A genetic model of salt-resistant hypertension has been developed recently through disruption of the guanylyl cyclase-A (GC-A) natriuretic peptide receptor gene (Lopez, M. J., Wong, S. K., Kishimoto, I., Dubois, S., Mach, V., Friesen, J., Garbers, D. L., and Beuve, A. (1995) Nature 378, 65–68). These genetically altered mice were used to determine which of the natural peptides with natriuretic peptide-like structures regulate blood pressure through the GC-A receptor. Atrial natriuretic peptide (ANP) or B-type natriuretic peptide (BNP) half-maximally relaxed precontracted aortic rings in wild-type mice at about 24 nM, but failed to relax such aortas in GC-A null mice, even at micromolar concentrations. C-type natriuretic peptide (CNP), in contrast, caused half-maximal relaxation at concentrations of 335 and 146 nM in aortas from either wild-type or null mice, respectively, suggesting that this peptide acted through a receptor other than GC-A. Since the in vitro results with aortic smooth muscle do not necessarily reflect the physiology of the smaller blood vessels important in blood pressure regulation, the blood pressures of conscious mice infused with the various peptides were determined. ANP caused decreases in blood pressure when infused at rates of 500 ng/kg/min, a rate which resulted in a plasma concentration of 0.8 nM. In the null mice, in contrast, ANP failed to lower blood pressure even at infusion rates of 50 µg/kg/min. Much higher infusion rates for CNP (50 µg/kg/min), which yielded final plasma concentrations of 18.3 nM, were required to lower blood pressure in wild-type mice, but the effects of CNP were not altered in GC-A null mice. Thus, two natriuretic peptides (ANP, BNP) act through GC-A whereas another (CNP) acts through another receptor to regulate blood pressure.

Three peptides of similar structure have been named natriuretic peptides: atrial natriuretic peptide (ANP), 1 B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). The three peptides are characterized by a 17-amino acid disulfide ring which contains a number of invariant amino acids (1, 2). Synthesis of the peptides occurs in various regions of the body, but ANP and BNP appear to be synthesized principally in the heart and to circulate, whereas CNP is not found in appreciable amounts in the blood and, therefore, may act locally (2). Members of the guanylyl cyclase family (the ANP clearance receptor is considered a truncated member) appear to represent the receptors for these peptides (1, 2). These plasma membrane forms of guanylyl cyclase are predicted to contain highly conserved intracellular protein kinase-like and cyclase catalytic domains, a single transmembrane segment, and a more variable extracellular ligand-binding domain. ANP and BNP bind with highest affinity to guanylyl cyclase-A (GC-A) and CNP with highest affinity to guanylyl cyclase-B (GC-B) (3–7). In addition to these cyclases, the receptor for heat-stable enterotoxins (8) and four presumed orphan cyclase receptors have been discovered in the mammal (9–11, 13).2 Recently, Yu et al. (14) have found at least 29 guanylyl cyclase sequences in Caenorhabditis elegans, many of which are expressed in sensory neurons, and thus guanylyl cyclases other than GC-A or GC-B, which can mediate the actions of the 17-amino acid natriuretic peptide-like structures, may exist. Gene disruption offers one potential powerful method by which to address the functions of a particular receptor, and here we make use of a mouse genetic model where the GC-A gene is disrupted. Such mice display a salt-resistant form of elevated blood pressure (15). Studies on aortic rings from these mice demonstrated that both ANP and BNP are ineffective in the null animals, whereas CNP retains the ability to relax the aorta. To determine whether the aortic ring studies reflected in vivo effects on blood pressure, ANP and CNP were infused in conscious animals. Although ANP caused a decrease in blood pressure in the wild-type mice, it failed to lower blood pressure in GC-A null mice. CNP, in contrast, although not nearly as potent as ANP, lowered blood pressure in both wild-type and null animals. Thus, CNP potentially regulates blood pressure at the local level. However, given that blood pressure is elevated in the GC-A null mice, as well as in ANP null mice (15, 16), CNP appears unable to maintain a normal blood pressure in the absence of ANP or GC-A. The same appears to be the case with NO, since blood pressures are elevated in eNOS gene-disrupted animals despite the presence of GC-A/ANP and in GC-A gene-disrupted mice despite the presence of NO synthase (15, 17).

MATERIALS AND METHODS

Animals—GC-A-deficient mice were produced (15) and bred under standard conditions in the Animal Resource Center, University of Texas Southwestern Medical Center where all animal protocols were approved by the Animal Care Committee (IACRAC). Wild-type and mutant homozygous null mice used in these studies were siblings (3–5 months of age) from heterozygous breedings within the GC-A gene-deficient breeding colony.

The Guanylyl Cyclase-deficient Mouse Defines Differential Pathways of Natriuretic Peptide Signaling*  

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The abbreviations used are: ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; PSS, physiological saline solution; KC-PO₄, physiological saline solution containing KCl instead of NaCl; GC-A, guanylyl cyclase-A; GC-B, guanylyl cyclase-B; eNOS, endothelial nitric-oxide synthase.  

2 S. Schulz, B. W. Wedel, A. Matthews, and D. L. Garbers, submitted for publication.
Buffers and Chemicals—The buffers used were a physiological saline solution (PSS) containing 119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂·2H₂O, 1.17 mM MgSO₄·7H₂O, 25 mM NaHCO₃, 1.18 mM KH₂PO₄, 0.027 mM EDTA, and 5.5 mM glucose, or PSS containing KCl (40 mM) substituted on a molar basis for NaCl (KCl-PSS). Rat ANP (6-23) (same sequence as mouse), mouse BNP-43, and rat CNP-22 (same sequence as mouse) were obtained from the American Peptide Co., Sunnyvale, CA. (-)-Norepinephrine was from Sigma. All peptide solutions were in PSS for the aortic ring studies or in Lactated Ringer’s solution (Fisher) for the infusion studies. Xylazine, ketamine, and heparin were obtained from Burns Veterinary Supply, Rockville Center, NY. Radioimmunoassay kits for rat ANP and CNP were from Peninsula Laboratories, Belmont, CA.

Aortic Ring Preparation—The thoracic aortas were removed from wild-type or homozygous null mice (euthanized without anesthesia) and placed on ice in chilled, oxygenated (95% O₂, 5% CO₂) PSS (4 °C). The aortas were gently washed with the PSS and then placed into freshly chilled and oxygenated PSS. The surrounding connective tissue was gently stripped away, and the aorta was divided into thirds with the middle portion taken for use in the Mulvany myograph (model 410A, J. P. Trading, Denmark) (18, 19). The chamber has a 10-mL capacity with two mounts for simultaneous evaluation of two aortic ring preparations. The midthoracic aorta was further divided into two equal halves. Aortic rings from both a wild-type and a GC-A null mouse were threaded over two 40-m wires (cut to 2.2-cm lengths) in oxygenated PSS at ambient temperature. The bath was then allowed to gradually warm to 37 °C. Continuous oxygenation with 95% O₂, 5% CO₂ was accomplished throughout the experiments. The length of each segment was measured with a calibrated eyepiece, and the correlation between force and internal circumference was determined using the MyoVista computer program (J. P. Trading, Denmark) to normalize the vessels to an internal diameter of 100 mm Hg (IC100). Experiments were on vessels held at an internal circumference equal to 90% of the IC100 (18, 19). After the normalized internal diameter was determined, the aortic rings were allowed to equilibrate in fresh PSS for 15 min after which norepinephrine (10 µM) was added to obtain an estimate of maximal force. The rings were washed twice with oxygenated PSS (37 °C) and allowed to equilibrate for an additional 15 min. The vessels were then precontracted with KCl-PSS (40 mM KCl) and allowed to reach a stable contraction plateau for 10 min. Natriuretic peptides were added in increasing concentrations every 3 min if no relaxation was evident or if relaxation was evident after a stable force (millinewtons) was present for 2 min. At the end of every experiment norepinephrine (10 µM) was added, after wash out of the natriuretic peptides, to determine whether the aortic rings were still responsive. The effect of each peptide was evaluated in two aortic rings from 6 wild-type and 6 null mice. The effect of BNP was assessed in two aortic rings from 2 wild-type and 2 GC-A null mice (total of four experiments per genotype). The responses were analyzed by nonlinear regression and analysis of variance for repeated measures with Tukey’s post hoc test of the means (20). Data are reported as mean ± S.E.

Blood Pressure Measurement—Measurements were in conscious mice by a tail cuff method with a computer automated system (Softern, Tokyo, Japan) as described previously (15, 21, 22). The mice were trained daily for a minimum of 7 days prior to beginning the study. At the end of the training period, mice were anesthetized with an intra-peritoneal injection of xylazine (1 mg/g body weight) and ketamine (100 µg/g body weight), and the right jugular vein was exposed and cannulated with a 10-cm length of sterile Tygon-tubing (inside diameter 0.10 in) in the catheter was filled with 10 µL of Lactated Ringer’s solution containing 100 units/ml of heparin sodium and closed by heating its tip. The catheter was then passed subcutaneously to emerge caudal to the cervical spine where it was fixed to the skin with a silk suture. The mice were allowed to recover for 48 h after surgery prior to initiating experimental studies.

During each infusion study, the jugular catheter was connected to a microinfusion pump (Harvard Apparatus). Lactated Ringer’s solution was infused at a rate of 60 µL/h. After an initial control period of 30 min, the synthetic peptides were included in the infusion solution and infused for 60 min. The total volume contained in the connecting catheter was 20 µL, and thus the infused peptides entered the bloodstream approximately 20 min after the start of the infusion period. Systolic and diastolic blood pressure and heart rate were monitored continuously throughout the experimental period (90 min). Measurements were taken every 2–3 min and from these the mean values for 15-min time intervals were calculated. Differences in blood pressure and heart rate between the initial control period (infusion of Lactated Ringer’s solution) and the subsequent peptide-infusion periods (treatment periods) were analyzed with one-way repeated-measures analysis of variance and Fisher’s protected least-significant-difference test (23). Values are expressed as mean ± S.E.

At the end of the infusion period, some mice were sacrificed, and 0.2 ml of blood obtained by cardiac puncture was placed in potassium-EDTA. Plasma ANP and CNP levels were determined with commercially available radioimmunoassay kits.

RESULTS AND DISCUSSION

Relaxation of Aortic Rings by Natriuretic Peptides—One explanation for the elevated blood pressure in the GC-A gene-disrupted mice is that its absence results in an inability of one or more natriuretic peptides to chronically relax vascular smooth muscle. Previous studies have established that ANP can lower blood pressure in animals or induce vasorelaxation in vascular rings (19, 24–27). However, it has been debated whether these vasorelaxant effects are caused through activation of GC-A or via other ANP-binding proteins, such as the clearance receptor (2, 26). Several studies have positively correlated increased cGMP levels and vasorelaxation in response to ANP (28, 29), whereas others have apparently separated vasorelaxation from cGMP elevations (30). We initially examined the responses of midthoracic aortic rings to ANP in both wild-type (Fig. 1A) and GC-A-deficient mice (Fig. 1A). ANP at 100 nM concentrations caused maximal relaxation of KCl-precontracted aortas of wild-type mice but failed to relax aortas of the GC-A null mice (Fig. 1B). ANP treatment results in a concentration-dependent relaxation in wild-type mice as shown by nonlinear regression analysis of mean contraction (% relaxation) versus log[ANP] (r² = 0.977, p < 0.05). The EC₅₀ for ANP in wild-type mice was 24.6 ± 1.6 nM. These results clearly establish that ANP acts through GC-A to relax aortic smooth muscle. Although there may be effects of ANP mediated through the clearance or other receptors, these do not appear to account for vasorelaxation. Our results, together with those recently described by Kishimoto et al. (31) for the kidney, suggest that GC-A is the sole receptor for the acute effects of ANP on vascular tone and natriuresis/diuresis.

BNP appears to be released principally from the ventricles of the heart through constitutive pathways (32). BNP binds to GC-A with higher affinity than GC-B, and based on these studies and the failure to identify another potential receptor for BNP, GC-A has been suggested as the BNP receptor (2, 33). BNP was evaluated in aortic rings from wild-type and 2 null mice, and the effect was similar to that observed for ANP. In wild-type mice, BNP treatment results in a concentration-dependent effect on vasorelaxation (r² = 0.9601, p < 0.05) and an EC₅₀ (23.9 ± 2.5 nM) similar to that observed with ANP. In GC-A-deficient mice there was no relaxation of the aortic rings (data not shown). Thus, GC-A, in fact, appears to represent the receptor for BNP, at least with respect to relaxation of aortic smooth muscle.

Previously it has been shown that CNP can lower blood pressure in anesthetized dogs but not in conscious sheep at similar doses (24, 34, 35). CNP can relax vascular preparations from pig, dog, human, and rat (27, 36–39). In the in vitro experiments, the vasorelaxant effect of CNP was observed at higher concentrations than required for the same effect with ANP (27, 36, 38) and at a potency consistent with activation of GC-A (2, 7, 27, 36, 38). Plasma levels of endogenous ANP and BNP rise during CNP infusion even at very low infusion rates (24). Thus, the lowering of blood pressure by CNP could be a direct effect or an indirect effect mediated through the elevations of ANP and BNP (2, 36). CNP-induced relaxation of KCl-precontracted aortic rings occurs in wild-type (Fig. 2A) or GC-A null (Fig. 2A) mice. The summary data for 6 mice (two
experiments for each mouse) of each genotype are shown in Fig. 2B. The EC₅₀ for CNP relaxation in wild-type mice was 335 ± 126 nM and in GC-A-deficient mice was 146 ± 43 nM (no significant difference). The potency of CNP relative to that of ANP is similar to that reported for rat aorta (EC₅₀: CNP, rat, 10.7 nM; ANP, rat, 1 nM (36)). This relative potency of CNP is also similar to that described for cell culture systems expressing GC-B (7, 33). Our results demonstrate that CNP does not require GC-A and suggest that CNP acts through GC-B (36).

Hemodynamic Effects of Natriuretic Peptides in Conscious Mice—Since the physiology of the aorta does not necessarily reflect that of the small resistance vessels, blood pressure was determined in the GC-A wild-type and null mice in response to the natriuretic peptides. We first infused either ANP or CNP into conscious wild-type mice (initial control infusion, n = 22; mean systolic blood pressure, 103 ± 21 mm Hg; diastolic blood pressure, 68 ± 16 mm Hg; heart rate, 542 ± 14 beats/min) or GC-A-deficient mice (initial control infusion, n = 12; systolic blood pressure, 119 ± 2.7 mm Hg; diastolic blood pressure, 76 ± 1.4 mm Hg; heart rate, 553 ± 14 beats/min). Initial blood pressures, but not heart rates, were significantly different between wild-type and GC-A-deficient mice. ANP reduces blood pressure in rats at infusion rates of 500 ng/kg/min or lower (40) and in this strain of mouse induces diuresis/natriuresis at this dose (31). ANP infusion (500 ng/kg/min) reduced systolic blood pressure from a basal level of 107 ± 4 mm Hg during the control infusion to 89 ± 4.8 mm Hg (after 30 min of ANP infusion, n = 5) (Fig. 3A). In contrast, CNP infused at the same dose (5 mice) or even at a 10-fold higher dose (5000 ng/kg/min, n = 5) failed to significantly affect blood pressure (Fig. 3B). Only at a 100-fold higher CNP dose (50,000 ng/kg/min) was blood pressure significantly reduced in wild-type conscious mice (101 ± 3.3 mm Hg reduced to 84 ± 3.3 mm Hg after a 30-min CNP infusion, n = 7, p < 0.05) (Fig. 3B). The magnitude of change was similar to that following ANP infusion (about 15 mm Hg reduction) (Fig. 3). Diastolic pressure paralleled the
reduction in systolic blood pressure. In wild-type mice, ANP (500 ng/kg/min) reduced diastolic blood pressure from 71 ± 2.3 mm Hg (control period) to 62.3 ± 2.7 mm Hg (after a 30-min infusion, p < 0.05). CNP had no effect at doses of either 500 or 5000 ng/kg/min, but caused a significant reduction at 50,000 ng/kg/min (68.7 ± 2.7 mm Hg to 58.6 ± 1.6 mm Hg after a 30-min infusion, p < 0.05). There were no significant changes in heart rate during ANP or CNP infusions.

The results suggested that CNP could be activating GC-A in the wild-type mice, given the high infusion rates required to affect blood pressure. CNP, therefore, was infused in the GC-A-deficient mice at 50,000 ng/kg/min. CNP infused at this rate caused a significant reduction in systolic blood pressure from 120.6 ± 4.2 mm Hg (control period) to 105 ± 3.6 mm Hg (after a 30-min infusion, n = 7, p < 0.05) (Fig. 4). Indeed, the time course and magnitude of systolic and diastolic blood pressure reduction were nearly identical in wild-type and GC-A-deficient mice with the exception of the difference in initial blood pressures (Figs. 3, 4, and 5). To assess the possibility that the lowering of blood pressure in response to these high dose infusions of CNP results from nonspecific effects of natriuretic peptides, we infused the same dose of ANP. Even at this high rate of infusion, ANP failed to affect blood pressure in the null mice (n = 5) (Fig. 4).

Circulating Levels of Natriuretic Peptides—Blood samples were obtained from four animals in each treatment group at the end of the experimental period. The plasma level of immunoreactive ANP (500 ng/kg/min) was 2500 ± 530 pg/ml (0.8 ± 0.17 nM) and that of immunoreactive CNP (50,000 ng/kg/min) was 40.3 ± 8.0 ng/ml (18.3 ± 3.3 nM). The concentration of CNP was about 20-fold higher than that of ANP, but considerably less than the 100-fold difference in infusion rate. Basal concentrations of circulating CNP were below the detectable limit in both wild-type and GC-A-deficient mice, whereas basal concentrations of ANP were about 159 ± 111 pm (n = 4) in wild-type and 279 ± 109 pm (n = 5) in GC-A null mice.

The in vivo infusion studies extend the aortic ring findings to the whole animal, demonstrating that CNP, in fact, lowers blood pressure in conscious mice deficient in GC-A. ANP, in contrast, fails to lower blood pressure even at high infusion rates. Thus, the actions of CNP in the vasculature are not mediated by GC-A.

If CNP normally regulates blood pressure, it likely does so at the local level since little if any circulates in the bloodstream. Endothelial cells express CNP in culture (41, 42) and in cocultures with vascular smooth muscle cells cause elevations of cGMP (41). Arteries and veins from pig, dog, human, and rat...
are known to relax in response to CNP (36–39). The vasorelaxant effect of CNP is observed at higher concentrations than required for the same effect with ANP, which may be more readily achieved near the sites of secretion of CNP. The production of CNP in endothelial cells as well as very low basal circulating levels (usually undetectable) suggest that local production and action characterize the CNP effector system.

The apparent inability of the CNP/GC-B signal transduction system to compensate for the elevated blood pressure observed in the absence of ANP/GC-A effector system is similar to the situation with two major mechanisms for vascular relaxation that have been described previously, the ANP/GC-A and nitric-oxide (NO) synthase/NO effector systems (2, 12, 17, 26).

In conclusion, these studies represent the first examination of the effects of natriuretic peptides on blood pressure regulation in mice lacking the GC-A receptor. We have shown that ANP or BNP regulate blood pressure exclusively through GC-A. CNP, under acute conditions. We also have shown that CNP is able to regulate blood pressure in the absence of GC-A. CNP, therefore, acts through another receptor, most likely GC-B.

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