Water deprivation enhances the inhibitory effect of natriuretic peptides on cAMP synthesis in rat renal glomeruli

Geoffrey E. Woodard, Xiaohong Li, and Juan A. Rosado

1National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-1752; 2Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029; and 3Department of Physiology, University of Extremadura, 10071 Cáceres, Spain

Submitted 2 March 2004; accepted in final form 27 April 2004

Water deprivation enhances the inhibitory effect of natriuretic peptides on cAMP synthesis in rat renal glomeruli. Am J Physiol Renal Physiol 287: F418–F426, 2004. First published May 4, 2004; 10.1152/ajprenal.00069.2004.—This study investigates the effect of water deprivation on the expression of atrial natriuretic peptide (ANP)1-28 binding sites in rat kidney. Water deprivation increased the Bmax of glomerular binding sites for ANP1-28, BNP, CNP1-22, and the synthetic des[Gln18,Ser19,Gly20,Leu21, Gly22]ANP4-23-amide (C-ANF) (13, 18). C-ANF is virtually without affinity for NPR-A or NPR-B (8, 10, 18, 21). The NPR-C function is still not fully understood and might be responsible for the lysosomal clearance of ANP1-28 (1). In addition, NPR-C also inhibits adenyl cyclase through the activation of a Gi (4, 20), although this effect has not received full acceptance since the discovery of a cGMP-dependent phosphodiesterase of cAMP that is known to account for the ability of ANP1-28 to reduce cAMP levels in some tissues (16, 21). Two NPR-C-like proteins, of 67 and 77 kDa, have been identified in rat glomerular membranes, and their electrophoretic mobilities with and without dithiothreitol suggest that both form disulfide-bridged dimers (11). Both proteins have high affinities for ANP1-28 and C-ANF; however, the 67-kDa protein has a high affinity for CNP1-22, whereas the 77-kDa protein hardly binds this peptide (11). The 67-kDa protein decreases the cAMP levels in rat glomeruli, an effect that was observed in the absence and presence of isobutylmethylxanthine (IBMX), an inhibitor of cGMP-dependent cAMP phosphodiesterase, suggesting that the 67-kDa protein inhibits cAMP synthesis rather than inducing cAMP degradation (11). On the other hand, the 77-kDa protein is involved in natriuretic peptide internalization (11). If the 67- and 77-kDa NPR-C-like proteins of rat glomerular membranes are indeed functionally distinct receptors, it is likely that their expression will be differentially regulated. On the basis of the fact that both NPR-C-like proteins bind ANP1-28 but only the 67-kDa protein has high affinity for CNP1-22, here we show that water deprivation increases the expression of the 67-kDa NPR-C-like protein in rat glomeruli and, therefore, inhibits cAMP synthesis, without affecting ligand binding to the 77-kDa NPR-C-like protein or natriuretic peptide internalization. Furthermore, we show that the NPR antagonist HS-142 binds to the 77- but not the 67-kDa NPR-C-like protein. In addition, we provide further evidence in favor of a role for a G protein in the inhibitory effect of the 67-kDa receptor induced on cAMP synthesis in renal glomeruli.
WATER DEPRIVATION INCREASES NPR-C EXPRESSION

MATERIALS AND METHODS

Materials. Unlabeled peptides and [125I]-tyr-CNP1-22 (1,300–1,900 Ci/mmol) were from Peninsula Laboratories (St. Helens, Merseyside, UK). [125I]-ANP1-28, [125I]-CNP1-22, [3H] standard, Hyperfilm H2 for autoradiography, and cAMP radioimmunoassay commercial kits were from Amersham (Little Chalfont, UK). Bisis(sulfosuccinimidyl) suberate (BS3) was from Pierce and Warriner (Chester, UK). GRI-AX film was from Genetic Research Instrumentation (Dunmow, Essex, UK). All other reagents were from Sigma (Poole, UK).

Animals. Wistar rats (250–300 g) were obtained from a certified commercial supplier (Charles River). Control groups were allowed free access to food and water. Matching groups were kept under identical conditions, except that they received no water for 4 days. Rats were anesthetized with ether and killed by rapid exsanguination.

The APS’s Guiding Principles in the Care and Use of Laboratory Animals were followed.

Competition experiments in isolated glomeruli. Isolation of rat glomeruli was performed as described previously (24). Briefly, kidneys were removed and placed in ice-cold Hank’s balanced salt solution (HBSS) containing (in mM) 137 NaCl, 10 HEPES, 5.4 KCl, 0.4 MgSO4, 0.34 Na2HPO4, 1.26 CaCl2, 4.17 NaHCO3, 0.44 K2HPO4, 0.49 MgCl2, and 5.56 glucose, pH 7.2, as well as 0.2% (wt/vol) BSA. The cortexes were minced, and glomeruli were isolated by differential sieving. Aliquots of glomeruli were preincubated with 1 mM phenanthroline for 6 min at 4°C and then incubated with 400 pM [125I]-ANP1-28 or [125I]-CNP1-22 in the presence of increasing concentrations of unlabeled peptides at 20°C for 10 min. Preliminary experiments showed that specific binding of radioligand reached equilibrium at 10 min (not shown). Incubations were stopped by centrifugation, and [125I] labeling was determined using a Packard Gamma Counter.

Binding assay of particulate ANP receptors in membranes. Freshly isolated renal papilla was homogenized and centrifuged at 1,000 g for 10 min at 4°C. The supernatant was then centrifuged for 60 min at 4°C at 40,000 g, and the pellet was washed, sonicated, and stored. Aliquots (10 μg) of papillary membrane were incubated for 1 h or 40 min with 200 pM [125I]-ANP1-28 or [125I]-CNP1-22 in the absence or presence of increasing concentrations of unlabeled natriuretic peptides in Tris buffer containing (in mM) 50 Tris, 4 MgCl2, and 1,1,10-phenanthroline, pH 7.6, as well as 0.4% (wt/vol) BSA. Incubation was stopped by filtration. The filter-bound radioactivity was determined in a Packard Gamma Counter. The nonspecific binding was measured in the presence of 1 μM ANP1-28.

Affinity cross-linking studies. Freshly isolated glomeruli were homogenized and centrifuged at 1,000 g for 10 min at 4°C. The supernatant was then centrifuged for 60 min at 4°C at 40,000 g, and the pellet was washed, sonicated, and stored. Aliquots (10 μg) of glomerular membranes (50 μg) were incubated with [125I]-ANP1-28 in the presence or absence of the indicated unlabeled peptides and inhibitors at 4°C for 2 h. Preliminary experiments showed that specific binding of the radioligand reached equilibrium after 2 h. The bound [125I]-ANP1-28 was then cross-linked to its receptor by 500 μM BS3 for 40 min at 4°C. The cross-linking reaction was stopped, and the samples were resuspended in Laemmli’s buffer (19) and separated by 10% SDS-PAGE. The autoradiographs were exposed for 31 days at −80°C.

Autodigestive studies. Kidney slides from male spontaneously hypertensive and Wistar-Kyoto rats in PBS containing (in mM) 120 NaCl, 21.6 Na2HPO4, 8.4 NaH2PO4, pH 7.2, with 1,10-phenanthroline were incubated with 100 pM [125I]-ANP1-28 or 200 pM [125I]-CNP1-22 (2,000 Ci/mmol) with or without unlabeled peptides at 20°C for 15 min. After incubation, the sections were exposed to Hyperfilm H2 for 31 days.

cAMP accumulation. Aliquots of 100 glomeruli (7.6 mg protein) from control and water-deprived rats were suspended in HBSS plus 0.2% BSA with 1 mM IBMX and then incubated for 10 min at 20°C with different agonists in the absence or presence of natriuretic peptides and/or HS-142 as indicated. Incubations were terminated by ice-cold trichloroacetic acid as previously described (10, 11). Aliquots were then centrifuged at 4,000 g for 10 min, and the supernatants were extracted with ether and radioimmunoassayed for cAMP as previously described (10, 11).

cGMP assay. cGMP production in glomerular membranes was determined as described previously (36). Glomerular membranes were diluted with Tris buffer such that 20 μl of this mixture solution contained 3–5 μg of protein. Guanylate cyclase activity was measured at 37°C in a reaction mixture containing (in mM) 50 Tris-HCl, 4 MgCl2, 1 IBMX, 1 GTP-Mg2+, 15 creatine phosphate, and 1 ATP as well as 20 U/ml creatine phosphokinase, pH 7.6. The reaction was started with the addition of the membrane suspension and stopped 20 min later with ice-cold 50 mM sodium acetate (pH 5.8) followed by boiling. The samples were then centrifuged at 4,000 g for 10 min, and cGMP was determined by radioimmunoassay.

Ligand internalization. [125I]-ANP1-28 internalization was studied in glomeruli from control and 4-day water-deprived rats. Aliquots of 2,400 glomeruli were incubated in HBSS containing 0.2% BSA, 1 mM 1,10 phenanthroline, and 1 nM [125I]-ANP1-28 (specific activity 500 Ci/mmol) with or without 1 μM ANP1-28, 10 μM C-ANF, or 30 ng/ml HS-142 for 1 h at 4°C and then for 10 min at 37°C. The incubation was stopped by centrifugation at 1,000 g for 2 min at 0°C, and internalized and surface-bound radioligands were measured. Internalized radioligand was measured after the addition of acetic acid-NaCl [final concentration of 0.2 M acetic acid-0.2 M NaCl (pH 2.5)] for 6 min at 4°C followed by centrifugation at 1,000 g for 2 min at 4°C and analysis of [125I] associated with sedimented glomeruli.

Data analysis. Data were analyzed using the LIGAND program. Analysis of statistical significance was performed using Student’s t-test. The significance level was P < 0.05.

RESULTS

Effects of water deprivation on renal binding of natriuretic peptides. Comparison of autoradiography with the corresponding stained tissue sections revealed a similar anatomic distribution of specifically reversible binding sites for ANP in control and water-deprived rats. [125I]-ANP1-28 and [125I]-CNP1-22 bound mostly to glomeruli. Radioligand binding to these structures was virtually abolished in the presence of 1 μM unlabeled ANP1-28, CNP1-22, and C-ANF (Fig. 1; n = 6).

Competitive inhibition of [125I]-ANP1-28 binding by ANP1-28 was further examined in control and water-deprived rats. As shown in Fig. 2, ANP1-28 displaced [125I]-ANP1-28 in a concentration-dependent manner in both control and water-deprived rats. Water deprivation increased the Bmax of glomerular binding sites for ANP1-28 without significantly altering their affinity for this peptide (Fig. 2, Table 1). A similar effect was observed when CNP1-22 binding was tested. Water deprivation increased the Bmax of the glomerular binding sites for CNP1-22 without significantly modifying its affinity (Table 1).

In contrast, we have found that water deprivation had no significant effects on ANP1-28 binding to the papilla (Fig. 3), which expresses NPR-A but does not detectably express NPR-C in the rat (9).

Interestingly, C-ANF abolished the difference observed in ANP1-28 glomerular binding in control and water-deprived rats. Thus the specific glomerular binding of 125I-ANP1-28 in the absence of C-ANF was 377 ± 26 and 625 ± 99 fmol/mg protein, respectively, in control and water-deprived rats (P < 0.001), whereas the corresponding figures were 134 ± 61 and 169 ± 72 fmol/mg protein with C-ANF. These findings suggest that water deprivation increases the expression of the
NPR-C-like binding sites in renal glomeruli without affecting the expression of NPR-A, in agreement with previous studies (6, 14, 17).

Affinity cross-linking of ANP receptors in glomerular membranes from control and water-deprived rats. Cross-linking studies using glomerular membranes from control and water-deprived rats revealed that 125I-ANP 1-28 was covalently incorporated into three bands with apparent molecular masses of 120, 77, and 67 kDa (Fig. 4). The band with a molecular mass of 120 kDa corresponds to NPR-A (29). In control and water-deprived rats, labeling of the 120-kDa band was completely displaced by incubation with 1 μM unlabeled ANP 1-28, ANP 1-28 and CNP 1-22 (C and c), and C-ANF (D and d) at 20°C for 15 min. The labeling of the 120-kDa protein was partially displaced by increasing concentrations of unlabeled CNP 1-22 (Fig. 4), demonstrating the specificity of the NPR-A receptor. The labeling of the 67- and 77-kDa bands was progressively displaced by increasing concentrations of unlabeled CNP 1-22 (Fig. 4) or C-ANF (not shown). These proteins, therefore, corresponded to the 67- and 77-kDa NPR-C-like proteins in the rat glomerulus. Although the labeling intensity of NPR-A and the 77-kDa NPR-C-like protein was not affected by water deprivation (Fig. 4), that of the 67-kDa NPR-C-like protein was clearly increased by water deprivation (Fig. 4), consistent with the results reported in the previous section, which suggest that water deprivation increases the B_{max} of the 67-kDa NPR-C-like protein.

Glomerular cAMP production in control and water-deprived rats. The basal rate of glomerular cAMP accumulation in the presence of IBMX was 4.8 ± 0.3 and 4.7 ± 0.3 fmol/mg protein in control and water-deprived rats, respectively. Treatment of renal glomeruli with 10 μM forskolin significantly

Fig. 1. Autoradiographs of renal binding of 100 pM 125I-labeled atrial natriuretic peptide (ANP) 1-28 and 200 pM 125I-C-type natriuretic peptide (CNP) 1-22 in control and water-deprived rats. Left: sections of kidney from control (A–D) or water-deprived (a–d) rats were incubated with 100 pM 125I-ANP 1-28 in the absence (A and a) and presence of 1 μM ANP 1-28 (B and b), CNP 1-22 (C and c), and C-ANF (D and d) at 20°C for 15 min. Right: sections of kidney from control (A–D) or water-deprived (a–d) rats were incubated with 200 pM 125I-CNP 1-22 in the absence (A and a) and presence of 1 μM CNP 1-22 (B and b), ANP 1-28 (C and c), and C-ANF (D and d) at 20°C for 15 min. Autoradiography was performed as described in MATERIALS AND METHODS.
increased glomerular cAMP production in control and water-deprived rats to 14/11006 1.2 and 13.8/11006 0.9 fmol/mg protein, respectively (P/11021 0.05; n/11005 6). We have found that treatment of renal glomeruli with CNP 1-22 reduced basal cAMP production in a concentration-dependent manner (Fig. 5A; n/11005 12). These effects were significantly greater in glomeruli from water-deprived rats (Fig. 5A; P/11021 0.05). ANP 1-28 and C-ANF also inhibited cAMP production by forskolin (Fig. 5A).

Table 1. Binding constants for the specifically reversible binding of ANP<sub>1-28</sub> or CNP<sub>1-22</sub> in the glomeruli of control and 4-day water-deprived rats

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Control Rats</th>
<th>Water-Deprived Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pK&lt;sub&gt;a&lt;/sub&gt;</td>
<td>K&lt;sub&gt;d&lt;/sub&gt;, nM</td>
</tr>
<tr>
<td>HA+A</td>
<td>8.63±0.61</td>
<td>2.31</td>
</tr>
<tr>
<td>HA+C</td>
<td>8.33±0.02</td>
<td>4.63</td>
</tr>
<tr>
<td>HC+C</td>
<td>8.63±0.30</td>
<td>2.34</td>
</tr>
</tbody>
</table>

Values are means ± SE. ANP and CNP, atrial and C-type natriuretic peptide, respectively; HA, 125I-ANP<sub>1-28</sub>; A, ANP<sub>1-28</sub>; HC, 125I-CNP<sub>1-22</sub>; C, CNP<sub>1-22</sub>. The maximum binding capacities (B<sub>max</sub>) and pK<sub>a</sub> were assessed from the competitive inhibition of the binding of 100 pM 125I-ANP<sub>1-28</sub> and 200 pM 125I-CNP<sub>1-22</sub> by various concentrations of unlabeled ANP<sub>1-28</sub> or CNP<sub>1-22</sub>. Apparent dissociation constants (K<sub>d</sub>) are also given. *P < 0.05 relative to controls.
greater inhibitory effect was observed when agonists such as histamine and 5-hydroxytryptamine were used to increase cAMP synthesis. ANP1-28, CNP1-22, and C-ANF significantly reduced cAMP production by histamine and 5-hydroxytryptamine in control and water-deprived rats, although the inhibitory effect was found to be significantly greater in water-deprived rats (Fig. 5, B and C; P < 0.05). These findings suggest that water deprivation increases the glomerular expression of the 67-kDa NPR-C-like protein. The greater inhibitory effect of natriuretic peptides on cAMP production stimulated by forskolin or the physiological agonists might be due to the different degree of activation of adenylate cyclase. Whereas cAMP levels in renal glomeruli from control and water-deprived rats were increased to 14 fmol/mg protein after treatment with 10 μM forskolin, stimulation with 10 μM histamine or 5-hydroxytryptamine increased cAMP levels to 6.0 ± 0.5 and 6.8 ± 0.3 fmol/mg protein, respectively. These findings support the physiological significance of the inhibitory effect of natriuretic peptides on cAMP production in renal glomeruli.

Effects of HS-142 on ligand binding, internalization, and cyclic nucleotide production. HS-142 is a competitive antagonist of ANP1-28 binding sites (25). Consistent with this, we have found that treatment with HS-142 abolishes ANP1-28-stimulated cGMP production (Fig. 6A). Our results indicate that HS-142 inhibited the labeling of NPR-A and the 77-kDa NPR-C-like protein by 100 pM 125I-ANP1-28, reaching complete inhibition at 40 μg/ml (Fig. 6B). In contrast, the affinity of the 67-kDa NPR-C-like protein for HS-142 was very low because no significant decrease in labeling of this protein was detected even at 100 μg/ml HS-142 (Fig. 6B).

Specific binding of 125I-ANP1-28 to glomerular sites might be removed by acidic washing, and only the labeling of internalized peptides would remain. Our results indicate that most of the specific binding of 125I-ANP1-28 was removed by acidic washing, but a significant acid-resistant component, which could be abolished in the presence of 10 μM C-ANF, remained (not shown). The magnitude of this acid-resistant component was similar in glomeruli from control and water-deprived rats (not shown). Treatment of renal glomeruli with 30 μg/ml HS-142 significantly reduced the acid-resistant component (Fig. 7A) without affecting the inhibitory effect of ANP1-28 on the histamine-induced increase in cAMP production (Fig. 7B).

Effects of nucleotides on ligand binding. Quantitative in vitro autoradiography demonstrated that increasing concentrations of GTPγS progressively inhibited the specific binding of 100 pM 125I-CNP1-22 to renal glomeruli (Fig. 8A). As shown in Fig. 8B, GTPγS but not GDP reduced 125I-ANP1-28 binding to glomerular sites in a concentration-dependent manner. In addition, GTPγS prevented labeling of the 67-kDa NPR-C-like protein by 125I-ANP1-28 without significantly affecting the radiolabeling of NPR-A or the 77-kDa protein (Fig. 8B). ATPγS had no such selective action. These results confirm that the specific binding site of subnanomolar concentrations of 125I-CNP1-22 in the rat kidney is the 67-kDa NPR-C-like protein, whose action is modulated by GTP-binding proteins.

DISCUSSION

Our results indicate that water deprivation increases the expression of the 67-kDa NPR-C-like protein in renal glomeruli, where dense localization of NPR-C has been previously demonstrated by immunohistochemistry and in situ hybridization (26, 33). As a result, ANP1-28, CNP1-22, and C-ANF show a greater inhibitory effect on cAMP production stimulated by forskolin or the agonists histamine and 5-hydroxytryptamine in glomeruli from water-deprived rats compared with those from controls.

The present study supports the idea that the 67-kDa NPR-C-like protein modulates glomerular cAMP synthesis. This hypothesis is based on our observation that subnanomolar CNP1-22 concentrations inhibited forskolin-induced cAMP synthesis in rat glomeruli. Previous studies have demonstrated that the 67-kDa protein shows high affinity for CNP1-22 in glomeruli from normal rats (11), and the present in vitro results
were consistent with that conclusion in both control and water-deprived rats; therefore, this protein is the only one that can account for the effects of subnanomolar concentrations of CNP1-22. cAMP levels were examined in the presence of IBMX, a phosphodiesterase inhibitor, which completely inhibits the cGMP-activated phosphodiesterase that may account for the effects of natriuretic peptides on cAMP levels (7). Therefore, our findings suggest that natriuretic peptides induce a greater reduction of cAMP levels in glomeruli from water-deprived rats by decreasing the rate of cAMP synthesis more effectively in these glomeruli. Our results indicate that natriuretic peptides induce a greater inhibition of cAMP production stimulated by physiological agonists, such as histamine or 5-hydroxytryptamine, which further suggests the physiological significance of this function.

The effects of GTPγS further implicate the 67-kDa protein in the control of glomerular adenylate cyclase activity. Both ANP1-28 and C-ANF inhibit adenylate cyclase activity in rat heart and kidney only if GTP is present (2, 5). This effect is inhibited by pertussis toxin in association with the ADP ribosylation of a 40-kDa protein, which is probably G_{i} (3). In agreement with the fact that the ligand affinity of G protein-coupled receptors is reduced by guanine nucleotides (15), we have found that GTPγS inhibited ligand binding to glomerular sites, specifically to the 67-kDa receptor, whereas GDP and ATPγS had no effect, suggesting that a G protein may interact with the 67-kDa glomerular protein.

Water deprivation approximately doubled the B_{max} of ANP1-28 and CNP1-22 binding sites, corresponding to NPR-C in renal glomeruli, without affecting ligand affinity. Our results indicate that water deprivation did not significantly alter 125I-ANP1-28 internalization, which involves the 77-kDa protein because 125I-CNP1-22 at subnanomolar concentrations, which only label the 67-kDa receptor, are not internalized, and that low CNP1-22 concentrations abolish 125I-ANP1-28 binding to the 67-kDa receptor without affecting its internalization. This evidence indicates that the 77-kDa NPR-C-like receptor is the only one involved in peptide internalization, and therefore the

---

**Fig. 5.** Effect of natriuretic peptides on cAMP generation in glomeruli from control and water-deprived rats. Glomeruli from control and water-deprived rats were stimulated with 10 μM forskolin (A), 10 μM histamine (B), or 10 μM 5-hydroxytryptamine (C) in the absence and presence of ANP1-28, CNP1-22, or C-atrial natriuretic factor (C-ANF). cAMP synthesis was measured as described in MATERIALS AND METHODS. Values are means ± se of 12 separate experiments expressed as percentage of control (agonist-treated glomeruli). *p < 0.05 compared with the effect observed in control rats.
increased NPR-C $B_{\text{max}}$ induced by water deprivation might be explained by an increase in 67-kDa protein expression. The conclusion that the 77-kDa NPR-C-like receptor is the only component involved in ligand internalization is supported by the results obtained using HS-142, an ANP-receptor antagonist. We have found that HS-142 abolished $^{35}$S-ANP$_{1-28}$ binding to NPR-A and the 77-kDa NPR-C-like protein; however, HS-142 did not modify ANP$_{1-28}$-induced inhibition of forskolin-dependent cAMP production. This observation confirms that the 77-kDa protein is not involved in regulating the synthesis of cAMP. It is likely that, as reported above, the 67-kDa protein is the only glomerular natriuretic peptide receptor to account for the effects of these peptides on cAMP production in the presence of HS-142.

The increased expression of the 67-kDa NPR-C-like protein in water-deprived rat kidney suggests that this receptor might be involved in the regulation of body fluid. There is a body of candidates for peptide internalization, NPR-A and the 77-kDa protein. The use of C-ANF, which has no affinity for NPR-A, clarifies this issue, because it abolished ligand internalization and therefore presents the 77-kDa NPR-C-like protein as being responsible for peptide internalization in renal glomeruli. In addition, we have found that HS-142 abolished $^{125}$I-ANP$_{1-28}$ binding to NPR-A and the 77-kDa NPR-C-like protein; however, HS-142 did not modify ANP$_{1-28}$-induced inhibition of forskolin-dependent cAMP production. This observation confirms that the 77-kDa protein is not involved in regulating the synthesis of cAMP. It is likely that, as reported above, the 67-kDa protein is the only glomerular natriuretic peptide receptor to account for the effects of these peptides on cAMP production in the presence of HS-142.

Fig. 6. Effect of HS-142 on ANP$_{1-28}$-induced cGMP production and specific $^{125}$I-ANP$_{1-28}$ binding. A: renal glomeruli were incubated with 1 μM ANP$_{1-28}$ in the presence or absence of 40 μg/ml HS-142, and cGMP production was determined as described in MATERIALS AND METHODS. B: renal glomeruli were incubated with 100 μM $^{125}$I-ANP$_{1-28}$ in the presence or absence of increasing concentrations of HS-142 as indicated. Affinity cross-linked proteins were dissolved in the presence of DTT and then separated by 10% SDS-PAGE. The autoradiographs were exposed for 31 days at −80°C. Each autoradiogram is representative of 6 separate determinations in different rats.

Fig. 7. Effect of HS-142 on peptide internalization and cAMP production. A: specific binding of 200 μM $^{125}$I-ANP$_{1-28}$ was analyzed in the absence or presence of 40 μg/ml HS-142. B: glomeruli from control rats were stimulated with 10 μM histamine in the absence and presence of ANP$_{1-28}$ and HS-142. cAMP synthesis was measured as described in MATERIALS AND METHODS. Values are expressed as percentage of control (agonist-treated glomeruli) and are presented as means ± SE of 6 separate experiments. *P < 0.05 compared with the effect induced by histamine.
evidence suggesting that natriuretic peptides are involved in the regulation of electrolyte balance, extracellular fluid volume, and blood pressure (22, 23, 27). Water deprivation has been shown to increase the density of glomerular NPRs in rats, which is accompanied by a decrease in plasma ANP (14, 17).

The greater total receptor density observed in water-deprived rats was caused by a striking increase in NPR-C density, whereas NPR-A density was not affected (17). Consistent with this, our results show an increased expression of NPR-C in water-deprived rats, which is due to the enhanced expression of the 67-kDa NPR-C-like protein. The activation of this protein by natriuretic peptides induces inhibition of cAMP production. A number of studies have reported that vasoactive agents, such as histamine or prostaglandins, increase cAMP generation in mesangial cells, which occupy a central position in the renal glomeruli, leading to cell relaxation and an increase in renal filtration and diuresis (30, 31). Therefore, the enhanced expression of the 67-kDa NPR-C-like protein induced by water deprivation might have a physiological role in the regulation of fluid homeostasis through the inhibition of cAMP synthesis and diuresis.
In summary, our observations demonstrate that water deprivation increases the expression of the 67-kDa NPR-C-like protein in rat glomeruli, without affecting the 77-kDa NPR-C-like protein receptor. Our findings support the hypothesis that the 77-kDa protein is involved in peptide internalization, whereas the 67-kDa NPR-C-like protein inhibits adenylate cyclase activity; therefore, water deprivation enhanced the inhibitory role of natriuretic peptides in cAMP synthesis, which is involved in the regulation of renal physiology (32, 37). These results suggest that the inhibition of adenylate cyclase by water deprivation may be one of the mechanisms through which natriuretic peptides regulate kidney functions.

ACKNOWLEDGMENTS

The British Heart Foundation supported this work.

REFERENCES


