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The heart communicates with the kidney exclusively through the guanylyl cyclase-A receptor: Acute handling of sodium and water in response to volume expansion

(hypertension/atrial natriuretic peptide/cyclic GMP/natriuresis/diuresis)

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ABSTRACT **Disruption of guanylyl cyclase-A (GC-A) results in mice displaying an elevated blood pressure, which is not altered by high or low dietary salt. However, atrial natriuretic peptide (ANP), a proposed ligand for GC-A, has been suggested as critical for the maintenance of normal blood pressure during high salt intake. In this report, we show that infusion of ANP results in substantial natriuresis and diuresis in wild-type mice but fails to cause significant changes in sodium excretion or urine output in GC-A-deficient mice. ANP, therefore, appears to signal through GC-A in the kidney. Other natriuretic/diuretic factors could be released from the heart. Therefore, acute volume expansion was used as a means to cause release of granules from the atrium of the heart. That granule release occurred was confirmed by measurements of plasma ANP concentrations, which were markedly elevated in both wild-type and GC-A-null mice. After volume expansion, urine output as well as urinary sodium and cyclic GMP excretion increased rapidly and markedly in wild-type mice, but the rapid increases were abolished in GC-A-deficient animals. These results strongly suggest that natriuretic/diuretic factors released from the heart function exclusively through GC-A.**

Since the discovery that mammalian heart atria synthesize and secrete atrial natriuretic peptide (ANP) (1, 2), the heart has been inexorably linked to the regulation of kidney function by a direct hormonal axis. ANP, stored as pro-ANP within granules of the heart, is released in response to atrial stretch (3, 4). Upon secretion, pro-ANP is cleaved by a processing enzyme to yield the peptide normally referred to as ANP [ANP-(99–126)] and the amino-terminal region [pro-ANP-(1–98)], both of which circulate. Acute infusion of ANP causes such responses as diuresis, natriuresis, hypotension, and inhibition of aldosterone secretion (5). The complete composition of the granules of the heart is not known, but B-type natriuretic peptide (BNP), a peptide synthesized principally within the ventricles of the heart (6), is also found within the atrial granules, although at much lower levels than ANP (7).

Both the ANP-clearance receptor (ANP-CR) and guanylyl cyclase-A (GC-A) have been suggested as the molecular targets for ANP action (8), and since no other receptors with high affinity for BNP have been discovered, GC-A and ANP-CR also have been suggested as the BNP receptors.

GC-A appears to contain a single transmembrane region that separates an extracellular ligand binding domain from two intracellular domains, one homologous to protein kinase catalytic domains and the other homologous to cyclase catalytic domains (9). The ANP-CR, in contrast, contains an extracellular ligand binding domain that is homologous to the same domain found in GC-A, a single transmembrane region, and a short cytoplasmic segment of about 37 amino acids (10). ANP-CR has been reported to function in two manners: to clear ANP from the circulation (11, 12) and to signal through interactions with G proteins (13).

Disruption of the gene for ANP, which also resulted in no atrial granules in homozygous null mice, led to animals who displayed a salt-sensitive elevation in blood pressure (14). However, disruption of the gene for GC-A resulted in an elevated, but salt-resistant, blood pressure (15). The differential phenotypes suggested that ANP might act through a receptor other than GC-A or that the ANP gene disruption also eliminated other natriuretic factors present either within the granules of the atria or within the amino-terminal regions of pro-ANP.

Using GC-A-deficient mice, we now directly demonstrate that GC-A is essential for ANP-induced acute regulation of diuresis and natriuresis. Furthermore, acute vascular volume expansion, which causes release of cardiac atrial granules, demonstrates that no natriuretic/diuretic response is seen in the absence of GC-A. Thus, GC-A appears to be the kidney receptor for natriuretic/diuretic factors released from granules of the heart, whether they be ANP, BNP, or others.

METHODS

Surgical Procedures. All studies were carried out under protocols reviewed and approved by the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center. The generation of GC-A-deficient mice has been described elsewhere (15). Studies were carried out in young adult GC-A-deficient mice (average body weight = 27.2 ± 1.1 g, n = 12) and their age-matched, wild-type litter mates (average body weight = 29.4 ± 1.3 g, n = 12). The mice were anesthetized with methoxyflurane (Pitman-Moore, IL) and placed on a heating pad (Harvard Apparatus, MA) to maintain rectal temperatures at 37°C. A femoral vein was cannulated with a tapered polyethylene catheter (intramedic PE-10 tubing; Becton Dickinson, MD) for intravenous infusion. The bladder was exposed and catheterized through a suprapubic incision with PE-10 tubing. Urine was collected into preweighed plastic tubes via the bladder catheter. On completion of the surgery, 360 μl of lactated Ringer's solution (Baxter, IL) containing 2% bovine serum albumin (Sigma, MO) was infused intravenously over 30 min followed by constant infusion of the same solution at 120 μl/hr throughout the duration of the study. These infusions were carried out with a microinfusion pump (Harvard Appa-

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Abbreviations: ANP, atrial natriuretic peptide; GC-A, guanylyl cyclase-A; ANP-CR, ANP clearance receptor; BNP, B-type natriuretic peptide.

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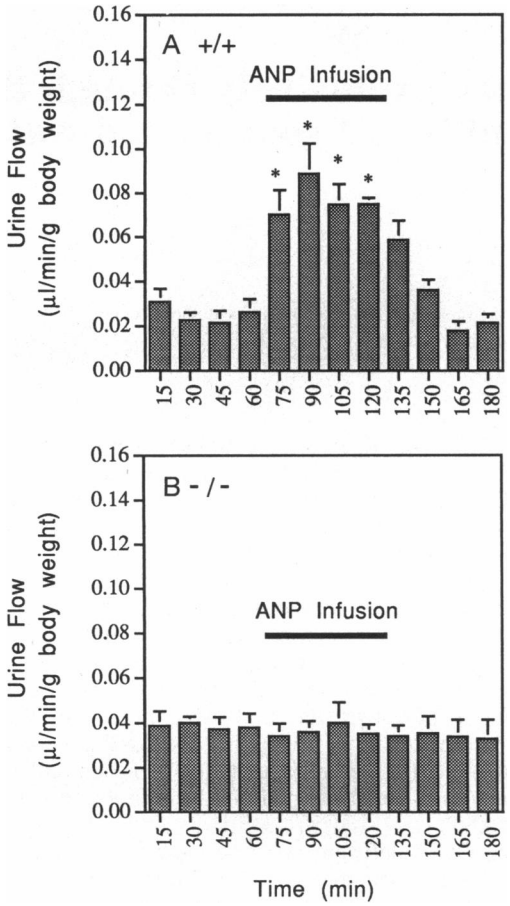

FIG. 1. Effects of ANP infusion on urine output of wild-type (*A*) or GC-A-deficient (*B*) mice. After mice were infused with lactated Ringer's solution containing 2% bovine serum albumin at a rate of 120 μl/hr for 1 hr, synthetic mouse ANP (500 ng per kg per min) was added to the infusion solution for 1 hr. After 1 hr, the infusion was continued with the buffered solution not containing ANP. Urine was collected and weighed every 15 min. There were significant differences in urine output between wild-type (n = 6) and GC-A-deficient (n = 5) mice during ANP infusion (*, P <0.05).

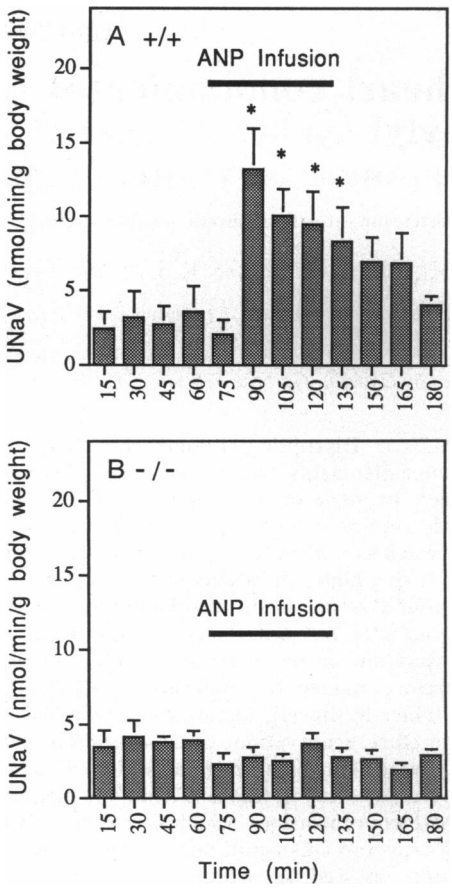

FIG. 2. Effects of ANP infusion on urine sodium excretion of wild-type (*A*) or GC-A-deficient mice (*B*). Mice were treated as described in Fig. 1. Urine sodium excretion was calculated every 15 min. There were significant differences in urinary sodium excretion between the genotypes (n = 5 of each genotype) beginning 15 min after initiation of ANP infusion (*, P <0.05). UNaV, uniary sodium excretion.

ratus, MA). After an equilibration period, urine was collected at 15-min intervals throughout the experiment.

ANP Infusion. Mice were infused with lactated Ringer's solution containing 2% bovine serum albumin at a rate of 120 μl/hr until constant urine output was achieved for 1 hr (stable urine output over four consecutive 15-min collections). Synthetic mouse ANP-(1–28) obtained from Peninsula Laboratories, Inc. was included in the infusion solution at a concentration of 500 ng per kg (body weight) per min for 1 hr, after which the buffered solution infusion was continued without ANP.

Volume Expansion. Basal urine output was established during the infusion with lactated Ringer's solution containing 4.5% bovine serum albumin at a rate of 4.3 μl per hr per g (body weight). After 1 hr, the rate was increased to 114 μl per hr per g (body weight) for 15 min to give a bolus of approximately 3% of body weight. The infusion was continued at 4.3 μl per hr per g (body weight) for 1 hr (urine collected each 15 min), and then a second bolus at the higher infusion rate was again given as described above. After 15 min, the rate of infusion was lowered to 4.3 μl per hr per g (body weight) for the remainder of the experiment.

Urinary Sodium and Cyclic GMP. Urine volume was determined gravimetrically. Urine sodium concentrations were measured directly using ion-specific electrodes (Beckman Astra, CA). After the acetylation of samples, urinary cyclic GMP concentrations were determined by radioimmunoassay as described (16). The product of these concentrations and urine flow rates yield sodium and cyclic GMP excretion rates.

Plasma ANP. During the volume expansion protocol as described above, blood was collected at intervals for the determination of ANP concentrations. Blood (100 μl) was drawn from the tail at the beginning of the experiment (basal), at 0.5 hr after the second bolus infusion (0.5 hr), and at the end of the experiment (2 hr). EDTA (1 mg/ml of blood; Sigma, MO) and aprotinin (500 KIU/ml of blood; Sigma, MO) were added to the blood samples, which were then centrifuged immediately at 4°C. Plasma samples were stored at -70°C until use. ANP concentrations of plasma were determined by commercially available radioimmunoassay (Peninsula, CA) according to the manufacturer's instruction. The assay was sensitive to 2 pg per tube.

Data Presentation and Statistical Analysis. Data are presented as group means ± SEM. Comparison between two groups was assessed by using the paired t test or unpaired t test assuming equal variance, where appropriate. A P value <0.05 was used to assign statistical significance.

RESULTS AND DISCUSSION

Administration of ANP normally causes a marked natriuresis and diuresis (17), and disruption of the ANP gene in mice results in salt-sensitive elevations of blood pressure (14). Thus, ANP appears to be an important hormone for blood volume

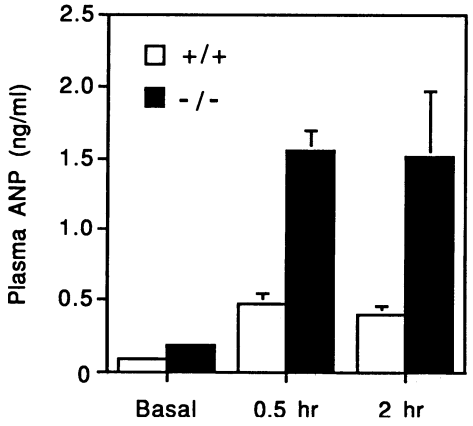

FIG. 3. Effect of volume expansion on plasma ANP levels of wild-type (n = 4, open bars) or GC-A-deficient mice (n = 5, solid bars). Blood samples were drawn at the beginning of the experiment (basal), at 0.5 hr after the second bolus infusion (0.5 hr), and at the end of the experiment (2 hr). Plasma ANP concentrations were determined by radioimmunoassay. Plasma ANP levels after volume expansion were significantly higher than basal in both genotypes.

homeostasis and for the handling of dietary salt. GC-A-deficient mice, however, maintain a constant, although elevated, blood pressure on either high or low salt diets (15), suggesting normal kidney function in the absence of the putative receptor for ANP. One explanation for the salt resistance in the GC-A-deficient mice and the salt sensitivity in the ANP-deficient mice, is that ANP acts through a pathway independent of GC-A. We therefore determined whether or not sodium excretion and urine output are modulated by ANP in the GC-A-deficient mouse.

Prior to infusion of ANP, the urine output in null mice was not significantly different from that of wild-type controls (Fig. 1). Infusion of ANP at a dose of 500 ng per kg per min to wild-type mice caused a rapid increase in urine output within 15 min that peaked at levels 3–4 times higher than the preinfusion rate (Fig. 1*A*). In contrast, ANP infusion failed to increase urine output in the GC-A-null-mice (Fig. 1*B*). Basal urinary sodium excretion of wild-type and of GC-A-null mice was similar prior to infusion of ANP (Fig. 2), and the peptide caused an approximately 5-fold increase in sodium excretion in wild-type mice (Fig. 2*A*). In the GC-A-null mice, however, ANP failed to elevate sodium excretion (Fig. 2*B*). Thus, ANP regulation of both urine output and sodium excretion appears to be mediated by the GC-A signaling pathway.

Given that ANP appears to function through GC-A, the next question focused on whether or not a natriuretic/diuretic factor other than ANP exists within the heart. The many caveats of gene disruption are exemplified in the ANP-deficient mouse where electron microscopic analysis revealed that homozygous null mutants contained no detectable atrial secretory granules (14). Thus, a natriuretic factor(s) other than ANP within these secretory granules would also have been conceivably eliminated in the ANP gene knockout mouse. The coexistence of BNP and ANP within atrial secretory granules, for example, has been reported (7). Exogenous synthetic BNP causes natriuresis (18), and therefore, BNP is certainly a candidate natriuretic factor. In addition, the ANP gene disruption also eliminates the amino-terminal regions of pro-ANP, and some studies have suggested that amino-terminal fragments of pro-ANP possess natriuretic activity (19).

To examine these possibilities, plasma volume expansion was used as a means to cause release of the cardiac granules containing ANP, and possibly other natriuretic factors. To confirm that a bolus infusion of an isooncotic solution causes release of the granules from the heart, plasma ANP concentrations were estimated before and after volume expansion.

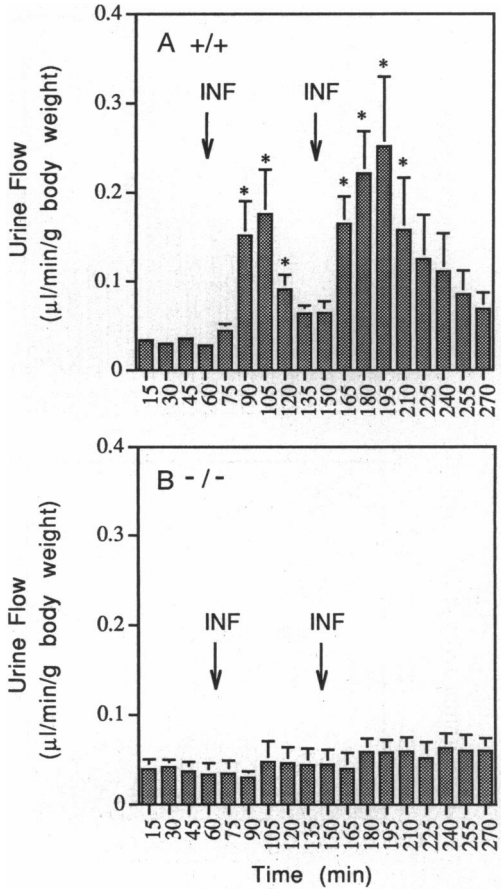

FIG. 4. Effect of volume expansion on urine flow of wild-type (*A*) or GC-A-deficient (*B*) mice. After mice were infused with lactated Ringer's solution containing 4.5% bovine serum albumin at a rate of 4.3 µl per hr per g (body weight) for 1 hr, the same solution was infused at a rate of 114 µl per hr per g (body weight) for 15 min (approximately 3% of body weight). The rate of infusion was then changed to the basal rate until four 15-min urine collections were made. Then the second bolus infusion was given to the mice at a rate of 114 µl per hr per g (body weight) for 15 min, after which the rate of infusion was again changed to 4.3 µl per hr per g (body weight). Urine was collected and weighed every 15 min. (*, $P < 0.05$ versus urine flow of GC-A-deficient mice, n = 8 of each genotype).

The basal ANP levels in wild-type and GC-A-null mice were not significantly different (Fig. 3), but 30 min after a second bolus infusion, ANP levels were about 5-fold higher in wild-type and 9-fold higher in GC-A-null mice. The high levels of ANP were maintained for up to 2 hr after the second bolus infusion in either genotype (Fig. 3). These results demonstrate that in either wild-type or GC-A-null mice, volume expansion causes a release of granules from the heart.

We could now ask the question of whether or not granule release would result in natriuresis/diuresis in the GC-A-null mice. In wild-type mice, after the second infusion to cause volume expansion, urine output reached a value 12-fold higher than during the preinfusion period (Fig. 4*A*). However, volume expansion in the GC-A-null mice caused little, if any, effect (Fig. 4*B*). In wild-type mice, volume expansion also caused rapid and dramatic increases in sodium excretion (about 6-fold higher) as shown in Fig. 5*A*. Instead of a rapid natriuretic response to volume expansion, seen in wild-type mice, there was only a slight and gradual increase in sodium output after volume expansion in the null mice (Fig. 5*B*).

An association between increases in plasma ANP and urinary cyclic GMP excretion during acute volume expansion is well documented (20, 21). Furthermore, a variety of other hormones are known to increase urinary cyclic GMP (22). We

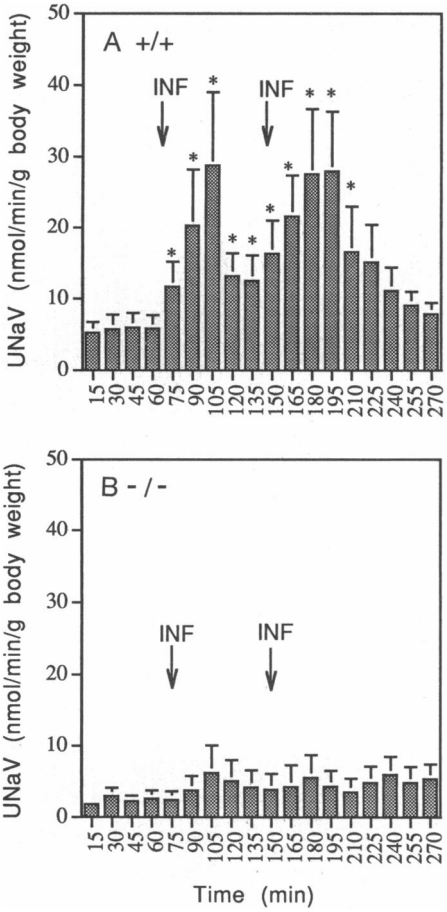

FIG. 5. Effect of volume expansion on urine sodium excretion of wild-type (*A*) or GC-A-deficient (*B*) mice. The mice were treated as described in Fig. 4, and urine sodium excretion was calculated every 15 min. (*, $P < 0.05$ versus urine sodium excretion of GC-A-deficient mice, $n = 7$ of each genotype). UNaV, urinary sodium excretion.

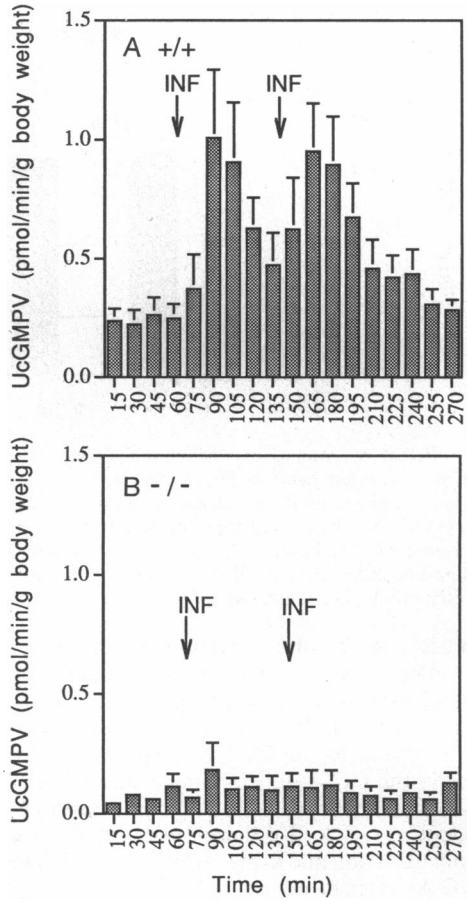

FIG. 6. Effect of volume expansion on urinary cyclic GMP excretion of wild-type mice (*A*) or GC-A-deficient mice (*B*). After mice were infused with lactated Ringer's solution containing 4.5% bovine serum albumin at a rate of 4.3 μl per hr per g (body weight) for 1 hr, the same solution was infused at a rate of 114 μl per hr per g (body weight) for 15 min. The rate of infusion was then changed to the rate of 4.3 μl per hr per g (body weight) until four 15-min urine collections were made. A second bolus infusion was given to the mice at a rate of 114 μl per hr per g (body weight) for 15 min, and then the rate of infusion was again changed to 4.3 μl per hr per g (body weight). Urinary cyclic GMP excretion was calculated every 15 min. Cyclic GMP excretion of GC-A-deficient mice was statistically lower than those of wild-type mice at all time points ($P < 0.05$, $n = 7$ of each genotype). UcGMPV, urinary cyclic GMP excretion.

could therefore determine whether or not the levels of this second messenger are altered by the release of granules from the heart in the GC-A-deficient mouse. Even under basal conditions, urinary cyclic GMP was significantly less in the GC-A-null mice (Fig. 6). Cyclic GMP excretion was about 30% of those found in wild-type mice, suggesting that GC-A activity accounts for 70% of the cyclic GMP found in urine under basal conditions. Whether or not a "tonic" modulator of GC-A (e.g., ANP or BNP) accounts for the normal basal urinary cyclic GMP is not known, but previous studies have suggested that there is no apparent correlation between basal circulating levels of ANP and urinary cyclic GMP (23). Since rates of sodium excretion and urine volume appeared normal in the GC-A-null mice, the results might suggest that a mechanism other than GC-A/cyclic GMP is important in the chronic handling of water or salt by the kidney. In this regard, it should be pointed out that GC-A-null mice are markedly hypertensive (15). Thus, it is possible that pressure natriuresis may compensate for the absence of the natriuretic peptide hormonal axis for the excretion of salt and water in chronic conditions.

Volume expansion caused an approximately 4-fold increase in urinary cyclic GMP of wild-type mice (Fig. 6*A*) in agreement with past observations (24). However, there was no urinary cyclic GMP response to volume expansion in GC-A-deficient mice (Fig. 6*B*). Thus, the elevations of cyclic GMP normally seen in urine after volume expansion appear principally due to GC-A.

It has been a classical approach to specifically inhibit or activate a signaling pathway to address its function. With the guanylyl cyclase receptors, specific inhibitors have not been available. Although one compound, HS-142–1, has been shown to diminish the effect of ANP during volume expansion (25), it is not a selective inhibitor of GC-A (26). Here, gene disruption was used to interrupt signaling by GC-A. These results strongly suggest that the heart communicates with the kidney exclusively through GC-A, at least with respect to the acute handling of sodium and water. Other heart hormones, such as BNP, also bind to GC-A, and therefore, multiple heart hormones may exist, but this single receptor seems to mediate their effects on the handling of salt and water within the kidney.

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