APRIL 1990
Volume 258/Number 4
Part 1 of Two Parts
ISSN-0002-9513

American Journal of Physiology

PUBLISHED BY
THE AMERICAN PHYSIOLOGICAL SOCIETY

American Journal of Physiology

APRIL 1990/Volume 258, Number 4

American Journal of Physiology: Cell Physiology

Irreversible inhibition of renal Na^+-P_i cotransporter by α-bromophosphonoacetic acid
M. Szczepanska-Konkel, A. Hoppe, J.-T. Lin, and T. P. Dousa C583

Effects of elevated temperature on protein breakdown in muscles from septic rats
M. Hall-Angerås, U. Angerås, P.-O. Hasselgren, and J. E. Fischer C589

Kinetic analysis of the downregulation of epidermal growth factor receptors in rats in vivo
S. Yanai, Y. Sugiyama, T. Iga, T. Fuwa, and M. Hanano C593

Kinetic mechanism of ATP action in Na^+-K^+-Cl^- cotransport of HeLa cells determined by Rb^+ influx studies
T. Ikehara, H. Yamaguchi, K. Hosokawa, and H. Miyamoto C599

Regulation of angiotensin II binding sites in neuronal cultures by protein kinase C
C. J. Kalberg and C. Sumners C610

Contractions of frog tonus fibers and their modification by length changes
E. Bozler C618

Loop diuretic and anion modification of NEM-induced K transport in human red blood cells
L. R. Berkowitz C622

Potassium conductances in tracheal epithelium activated by secretion and cell swelling
A. G. Butt, W. L. Clapp, and R. A. Frizzell C630

Comparison of leukotriene B_4-induced neutrophil migration through different cellular barriers
T. B. Casale and M. K. Abbas C639

Effects of phenylarsine oxide on stimulation of glucose transport in rat skeletal muscle
E. J. Henriksen and J. O. Holloszy C648

Calcium entry in rat parotid acini: activation by carbachol and aluminum fluoride
L. M. Mertz, V. J. Horn, B. J. Baum, and I. S. Ambudkar C654

Voltage-dependent gating of gap junction channels in embryonic chick ventricular cell pairs
R. D. Veenstra C662

Lipoprotein lipase: cellular origin and functional distribution
L. Camps, M. Reina, M. Llobera, S. Vilaró, and T. Olivecrona C673

Replacement of molecular species of phosphatidylcholine: influence on erythrocyte Na transport
B. Engelmann, J. A. F. Op Den Kamp, and B. Roelofsen C682

Atrial natriuretic factor receptors in cultured renomedullary interstitial cells
B. M. A. Fontoura, D. R. Nussenzveig, K. M. Pelton, and T. Maack C692

Alterations of bile acid and bumetanide uptake during culturing of rat hepatocytes
W. Föllmann, E. Petzinger, and R. K. H. Kinne C700

Effect of norepinephrine on Na^+-K^+ pump and Na^+ influx in sheep cardiac Purkinje fibers
S. W. Chae, D. Y. Wang, Q. Y. Gong, and C. O. Lee C713

Exercising mammals synthesize stress proteins
M. Locke, E. G. Noble, and B. G. Atkinson C723

Cover: stereotactic representation of cerebral glucose metabolism in living rats. From L. S. Hibbard and R. A. Hawkins. Three-dimensional reconstruction of metabolic data from quantitative autoradiography of rat brain. *Am. J. Physiol.* 247 (*Endocrinol. Metab.* 10): E412–E419, 1984.

(Contents continued)

3,9 Central nervous system injection of dynorphin-(1—13) overrides gastric satiety factors in sheep
M. A. Della-Fera, C. A. Baile, B. D. Coleman, J. L. Miner, and J. A. Paterson R946

Acetylcholinesterase inhibitor, pyridostigmine bromide, reduces skin blood flow in humans
L. A. Stephenson and M. A. Kolka R951

6,7 A distributed model of fluid and mass transfer in peritoneal dialysis
E. L. Seames, J. W. Moncrief, and R. P. Popovich R958

7,2,5 Differential osmoresponsiveness of periventricular neurons in duck hypothalamus
K. Kanosue, H. Schmid, and E. Simon R973

2,4 Relation of plasma norepinephrine and sympathetic traffic during hypotension in humans
R. F. Rea, D. L. Eckberg, J. M. Fritsch, and D. S. Goldstein R982

5,6 Ethanol attenuates endotoxin-enhanced glucose utilization
P. E. Molina, C. H. Lang, G. J. Bagby, and J. J. Spitzer R987

3,9,10 Phase-shifting the light-dark cycle resets the food-entrainable circadian pacemaker
J. E. Ottenweller, W. N. Tapp, and B. H. Natelson R994

Somatostatin and innervation of the heart of the snake *Elaphe obsoleta*
J. A. Donald, J. E. O'Shea, and H. B. Lillywhite R1001

Different effects of sodium or chloride depletion on angiotensin II receptors in rats
P. E. Ray, E. Castrén, E. J. Ruley, and J. M. Saavedra R1008

5,11,4 Assessing the characteristic between length of hypoxic exposure and serum erythropoietin levels
C. Cahan, P. L. Hoekje, E. Goldwasser, M. J. Decker, and K. P. Strohl R1016

Possible role of basolateral cell membrane in proximal renal tubule osmoregulation
D. Terreros, H. Kanli, and J. Coombs R1022

Hemodynamic responses to leukotriene receptor stimulation in conscious rats
D. E. Allen and M. Gellai R1034

4 Association of DOCA hypertension with induction of atherosclerosis in rats with short-term diabetes mellitus
R. A. Hebden, M. E. Todd, C. Tang, B. Gowen, and J. H. McNeill R1042

Ascending collaterals of medullary barosensitive neurons and C1 cells in rats
J. R. Haselton and P. G. Guyenet R1051

Temperature regulation in biotelemetered spontaneously hypertensive rats
R. M. Morley, C. A. Conn, M. J. Kluger, and A. J. Vander R1064

5 In vivo endotoxin and IL-1 potentiate insulin secretion in pancreatic islets
M. R. Yelich R1070

RAPID COMMUNICATIONS

4,5,7 Distribution of atrial natriuretic peptide receptor subtypes in rat brain
J. Brown and A. Czarnecki R1078

4,5,7 Dehydration increases the density of C receptors for ANF on rat glomerular membranes
M. C. Kollenda, A. M. Vollmar, G. A. McEnroe, and A. L. Gerbes R1084

Propranolol inhibits O_2-sensitive chemoreceptor activity in trout gills
M. L. Burleson and W. K. Milsom R1089

LETTERS TO THE EDITOR

Scaled creatine kinase model
R. J. Connett R1092

Information for Authors appears in the June and December issues

Dehydration increases the density of C receptors for ANF on rat glomerular membranes

MARGIT C. KOLLENDA, ANGELIKA M. VOLLMAR,
GLENN A. McENROE, AND ALEXANDER L. GERBES
Department of Medicine II, Klinikum Grosshadern and Institute of Pharmacology, Toxicology, and Pharmacy, Ludwig-Maximilians-University, 8000 Munich 70, Federal Republic of Germany; and California Biotechnology, Mountain View, California 94043

KOLLENDA, MARGIT C., ANGELIKA M. VOLLMAR, GLENN A. MCENROE, AND ALEXANDER L. GERBES. *Dehydration increases the density of C receptors for ANF on rat glomerular membranes.* Am. J. Physiol. 258 (Regulatory Integrative Comp. Physiol. 27): R1084–R1088, 1990.—The present study determined the presence of two types of binding sites for atrial natriuretic factor (ANF), the B and C receptor, on rat glomerular membranes. The effect of short-term salt loading and dehydration on these two receptor populations was investigated consecutively. Salt-loaded rats did not show significant changes in plasma ANF concentrations or in the number of ANF binding sites. Water-deprived rats presented significantly lower plasma ANF concentrations (22.0 ± 1.9 vs. 34.4 ± 3.8 fmol/ml, $P < 0.01$) and an increase in total receptor density (1,860 ± 398 vs. 987 ± 143 fmol/mg protein) as compared with the control group. Differentiation of both receptor populations showed that it was the C receptors that accounted for this increase (1,772 ± 369 vs. 901 ± 151 fmol/mg protein, $P < 0.05$), whereas B-receptor density was unchanged (89 ± 31 vs. 87 ± 44 fmol/mg protein). These data suggest that C receptors for ANF are affected by changes of body fluid volume.

water deprivation; salt loading; plasma atrial natriuretic factor concentration; B and C receptors for atrial natriuretic factor

ATRIAL NATRIURETIC FACTOR (ANF), a hormone synthesized and secreted by cardiac atrial myocytes (6) is involved in the regulation of body fluid and electrolyte balance. ANF is released into the circulation in response to atrial distension caused by volume expansion (11, 12, 16). Its natriuretic and diuretic activities are mediated by interaction with specific renal receptors. Previous studies demonstrated that two distinct receptor types are present in kidney, a high-affinity binding site (B receptor) and a low-affinity binding site (C receptor) (7, 21). It has been suggested that the B receptor, which is tightly coupled to particulate guanylate cyclase, acts as the biologically active receptor (5), whereas C receptors seem to function as a hormonal buffer system by modulating plasma concentrations of ANF (2).

Studies on rat glomerular receptor status during physiological changes in sodium and water metabolism demonstrated an inverse relationship between plasma ANF concentration and density of ANF receptors (3, 9, 10), indicating that both plasma ANF concentration and glomerular receptor density are influenced by alterations of body fluid volume. So far studies on the effect of changes in extracellular volume referred only to the total ANF binding sites. However, regarding the different properties of B and C receptors, the present study aimed at describing the influence of changes in body fluid volume on both receptor types in renal glomeruli.

METHODS

Animal preparation. Male Sprague-Dawley rats (280–320 g) were kept at constant room temperature and in controlled dark-light periodicity. They received regular pelleted chow and tap water ad libitum for 3 days before the experiment. Then the water-deprived group ($n = 12$) was left without water for 3 days but had free access to food. Salt loading ($n = 12$) was produced by offering 1% saline as a drinking solution for 3 days together with a regular diet. The controls ($n = 12$) were fed a standard rat chow and given tap water ad libitum for 3 days. The animals were decapitated, trunk blood was collected in precooled tubes with EDTA (1 mg/ml), and the kidneys were rapidly excised.

Radioimmunoassay (RIA) for ANF. Blood samples were centrifuged at 1,500 *g* for 10 min at 4°C, and the plasma was stored at −70°C until assayed. Extraction of plasma samples and RIA procedures were modified from (1, 11). Briefly, 200 μl plasma samples were extracted by adsorption to activated Amberlite XAD-2 adsorbent resin (particle size 0.3–1.0 mm, Serva, Heidelberg, FRG) and eluted with acetonitrile-0.1% trifluoracetic acid (80%-20%). Recovery was 70%, and the detection limit of the RIA was 0.5 fmol/tube.

Preparation of glomerular membranes. Rat glomeruli were isolated according to a graded sieving method (22) with some modifications. Kidneys were cut longitudinally, the medulla was removed, and the cortical tissue was filtered through 250-, 140-, and 75-μm sieves. Glomeruli were then suspended in ice-cold phosphate-buffered saline (PBS), pH 7.5, and centrifuged (10,000 *g*, 4°C, 10 min). Membranes were prepared by resuspending the glomeruli in 50 mM tris(hydroxymethyl)aminomethane (Tris)·HCl (pH 7.4) and 1 mM $NaHCO_3$ and by homogenizing with a Polytron (Kinematica, Littau, Switzerland) at 4°C, setting 6, for 3 × 20 s. The homogenate was diluted (1:1) with 50 mM Tris·HCl (pH 7.4), 1 mM EDTA, and 1 mM $MgCl_2$ and was sedimented at 30,000

g for 15 min at 4°C. The pellet was washed once and resuspended in 50 mM Tris·HCl (pH 7.4) and 250 mM sucrose. The membranes were frozen and stored at −70°C. Protein concentration was determined by the method of Lowry et al. (18) using bovine serum albumin (BSA) as standard.

Binding assay. Binding as a function of time at 4°C showed binding equilibrium after 4 h, which remained stable for at least 5 h more. Consequently, binding studies were performed in triplicate at 4°C for 5 h in a buffer containing 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.5, 5 mM $MgCl_2$, 0.2% BSA, 0.1% bacitracin and the following protease inhibitors: 4 μM aprotinin, 8 μg/ml phosphoramidon, 2 μg/ml bestatin, and 40 μg/ml chymostatin (Sigma, Deisenhofen, FRG). Incubation volume was 0.5 ml. For competitive binding studies (in triplicate) the unlabeled peptides of rat ANF (rANF) rANF-(99—126) (Novabiochem, Läufelfingen, Switzerland) or des[Gln^{18},Ser^{19},Gly^{20},Leu^{21},Gly^{22}]rANF-(4—23)-NH_2, a truncated ANF analogue that binds to C receptors only (21), were present in increasing concentrations (10^{-12}–10^{-6} M). Displacement with these two unlabeled ANF peptides allowed differentiation of both receptor types. ^{125}I-rANF-(99—126) (Biotrend, Cologne, FRG; specific activity 2,200 Ci/mmol) concentration was 20 pM for displacement curves and 10–2,000 pM for saturation studies. Specific binding of ANF increased with increasing protein concentration, showing linearity between 5 and 30 μg. Accordingly, binding reactions were initiated by adding 15 μg of glomerular membrane protein, a concentration well within this linear range. Reactions were stopped by dilution with 2.5 ml ice-cold 50 mM Tris·HCl (pH 7.4) and rapid filtration through 1% polyethylenimine-treated Whatman GF/C filters. Filters were washed with 5 ml of 50 mM Tris·HCl (pH 7.4), allowed to dry, and radioactivity was measured in an LKB 1261 Multigamma counter (Turku, Finland) with 80% efficiency.

Affinity cross-linking of ANF receptors. Glomerular membrane proteins (30 μg) were incubated for 5 h at 4°C with 60 pM ^{125}I-rANF-(99—126) in the presence or absence of unlabeled rANF-(99—126). After incubation, the membranes were washed, resuspended in ice-cold PBS buffer (pH 7.5), and incubated with 0.1 mM bis-(sulfosuccinimidyl)suberate (Pierce, Beijerland, Netherlands) for 25 min at 4°C. The reaction was quenched by adding 10 mM ammonium acetate, and the membranes were centrifuged in an Eppendorf centrifuge for 7 min. The pellet was resuspended in 30 μl sample buffer [62 mM Tris·HCl (pH 6.8), 2% sodium dodecyl sulfate (SDS), 10% glycerol, 0.001% bromphenol blue] containing 5% 2-mercaptoethanol and heated for 3 min at 100°C. The membranes were resolved on a 7.5% SDS polyacrylamide gel under constant voltage conditions (Hoefer Scientific Instruments, San Francisco, CA). Standard molecular weight markers were used for calculation of molecular weights of labeled bands (Serva). Gels were stained, dried, and exposed to Kodak X-OMAT AR film with a Kodak X-OMAT regular enhancing screen at −70°C for 14 days.

Analysis of data. The results are expressed as means ± SE. Binding data were analyzed using the LIGAND program (23) to determine the density and affinity of binding sites from competition experiments. The dissociation constant (K_d) and maximum binding sites (B_{max}) were calculated for each animal, and results from each group were compared by the unpaired Student's *t* test. Data from saturation experiments were analyzed by ENZFITTER (Elsevier, BIOSOFT, Cambridge, UK).

RESULTS

Receptor binding characteristics. Figure 1 illustrates that binding of ^{125}I-rANF to glomerular membranes was saturable in a biphasic manner. Scatchard analysis, as shown in the *inset* of Fig. 1, demonstrated the presence of a high-affinity binding site (K_d 8 pM), as reported for the B receptor, and a low-affinity binding site with K_d 500 pM, as shown for the C receptor (8, 21). Receptor density was 249 fmol/mg protein for the high-affinity binding site and 1,865 fmol/mg protein for the low-affinity binding site.

To verify the identity of these binding sites as B and C receptors, we characterized their molecular weight by affinity cross-linking studies. Autoradiography of the dried gel (Fig. 2) showed two bands with the apparent molecular masses of 65 kDa, as reported for the C receptor, and 130 kDa, as shown for the B receptor, respectively. The specificity of ^{125}I-rANF cross-linking was demonstrated by displacement with increasing concentrations of unlabeled ANF (Fig. 2). These data most probably identified the two ANF binding sites determined in our experiments as B and C receptors.

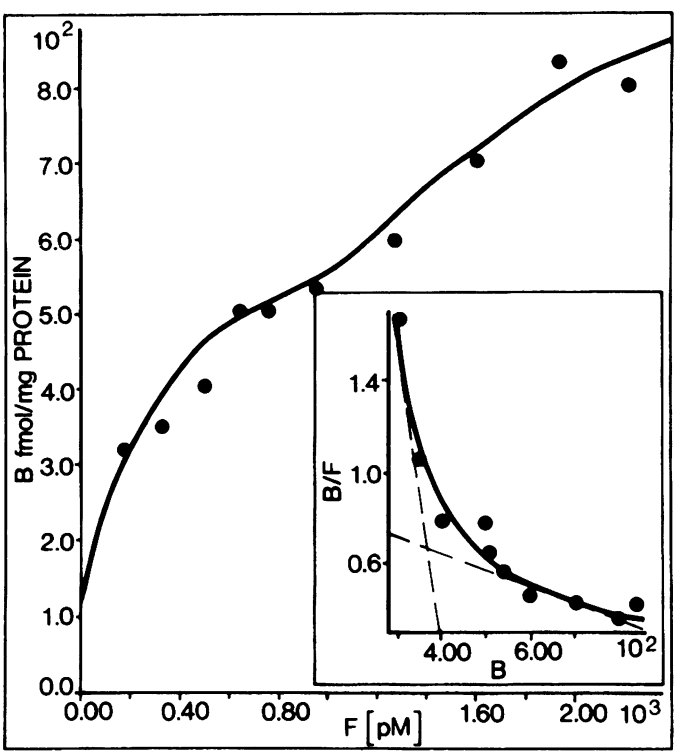

FIG. 1. Saturation binding curve of ^{125}I-rANF-(99—126) to renal glomerular membranes. Membranes were incubated in triplicate in presence of increasing concentrations of ^{125}I-rANF. *Inset*: Scatchard analysis of same data, suggesting two binding sites.

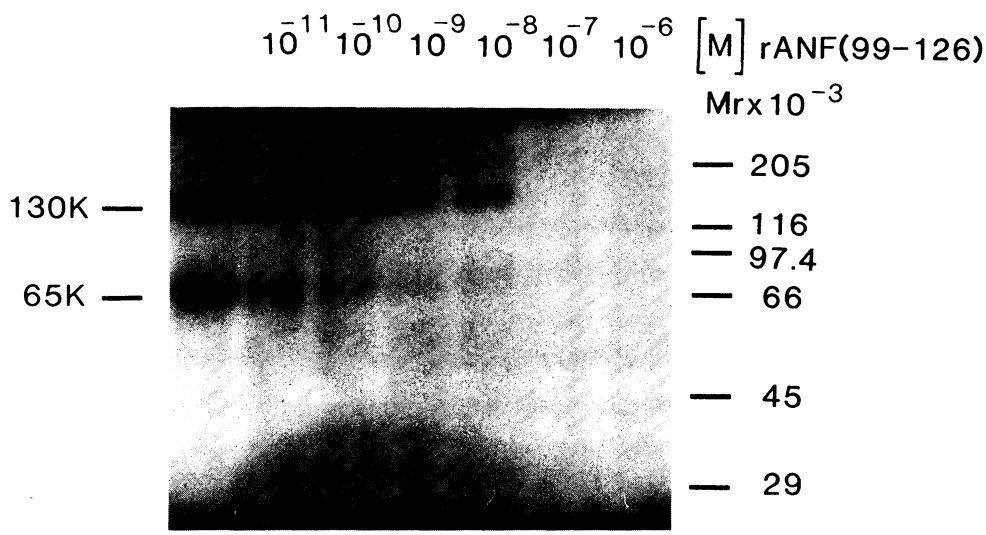

FIG. 2. Autoradiogram of SDS-polyacrylamide gel electrophoresis-resolved rat glomerular membrane protein cross-linked to ^{125}I-rANF in absence and presence of increasing concentrations of unlabeled rANF-(99—126). Specifically labeled bands are at 130 and 65 kDa. Mobilities of molecular weight (M_r) standards are shown.

Short-term salt loading. Salt loading for 3 days significantly increased body weight from 293 ± 11 to 335 ± 7 g ($P < 0.01$). Plasma ANF concentrations of the salt-loaded group did not change significantly as compared with the control group (Table 1). Displacement binding studies with rANF-(99—126) and des(18—22)-rANF-(4—23)NH_2, respectively (Fig. 3), showed no significant difference of B- or C-receptor density as compared with controls (Table 1). No change in receptor affinity to ANF was observed (Table 1).

Dehydration. Water-deprivation for 3 days significantly decreased body weight from 314 ± 12 to 277 ± 8 g ($P < 0.05$). As seen in Table 1 the plasma ANF concentration was reduced from 34.4 ± 3.8 to 22.0 ± 1.9 fmol/ml ($P < 0.01$). Analysis of competition binding curves (Fig. 3) showed that the total receptor density increased from 987 ± 143 to 1,860 ± 398 fmol/mg protein (Table 1). Quantification of both receptor types showed that the increase in receptor density only affected the C-receptor population, which increased significantly from 901 ± 151 to 1,772 ± 369 fmol/mg protein ($P < 0.05$), whereas the density of B receptors remained unchanged (Table 1). There was no significant difference in affinity of B or C receptors to ANF between control and dehydrated rats (Table 1).

TABLE 1. *Effects of salt loading and dehydration on plasma ANF concentrations, density, and affinity of glomerular ANF receptors*

	Controls	Dehydration	High Salt
Plasma ANF concentration, fmol/ml	34.4±3.8	22.0±1.9*‡	35.9±3.2*§
Total binding sites, fmol/mg protein	987±143	1,860±398	924±156†§
B receptor			
B_{max}, fmol/mg protein	87±44	89±31	55±14
K_d, pM	45±36	25±14	21±11
C receptor			
B_{max}, fmol/mg protein	901±151	1,772±369†‡	869±155†§
K_d, pM	311±30	380±104	325±67

Values are means ± SE; $n = 12$ rats. B_{max}, maximum binding sites; K_d, dissociation constant. * $P < 0.01$, † $P < 0.05$, compared with ‡: controls, §: dehydration.

DISCUSSION

In the present study we demonstrated in a first step the presence of B and C receptors for ANF on rat glomerular membranes. This conclusion is supported by the following observations. *1)* Saturation binding experiments and Scatchard analysis clearly demonstrated two distinct ANF binding sites differing in their affinity for ANF (Fig. 1). Dissociation constants calculated for the high- and low-affinity binding sites agreed with those reported for B and C receptors (8). *2)* SDS-polyacrylamide gel electrophoresis-resolved glomerular membranes cross-linked to ^{125}I-rANF showed two specifically labeled bands at 65 and 130 kDa (Fig. 2). Therefore, in accordance with previous studies (17, 24), these two ANF binding sites could be regarded as C receptors (65 kDa) and B receptors (130 kDa), respectively.

Binding studies with displacement by two different ligands were used to differentiate B and C receptors. The discrimination between these two receptor populations allowed us in a second step to address in greater detail the known influence of extracellular volume changes on glomerular ANF binding sites and on plasma ANF concentrations (3, 9, 10, 15, 25). Short-term salt loading, increasing body weight by 14%, and dehydration, decreasing body weight by 12%, were used to induce changes in extracellular volume. Salt loading did not change the total number nor the proportion of B and C receptors on glomerular membranes nor the plasma ANF concentration. In contrast, plasma ANF concentration after water deprivation was significantly reduced while the number of total ANF binding sites increased markedly. The significant increase in total receptor density observed in water-deprived rats was caused by a striking increase in C-receptor density, whereas B-receptor density was not affected (Table 1).

Our results agree with the autoradiographic demonstration that the total number of ANF binding sites was much more affected by dehydration than by salt loading (20). Our findings are in keeping with another observation, that 3 days of salt loading did not affect plasma ANF concentration (19) but are in contrast to other studies, showing that salt loading for between 2 and 14

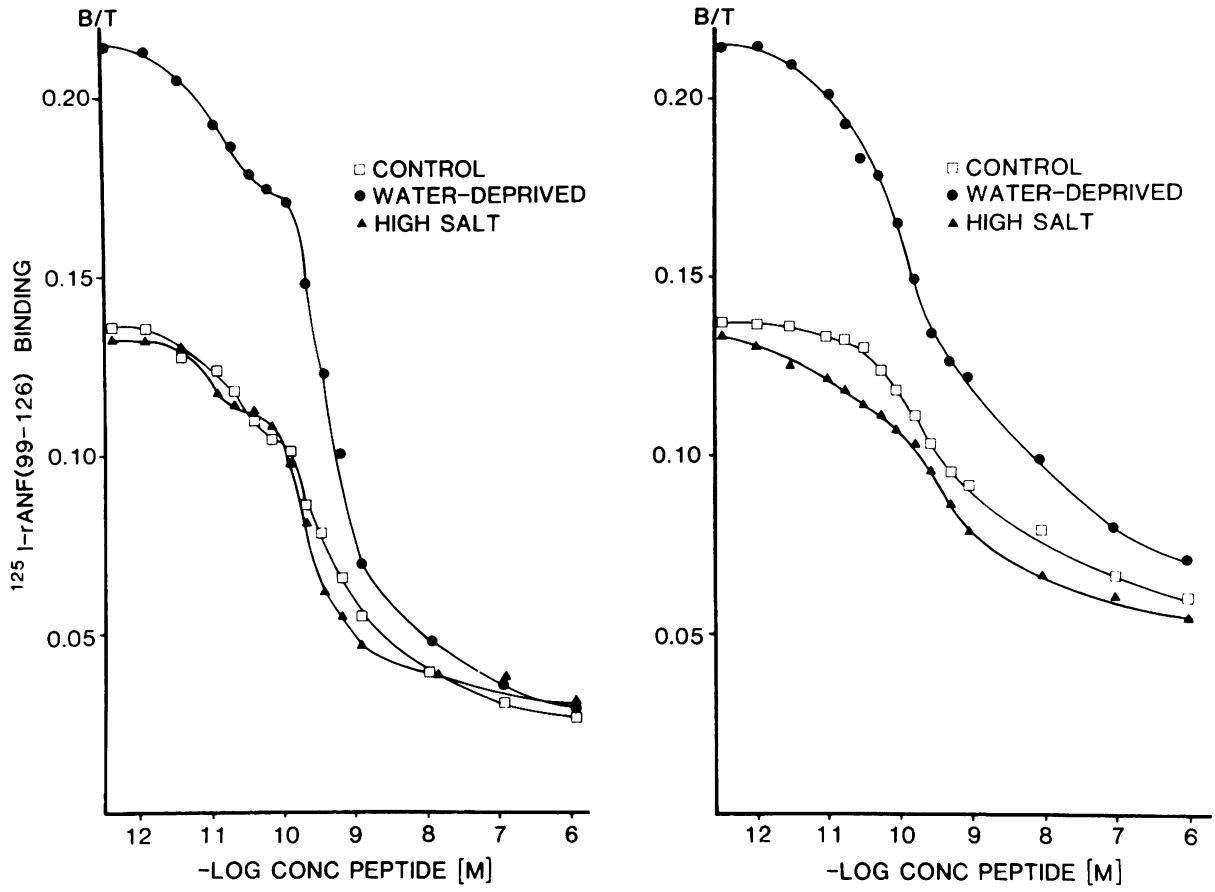

FIG. 3. Representative competition binding curves of ^{125}I-rANF-(99—-126) to glomerular membranes. Membranes from control, dehydrated, and salt-loaded rats were incubated in triplicate with increasing concentrations of unlabeled rANF-(99—126) or des[Gln^{18},Ser^{19},Gly^{20},Leu^{21},Gly^{22}]rANF-(4—23)-NH_2. Results are expressed as bound-total (B/T) vs. total (unlabeled peptide). *Left*: binding of ^{125}I-rANF in presence of unlabeled rANF-(99—126). *Right*: binding of ^{125}I-rANF in presence of unlabeled des(18—22)-rANF-(4—23)-NH_2.

days increased circulating ANF (3, 9, 13, 15, 25, 26). Therefore, whereas different results with a longer period of salt loading cannot be excluded, our results demonstrate that short-term dehydration affects the ANF system more markedly than short-term salt loading.

Our results show that dehydration leads to decreased plasma ANF concentrations and simultaneously to an increase in the number of C receptors. In an effort to teleologically explain these findings, we offer the following hypothesis. Dehydration induces changes in the ANF system (decrease of plasma concentration, increase of clearance receptors), which result in reduced ANF-mediated diuresis. An increase of circulating ANF, e.g., after drinking, during dehydration would cause less renal effects than in normal hydration, due to an augmented removal of ANF by the increased density of C receptors. This again would aim at replenishing body volume. Thus a regulation of plasma ANF concentration and C-receptor density in the way observed by us could reflect an adaptation system for maintaining fluid homeostasis.

Furthermore, our findings might explain several so far poorly understood observations of a blunted biological response to exogenous ANF despite an increase in total glomerular receptor density after various experimental procedures (3, 4, 14). Conditions such as dehydration, low-salt intake, or adrenalectomy would result in low extracellular volume with reduced ANF release. Because all these volume-depleted conditions have been found related to an increase in total glomerular receptor density, the lack of renal response to exogenously administered ANF could be explained by selective alterations of receptor subtypes. An enhancement of C receptors only, at unchanged density of the biologically active B receptors, could account for this seeming contradiction.

In conclusion, our results confirmed the presence of two distinct ANF receptor types on rat glomerular membranes. We demonstrated that after dehydration plasma ANF concentration decreased while total receptor density increased. Quantification of both receptor types showed that C-receptor density was enhanced, whereas the density of B receptors was unaffected, suggesting that C-receptor density might be regulated by plasma ANF concentration. This inverse relationship between plasma ANF concentration and C-receptor density might reflect a mechanism possibly involved in the control of body fluid volume.

The technical assistance of M. Bauch and M. Hummel is gratefully acknowledged. We thank Dr. R. M. Arendt for providing the antibody Toni III, Dr. A. de Léan for helpful discussions, Dr. P. J. Munson and

Prof. J. Remien for clarification of data analysis, and Prof. G. Paumgartner for continuous support.

This work was supported by the Deutsche Forschungsgemeinschaft (Ge 576/2-1).

Address for reprint requests: A. L. Gerbes, Med. Klinik II, Klinikum Grosshadern, Marchioninistrasse 15, 8000 Munich 70, FRG.

Received 27 November 1989; accepted in final form 23 January 1990.

REFERENCES

1. ARENDT, R. M., A. L. GERBES, D. RITTER, AND E. STANGL. Molecular weight heterogeneity of plasma-ANF in cardiovascular disease. *Klin. Wochenschr.* 64, *Suppl.* VI: 97–102, 1986.
2. ALMEIDA, F. A., M. SUZUKI, R. M. SCARBOROUGH, J. A. LEWICKI, AND T. MAACK. Clearance function of type C receptors of atrial natriuretic factor in rats. *Am. J. Physiol.* 256 (*Regulatory Integrative Comp. Physiol.* 25): R469–R475, 1989.
3. BALLERMANN, B. J., R. L. HOOVER, M. J. KARNOVSKY, AND B. M. BRENNER. Physiologic regulation of atrial natriuretic peptide receptors in rat renal glomeruli. *J. Clin. Invest.* 76: 2049–2056, 1985.
4. CACHOFEIRO, V., E. L. SCHIFFRIN, M. CANTIN, AND R. GARCIA. Glomerular and vascular atrial natriuretic factor receptors in adrenalectomized rats. *Am. J. Physiol.* 256 (*Regulatory Integrative Comp. Physiol.* 25): R1250–R1257, 1989.
5. CHINKERS, M., D. L. GARBERS, M.-S. CHANG, D. G. LOWE, H. CHIN, D. V. GOEDDEL, AND S. SCHULZ. A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor. *Nature Lond.* 338: 78–83, 1989.
6. DE BOLD, A. J. Heart atria granularity: effects of changes in water-electrolyte balance. *Proc. Soc. Exp. Biol. Med.* 161: 508–511, 1979.
7. DE LÉAN, A., P. VINAY, AND M. CANTIN. Distribution of atrial natriuretic factor receptors in dog kidney fractions. *FEBS Lett.* 193: 239–242, 1985.
8. FÉTHIÈRE, J., S. MELOCHE, T. T. NGUYEN, H. ONG, AND A. DE LÉAN. Distinct properties of atrial natriuretic factor receptor subpopulations in epithelial and fibroblast cell lines. *Mol. Pharmacol.* 35: 584–592, 1989.
9. GAUQUELIN, G., R. GARCIA, F. CARRIER, M. CANTIN, J. GUTKOWSKA, G. THIBAULT, AND E. L. SCHIFFRIN. Glomerular ANF receptor regulation during changes in sodium and water metabolism. *Am. J. Physiol.* 254 (*Renal Fluid Electrolyte Physiol.* 23): F51–F55, 1988.
10. GAUQUELIN, G., G. THIBAULT, M. CANTIN, E. L. SCHIFFRIN, AND R. GARCIA. Glomerular atrial natriuretic factor receptors during rehydration: plasma NH_2- and COOH-terminal levels. *Am. J. Physiol.* 255 (*Renal Fluid Electrolyte Physiol.* 24): F621–F625, 1988.
11. GERBES, A. L., R. M. ARENDT, R. GERZER, W. SCHNIZER, D. JÜNGST, G. PAUMGARTNER, AND H. WERNZE. Role of atrial natriuretic factor, cGMP and the renin-aldosterone system in acute volume regulation of healthy human subjects. *Eur. J. Clin. Invest.* 18: 425–429, 1988.
12. GERBES, A. L., AND A. M. VOLLMAR. Water immersion increases the concentration of the immunoreactive N-terminal fragment of proatrial natriuretic factor in human plasma. *Biochem. Biophys. Res. Commun.* 156: 228–232, 1988.
13. GUTKOWSKA, J., E. L. SCHIFFRIN, M. CANTIN, AND J. GENEST. Effect of dietary sodium on plasma concentration of immunoreactive atrial natriuretic factor in normal humans. *Clin. Invest. Med.* 9: 222–224, 1986.
14. HINKO, A., M. THIBONNIER, AND J. P. RAPP. Binding characteristics of atrial natriuretic factor and the production of cyclic GMP in kidneys of DAHL salt-sensitive and salt-resistant rats. *Biochem. Biophys. Res. Commun.* 144: 1076–1083, 1987.
15. HOLLISTER, A. S., I. TANAKA, T. IMADA, J. ONROT, I. BIAGGIONI, D. ROBERTSON, AND T. INAGAMI. Sodium loading and posture modulate human atrial natriuretic factor plasma levels. *Hypertension Dallas* 8, *Suppl.* II: 106–111, 1986.
16. LANG, R. E., H. THÖLKEN, D. GANTEN, F. C. LUFT, H. RUSKOAHO, AND T. UNGER. Atrial natriuretic factor—a circulating hormone stimulated by volume loading. *Nature Lond.* 314: 264–266, 1985.
17. LEITMAN, D. C., J. W. ANDRESEN, R. M. CATALANO, S. A. WALDMAN, J. J. TUAN, AND F. MURAD. Atrial natriuretic peptide binding, cross-linking, and stimulation of cyclic GMP accumulation and particulate guanylate cyclase activity in cultured cells. *J. Biol. Chem.* 263: 3720–3728, 1988.
18. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275, 1951.
19. LUFT, F. C., R. B. STERZEL, R. E. LANG, E. M. TRABOLD, R. VEELKEN, H. RUSKOAHO, Y. GAO, D. GANTEN, AND T. UNGER. Atrial natriuretic factor determinations and chronic sodium homeostasis. *Kidney Int.* 29: 1004–1010, 1986.
20. LYNCH, D. R., K. M. BRAAS, AND S. H. SNYDER. Atrial natriuretic factor receptors in rat kidney, adrenal gland, and brain: autoradiographic localization and fluid balance dependent changes. *Proc. Natl. Acad. Sci. USA* 83: 3357–3361, 1986.
21. MAACK, T., M. SUZUKI, F. A. ALMEIDA, D. NUSSENZVEIG, R. M. SCARBOROUGH, G. A. MCENROE, AND J. A. LEWICKI. Physiological role of silent receptors of atrial natriuretic factor. *Science Wash. DC* 238: 675–678, 1987.
22. MISRA, R. P. Isolation of glomeruli from mammalian kidneys by graded sieving. *Am. J. Clin. Pathol.* 58: 135–139, 1974.
23. MUNSON, P. J., AND D. RODBARD. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* 107: 220–239, 1980.
24. OLINS, G. M., D. R. PATTON, P. R. BOVY, AND P. P. MEHTA. A linear analog of atrial natriuretic peptide (ANP) discriminates guanylate cyclase-coupled ANP receptors from non-coupled receptors. *J. Biol. Chem.* 263: 10989–10993, 1988.
25. SAGNELLA, G. A., N. D. MARKANDU, A. C. SHORE, AND G. A. MACGREGOR. Plasma immunoreactive atrial natriuretic peptide and changes in dietary sodium intake in man. *Life Sci.* 40: 139–143, 1986.
26. SCHWARTZ, D., N. C. KATSUBE, AND P. NEEDLEMAN. Atriopeptin release in conditions of altered salt and water balance in the rat. *Biochem. Biophys. Res. Commun.* 137: 922–928, 1986.