

# A genetic model defines the importance of the atrial natriuretic peptide receptor (guanylyl cyclase-A) in the regulation of kidney function

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**Disruption of the atrial natriuretic peptide (ANP) receptor [guanylyl cyclase-A (GC-A)] gene yields mice with a salt-resistant form of hypertension, raising fundamental questions on the role of ANP in acute regulation of the kidney. Here, we show that water intake, food consumption, stool weight, urine volume, and sodium excretion are not significantly different between wild-type and GC-A null mice on standard rodent chow (0.7% NaCl) or a high-salt diet (8% NaCl). In conscious mice with an indwelling catheter, the infusion of a physiological saline solution containing 4% BSA resulted in a marked natriuresis/diuresis in wild-type mice but no response in GC-A null animals. When physiological saline was given by gavage, however, the kidney response of wild-type and null mice was equivalent, raising the possibility that the gastrointestinal tract can directly regulate kidney function. However, administration of 0.9% saline through an intraperitoneal route also resulted in equal kidney responses in wild-type and null mice. When 0.9% NaCl lacking protein was infused i.v., wild-type and null mice both responded at the kidney level. Thus, GC-A appears dispensable for regulation of sodium/water excretion in response to changes in dietary sodium concentration, but likely becomes critical in volume expansions where the isoosmotic pressure remains constant, such as head-out immersion or the initial and correctable stages of congestive heart failure.**

**A**trial natriuretic peptide (ANP) and B type natriuretic peptide are signaling molecules that can be released by the endocrine heart to stimulate natriuresis/diuresis in the kidney. The receptor for both peptides appears to be guanylyl cyclase-A (GC-A; natriuretic peptide receptor-A) based on binding affinities and disruption of the GC-A gene (1).

At least three prominent phenotypes are evident in GC-A null mice: elevated basal blood pressure, resistance to dietary salt-associated increases in blood pressure, and marked cardiac hypertrophy (2, 3). The salt-resistant elevation in blood pressure mimics the phenotype of at least 20 million Americans with essential hypertension (4–6) and suggests that GC-A is not a critical regulator of kidney function under chronic high- or low-salt diets. Yet, previous studies suggest that GC-A is essential for acute natriuresis associated with intravascular volume expansion (7). In those studies, when anesthetized mice were infused with a Lactated Ringer's solution containing 4% BSA, wild-type mice elevated sodium excretion and urine output by 5-fold, whereas no natriuretic response occurred in the GC-A null mice (7). One possible explanation for this disparity in response to dietary compared with i.v. salt would be direct communication between the gastrointestinal tract and the kidney, resulting in natriuresis during high dietary salt. Indeed, several laboratories have demonstrated a greater natriuresis in response to enteral or portal saline administration when compared with peripheral i.v. saline loading (8–13). Furthermore, one family of peptides (guanylin, uroguanylin) found in the intestine, and suggested to regulate the kidney, binds to another

guanylyl cyclase receptor, GC-C (14), which, at least in some animals, is expressed in the kidney (15).

Here, we show that GC-A appears unnecessary in the acute regulation of kidney function unless intravascular volume is increased at a constant protein concentration.

## Materials and Methods

The generation of GC-A-deficient mice has been described previously (2). Studies were carried out in adult mice that had been backcrossed for three to six generations into the C57BL/6 strain. The Animal Care and Use Committee of West Virginia University and the University of Texas Southwestern Medical Center approved all studies.

**Metabolic and Blood Pressure Responses to Dietary Salt.** Mice were acclimated to metabolic cages (Mouse Diuresis Cage; Nalge) for 2 days before data collection. They received a standard, powdered rodent chow (0.7% NaCl; Harlan Teklad, Madison, WI) and water *ad libitum* for the first 4 days of measurements and then were subsequently switched to the 8% NaCl diet (Harlan Teklad). Urine was collected every 24 hr for measurement of sodium (Beckman Astra, Richardson, TX) and cGMP (RIA). Blood pressure was measured by tail cuff (Softron, Tokyo) each afternoon as described previously (2).

**Acute Saline Loading by Gavage or Intraperitoneal Injection.** Animals were trained in the metabolic cages for 2 hr per day for 2 days before data collection. In addition, for gavage studies, mice received 2 days of sham gavage followed by 1 day of tap water gavage before data collection. After the training period, mice were given two 1-ml boluses of 0.9% sodium chloride separated by 1 hr, either by gavage or i.p. injection. Urine was collected in metabolic cages for a total period of 6 hr. Urine volume was recorded and then cages were rinsed with deionized water to maximize sodium recovery. Sodium was measured by flame photometry.

**Acute Saline Loading Through a Chronic Indwelling Jugular Catheter.** Mice were anesthetized with ketamine/xylazine. The jugular vein was cannulated with Micro-Renathane tubing (Bainbridge Scientific), and the tubing was exteriorized between the scapulae. Mice were allowed 2 days recovery before training. Chronically catheterized mice were trained in holders for 2 days. Beginning on the fifth postoperative day, mice received either

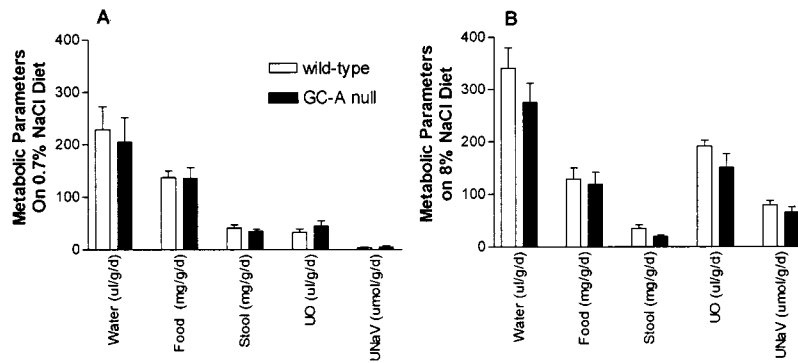
Abbreviations: GC-A, guanylyl cyclase-A; ANP, atrial natriuretic peptide.

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**Fig. 1.** Daily water intake, food consumption, stool weight, urine output (UO), and sodium excretion (UNaV) of wild-type and GC-A null mice on a 0.7% NaCl diet (A) or 8% NaCl diet (B). Data are presented as means  $\pm$  SE;  $n = 5$  for each genotype. No significant differences were noted between the genotypes for any of the parameters.

0.9% NaCl or 0.9% NaCl plus 4% BSA (Sigma) as two 1-ml i.v. boluses given over 15 min each and separated by 1 hr. Urine was collected in metabolic cages for the 6-hr time period as described above.

**Urinary cGMP.** Urine was collected from metabolic cages, centrifuged promptly to remove sediment, and stored at  $-80^{\circ}\text{C}$ . cGMP was measured by ELISA (Assay Designs, Ann Arbor, MI).

**Plasma ANP.** Under methoxyflurane anesthesia, 50  $\mu\text{l}$  of blood was collected from tails before or at 0.5, 2, and 4 hr after gavage with 0.9% NaCl. EDTA (1 mg/ml) and aprotinin (500 kallikrein inhibitor units/ml) were added to the samples before centrifugation and storage at  $-80^{\circ}\text{C}$ . ANP was measured by RIA (Peninsula Laboratories).

**Statistical Analyses.** Data are presented as the average of means  $\pm$  SEM. Data analyses involving all three genotypes were performed by one-way ANOVA (GraphPad, San Diego) or by ANOVA with repeated measures where indicated. Individual groups then were compared by using Tukey's method. Wild-type vs. null animals were compared by use of an unpaired, two-tailed  $t$  test.

## Results and Discussion

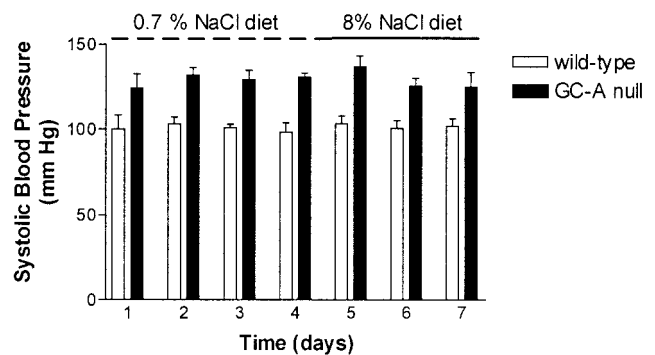
To determine whether the absence of GC-A modified eating or drinking behavior, we measured water intake, food consumption, stool weight, urine volume, and sodium excretion on standard rodent chow (0.7% NaCl) and on a high-salt diet (8% NaCl). There were no significant differences between genotypes with respect to the measured parameters on either diet (Fig. 1). Systolic blood pressure of the GC-A null mice was significantly higher compared with wild-type mice on the standard diet ( $129 \pm 2$  mm Hg for  $-/-$  vs.  $101 \pm 1$  mm Hg for  $+/+$ ;  $P < 0.0001$ ), but no increase in blood pressure in either genotype occurred on the high-salt diet ( $129 \pm 4$  mm Hg for  $-/-$  vs.  $102 \pm 1$  mm Hg for  $+/+$ ) (Fig. 2). Urine output was higher on the high-salt diet, but no difference between the genotypes with regard to sodium excretion was apparent (Fig. 3 A and B). Wild-type mice showed significant elevations in cGMP in response to high dietary salt (Fig. 3C), suggesting that the ANP/GC-A-signaling pathway was activated by high salt.

Several papers have suggested that ANP in the brain plays an important role in the central control of drinking behavior. Intracerebroventricular administration of synthetic ANP decreases the water intake of dehydrated rats (16–18), and neutralization of ANP with anti-ANP antiserum potentiates water

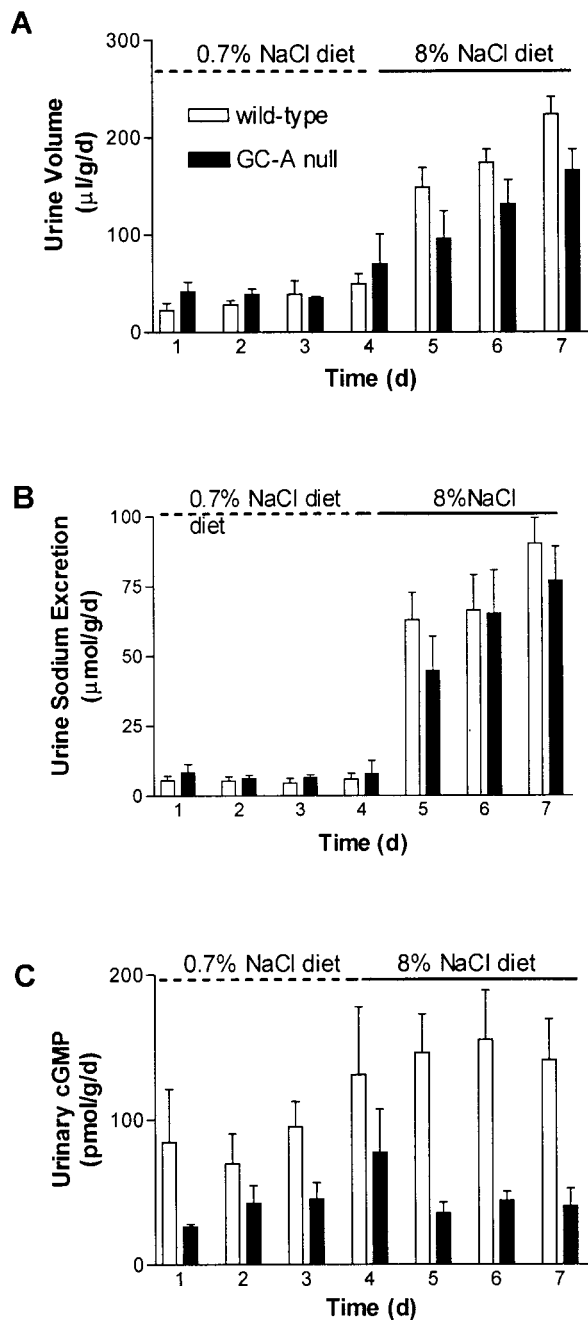
intake induced by water deprivation or angiotensin II injection (16, 19). These findings suggest that endogenous ANP in rat brain antagonizes the action of angiotensin and plays an important role in the maintenance of drinking behavior. Because there was no apparent difference between GC-A null mice and wild-type mice with respect to drinking behavior, the ANP-GC-A pathway appears to have little, if any, effect on drinking behavior when body fluid volume is not reduced.

To study further the gastrointestinal response to salt and to avoid the expected increase in serum osmolarity associated with the 8% NaCl diet, we administered isotonic 0.9% NaCl by gavage. Wild-type and GC-A heterozygous or null mice displayed similar urine output and sodium excretion in response to two 1-ml saline boluses given 1 hr apart (Fig. 4). Urinary cGMP was 2- to 3-fold higher in wild-type than null mice (data not shown). We next explored the consequences of sodium loading through an i.p. injection, thus bypassing the interaction of saline with the intestinal epithelium. Again, there was no difference between urine volume or sodium excretion between the genotypes (Fig. 5).

Previous studies from our laboratory revealed marked differences in urine output and sodium excretion between anesthetized wild-type and GC-A null mice infused with a bolus of 3% body weight of an isotonic, isooncotic solution (7). To remove confounding factors associated with anesthesia, in the present study we used conscious animals with an indwelling jugular catheter and expanded the cardiovascular volume with 0.9% NaCl containing 4% BSA (isooncotic). Whereas the wild-type

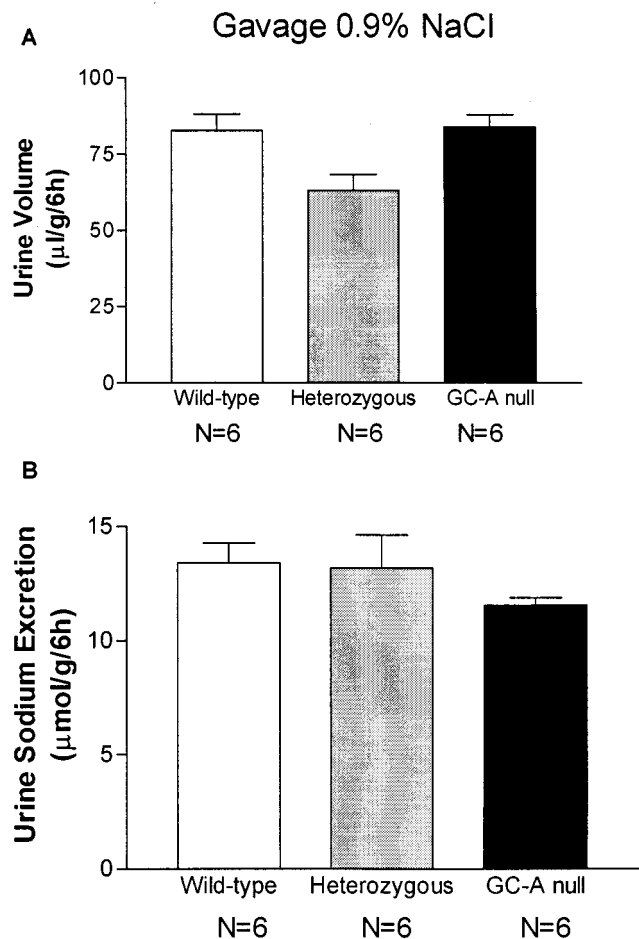


**Fig. 2.** Effect of dietary salt on systemic blood pressure of wild-type and GC-A null mice. Animals were fed normal chow (0.7% NaCl) on days 1–4 and then switched to a high-salt (8%) diet on day 5.



**Fig. 3.** Effect of high-salt diet on urine volume (A), urine sodium excretion (B), and urinary cGMP levels (C) in wild-type and GC-A null mice. Data are presented as means  $\pm$  SE;  $n = 5$  for each genotype. On days 1–4, mice were fed standard chow (0.7% NaCl) and then the diet was changed to 8% NaCl on day 5. Urine volume and sodium excretion increased in both genotypes in response to the high-salt diet ( $P < 0.0001$ , two-way ANOVA). Urine volume was significantly higher in wild-type mice compared with null ( $P < 0.05$ , two-way ANOVA), but there was no significant difference between the genotypes with respect to urine sodium excretion ( $P = 0.39$ , two-way ANOVA). Only wild-type mice showed significant elevations of urinary cyclic GMP in response to the high-salt diet ( $P < 0.05$ , repeated-measures ANOVA), and urinary cGMP levels were significantly higher in wild-type mice compared with null mice ( $P < 0.0005$ , unpaired *t* test).

mice had a marked increase in sodium excretion and urine volume in response to the infusion, heterozygote animals had about one-half the response and homozygous nulls failed to

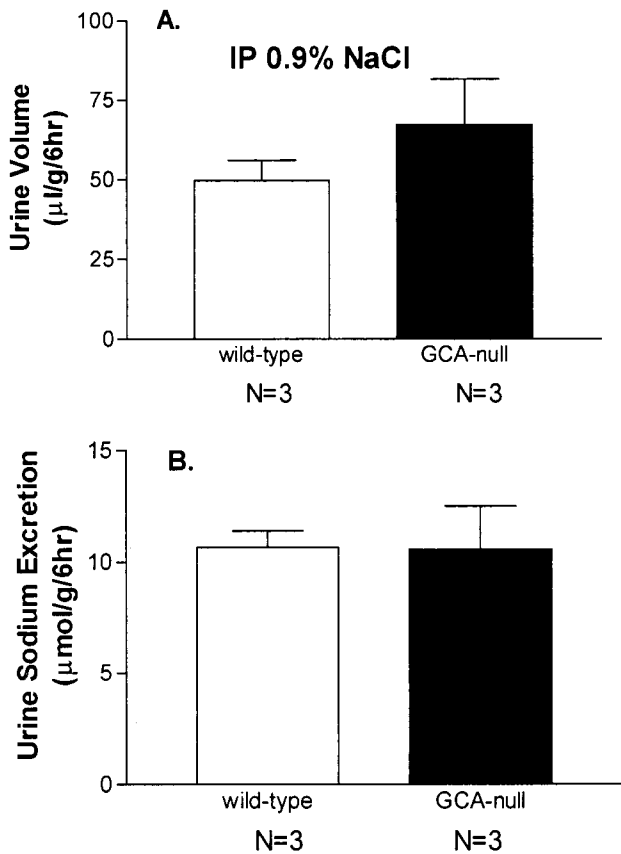


**Fig. 4.** Urine volume (A) and sodium excretion (B) in wild-type, heterozygous, and GC-A null mice in response to gavage with two 1-ml boluses of 0.9% NaCl separated by 1 hr. There was no significant difference between wild-type and GC-A null mice with respect to urine. Urinary cGMP levels were significantly less for null mice than for wild-type mice ( $P < 0.001$ ; data not shown).

respond (Fig. 6). Thus, under conditions of an isoosmotic volume expansion, the endocrine heart appears to be required for renal sodium excretion, and this regulation proceeds through the GC-A-signaling pathway.

Isoosmotic volume expansion is observed in some clinical settings such as congestive heart failure, renal failure, and liver cirrhosis with ascites (20). Studies in an experimental model of congestive heart failure have demonstrated that inhibition of ANP by either specific antibodies (21) or the ANP receptor antagonist, HS-142-1 (22), causes decreased urine flow or sodium excretion. These results together with our previous findings (7) demonstrate that GC-A plays a prominent role in natriuresis/diuresis after isoosmotic volume expansion and, thus, suggests that activation of GC-A could provide a therapeutic remedy for the disease.

When 0.9% NaCl lacking protein was used for the volume expansion of wild-type and null mice, natriuresis/diuresis in the null animals now exceeded that of the wild-type mice (Fig. 6). That GC-A null mice displayed increased sodium excretion under conditions of low protein volume expansion is consistent with data from many laboratories demonstrating exaggerated natriuresis in both animals and humans with hypertension (23–27). Various hypotheses for enhanced natriuresis have included an augmented effect of ANP on the kidney (25, 28). We have shown previously that ANP infusion does not cause natri-

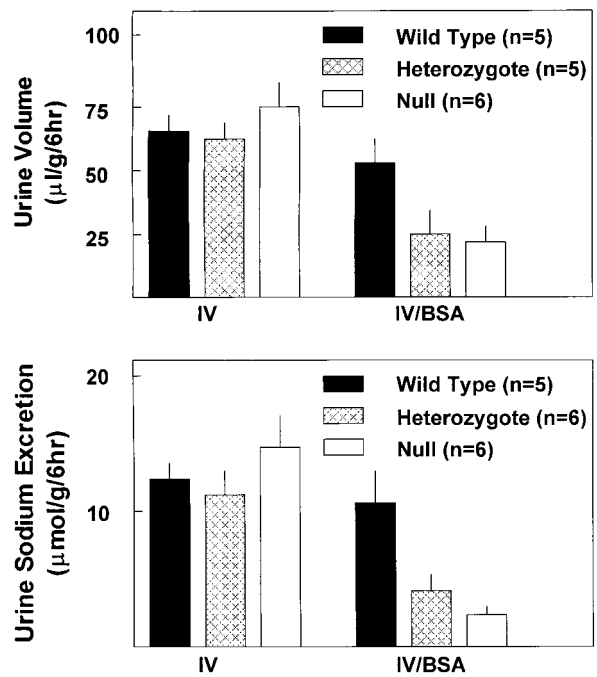


**Fig. 5.** Urine volume (A) and sodium excretion (B) in wild-type and GC-A null mice in response to i.p. injection of two 1-ml boluses of 0.9% NaCl separated by 1 hr. There was no significant difference between the genotypes with respect to urine volume or sodium excretion.

uresis in GC-A null mice, suggesting that ANP does not signal through another receptor for sodium excretion (7). Thus, other mechanisms must lead to exaggerated natriuresis in GC-A null mice.

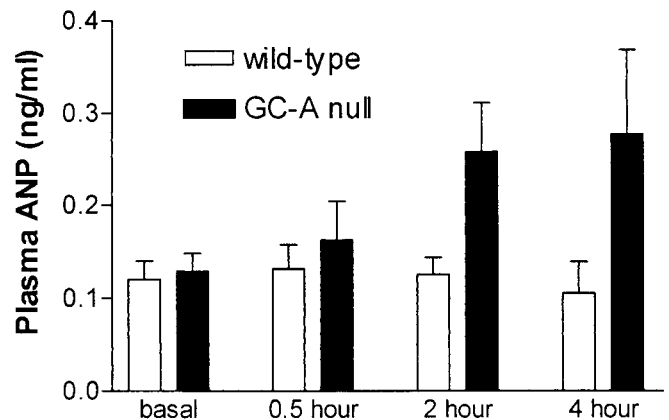
Although GC-A did not seem necessary for excretion of sodium after saline gavage or i.v. infusion of saline, urinary cGMP nevertheless was elevated in wild-type mice, and, therefore, GC-A could be responsible for some part of the natriuretic response to oral sodium ingestion in normal animals although clearly was not required. Plasma ANP levels, used as a measure of intravascular volume expansion, rise by 5-fold in wild-type mice after i.v. administration of an isotonic, isoosmotic fluid (7). Here, ANP levels did not change significantly, however, in the wild-type mice after gavage with 0.9% NaCl (Fig. 7).

The most striking finding of this study is that the ANP/GC-A-signaling pathway appears critical for regulation of acute kidney function in the case of an i.v. isotonic, isoosmotic volume expansion, but not for volume expansions where the oncotic pressure is altered. Various investigators have reported that proximal tubule reabsorption of sodium varies directly with protein concentration of infusate and, thus, capillary oncotic pressure (29–32). Ott *et al.* (32) demonstrated that protein concentration affected proximal sodium reabsorption in dogs only in the presence of extracellular volume expansion. We hypothesize that in the absence of GC-A, the mouse is unable to overcome the effect of increased proximal tubule sodium reabsorption that occurs under conditions of isoosmotic volume expansion. In humans, Loon *et al.* (33) examined filtration



**Fig. 6.** Urine volume (A) and sodium excretion (B) in response to i.v. infusion of two 1-ml boluses of either an isotonic saline solution (IV) or an isoosmotic saline solution (0.9% NaCl, 4% BSA; IV/BSA). The GC-A null mice that were infused with the isoosmotic solution showed significantly lower urine output and sodium excretion than either wild-type or heterozygous mice. The null mice also had a significantly elevated sodium excretion relative to wild-type animals.

dynamics and the natriuretic response to volume expansion with either 5% albumin in 0.9% NaCl or 0.9% NaCl alone. Subjects had a significantly higher sodium excretion rate during the saline infusion than the infusion containing 5% albumin. Patients were followed for 4 hr after the infusion and showed delayed sodium excretion such that subjects tended to return toward sodium balance with time. We did not measure sodium excretion in the days after the bolus, but would expect them to compensate with late natriuresis to avoid the sequelae of marked volume expansion such as uncompensated congestive heart failure or edema, neither of which was clinically evident.



**Fig. 7.** Plasma ANP levels before and 0.5, 2, and 4 hr after gavage with two 1-ml boluses of 0.9% NaCl. There was a significant effect of genotype (two-way ANOVA,  $P < 0.003$ ), but not time, on plasma ANP levels.

Several lines of evidence have suggested that resistance to ANP explains in part the maintenance of volume expansion in congestive heart failure, nephrotic syndrome, and cirrhosis. Patients with sodium-retaining disorders are resistant to the effects of ANP infusion or to endogenously released ANP during head-out immersion (28, 34, 35). It is reasonable to suggest that humans with GC-A deficiency are more vulnerable to volume expansion in such pathological conditions. Increased ANP levels also have been reported in patients with essential hypertension (36) and cardiac hypertrophy (35–37).

Sodium excretion was similar in wild-type and heterozygous mice after gavage, i.p., or i.v. bolus and was increased in the GC-A null mice after i.v. bolus compared with enteral saline, making it unlikely that an intestinal factor was responsible for natriuresis in absence of GC-A. Urinary cGMP excretion also was significantly less in GC-A null mice after saline gavage, again suggesting that a cGMP-dependent pathway (e.g., guanylin, uroguanylin) was not responsible for natriuresis in response to

enteral saline. Finally, the data also suggest that ANP/B type natriuretic peptide may activate GC-A in wild-type mice in response to high-salt diets, but GC-A activation is not an essential event, because the failure to elevate cGMP in null mice had no effect on normal kidney function.

In summary, the GC-A-deficient mouse demonstrates that the ANP/GC-A-signaling pathway is essential for acute natriuresis in response to an isoosmotic saline volume expansion. Thus, in situations such as head-out immersion, or the initial and correctable phases of congestive heart failure, the endocrine heart coupled to signaling through GC-A is likely essential for natriuresis and, perhaps, survival. Yet, under conditions that are not isoosmotic (administration of physiological saline by gavage, i.p. injection, or i.v. infusion), GC-A is not required for normal kidney function.

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