ×160.)](image-url)

![Fig. 3. Localization of adrenal gland ANF receptors. Adrenal gland tissue sections were incubated as described. (A) Incubation in 5 pM [125I]-ANF. (B) Incubation in 5 pM [125I]-ANF with 100 nM unlabeled ANF. Specific grains are found most densely in the zona glomerulosa of the gland (→) with moderate grains over the remainder of the cortex. No specific binding was found over the adrenal medulla (M).](image-url)
yields from these data is (C), of concentrations Rat -2I-ANF with in 3 ly 160% of control levels, hydrated binding to over the is found 100 containing 144 adrenal gland brain and kidney binding over the is adjacent to high-affinity binding to (D), approximately 25% of control levels were: 33 fmol/mg of protein and 144 pM, respectively.

Regulation of ANF Binding Sites. In water-deprived dehydrated rats, kidney receptor levels increased to approximately 160% of control levels in 5 days with increases detectable in 3 days (Figs. 7 and 8). Saturation analysis indicates an increase in $B_{\text{max}}$ with no change in the $K_d$ (Fig. 6). In adrenal glands, water deprivation augmented receptor levels with detectable effects at day 1 and an increase to approximately 125% of control levels by day 5. Salt-loading by substituting 2.0% NaCl for drinking water for 7 days lowered kidney receptor levels by 20% (Table 1). Similarly, receptor levels in diabetic rats were 80% of control values. Several endocrine treatments failed to alter ANF receptor levels substantially. These included adrenalectomy and treatment with desoxycorticosterone acetate. In addition, no differences in receptor levels were detected in either homozygous or heterozygous Brattleboro rats or in SHR (Table 1).

DISCUSSION

The localizations of ANF receptors we observe resemble the preliminary results of others (18-24, 29) and may explain the physiological actions of ANF. Receptors in renal glomeruli may mediate the natriuretic effects of ANF through an increased glomerular filtration rate (30-32). Other studies suggest that ANF acts on the renal medulla (33) and redistributes blood flow through blood vessels of the kidney (32). Such actions may be secondary to ANF-elicited glomerular
events. Alternatively, there may be two subtypes of renal ANF receptors as suggested by physiological experiments, indicating a subtype mediating natriuresis and a second that affects renal blood flow (34). The incubation conditions and relatively long wash times used in this study optimize high-affinity binding to glomeruli. Shorter wash times greatly increase low-affinity binding in the renal medulla and inner portion of the renal cortex (unpublished observations), and others have detected binding in the renal papilla (24). These sites may represent receptors with substantially lower affi-

Table 1. Regulation of renal and adrenal zona glomerulosa ANF receptors

<table>
<thead>
<tr>
<th>Rat treatment</th>
<th>125I-ANF bound, % of control</th>
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<tbody>
<tr>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>110 ± 3 (8)</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>119 ± 7 (6)*</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>119 ± 6 (6)*</td>
</tr>
<tr>
<td>Desoxycorticosterone acetate</td>
<td>102 ± 6 (6)</td>
</tr>
<tr>
<td>Salt-loading</td>
<td>80 ± 5 (6)*</td>
</tr>
<tr>
<td>NaCl injection</td>
<td>103 ± 6 (8)</td>
</tr>
<tr>
<td>LiCl injection</td>
<td>94 ± 5 (8)</td>
</tr>
<tr>
<td>Brattleboro</td>
<td></td>
</tr>
<tr>
<td>Homozygous</td>
<td>87 ± 5 (8)</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>89 ± 6 (8)</td>
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<tr>
<td>Diabetic</td>
<td>80 ± 4 (10)*</td>
</tr>
<tr>
<td>SHR</td>
<td>157 ± 6 (8)*</td>
</tr>
<tr>
<td>Water deprivation</td>
<td>108 ± 3 (10)</td>
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<tr>
<td>Adrenal gland</td>
<td></td>
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<tr>
<td>Diabetic</td>
<td>88 ± 10 (8)</td>
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<tr>
<td>Water deprivation</td>
<td>122 ± 4 (8)*</td>
</tr>
<tr>
<td>SHR</td>
<td>86 ± 4 (8)</td>
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</table>

Animals were treated and the tissue was prepared for autoradiography as described. Cryostat tissue sections (8 µm) of rat kidney or adrenal gland were incubated in 5–10 µM 125I-ANF, and autoradiograms were prepared by using Ultratome. The autoradiograms generated were quantitated by using a computerized image analysis system in the following manner. For kidneys, approximately 10 glomeruli were quantitated from each of two tissue sections from three to five separate rats for each experimental result. Results are expressed as the percentage of the appropriate control value ± SEM for n separate kidneys or adrenal glands shown in parentheses and are from a representative experiment repeated four times.

*P < 0.001.

The proposed receptor localizations and functions of ANF are compared to those proposed for angiotensin II. In the kidney, adrenal zona glomerulosa, blood vessels, posterior pituitary, subfornical organ, and area postrema, these hormones oppose each other. In the choroid plexus and ciliary body, ANF presumably acts independently of angiotensin II. In other regions, such as the adrenal medulla and anterior pituitary, angiotensin II has actions, while ANF has no apparent physiological effect. ADH, antidiuretic hormone; CSF, cerebrospinal fluid.
also found in the uterus (52) where ANF binding has not been reported.

We observe a dynamic relationship between plasma levels of ANF and ANF receptor numbers. Water deprivation, which depresses plasma ANF levels and ANF synthesis (16), markedly augments receptor number in the kidney and adrenal. Conversely, increased levels of circulating ANF in salt-loaded rats decrease receptor levels.

Spontaneously hypertensive rats show little change in ANF receptors even though these animals have increased levels of circulating ANF and increased responsiveness to ANF (21). The reason for this observation is unclear, although the SHR used in our study are younger than those used for many other studies (21). We do observe a small decrease in renal ANF receptors in animals made diabetic through streptozocin treatment, suggesting that levels of ANF receptors may be decreased in at least one form of hypertension.

We thank Dr. Errol deSouza for providing adenalec-tomized rats, Drs. Richard E. Mains, Elizabeth A. Epper, Victor May, and Ron B. Emeson for providing streptozocin-treated rats, and Virginia Wilson, Clark Venable, and Naomi Taylor for expert technical assistance. This work is supported by U.S. Public Health Service Grant DA-00266, RSA Award DA-00074 to S.H.S. and Training Grant GM-07309 to D.R.L.