Effects of severe hemorrhage on plasma ANP and glomerular ANP receptors

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Frajewicki, Victor, Luna Kahana, Haya Yechiel, Vera Brod, Ricardo Kohan, and Haim Bitterman. Effects of severe hemorrhage on plasma ANP and glomerular ANP receptors. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1623–R1630, 1997.—Atrial natriuretic peptide (ANP) plays an important role in blood volume and electrolyte homeostasis in normovolemia and in hypervolemia. The currently available information on the effects of hypovolemia on plasma ANP is contradictory. Moreover, possible regulation of ANP receptors during severe hemorrhagic hypovolemia has not been investigated. This study evaluated the effects of severe hemorrhage on plasma ANP and on the regulation of glomerular ANP receptor subtypes in anesthetized rats. Constant rate bleeding of 50% of total blood volume within 2 h induced a reproducible shock state characterized by marked decreases in blood pressure, heart rate, and hematocrit and an increase in plasma renin activity and aldosterone. Hemorrhaged rats exhibited a gradual significant increase in plasma ANP from 39.3 ± 2.9 to 114.7 ± 20.0 pg/ml/h after the bleeding (P < 0.001 from the initial value and P < 0.02 from the final value of sham-shock rats). Hemorrhage induced a significant decrease in total glomerular ANP binding sites (172 ± 25 vs. 363 ± 39 fmol/mg protein in hemorrhaged and sham-shock rats, respectively, P < 0.05). This decrease was mainly due to a significant decrease in ANPc receptors (132 ± 22 vs. 312 ± 40 fmol/mg protein in hemorrhaged and sham-shock rats, respectively, P < 0.05). Hemorrhage did not change glomerular ANPa receptor density. No significant differences in the affinity of the glomerular receptor subtypes for ANP were detected. Our data indicate that plasma ANP increases after prolonged severe hemorrhage. It is suggested that downregulation of renal ANPc receptors leads to reduced clearance of ANP and contributes to elevation of its plasma level after severe hemorrhage.

circulatory shock; bleeding; blood pressure; plasma renin activity; aldosterone

ATRIAL NATRIURETIC PEPTIDE (ANP) is an important regulatory hormone secreted primarily from mammalian atria in response to increased wall stretch (8). Hypervolemia and elevation of left atrial pressure or volume are the major known factors stimulating its release. ANP plays an important role in blood volume homeostasis by inducing rapid natriuresis and water excretion, facilitating transudation of plasma water to the interstitium, lowering blood pressure, and antagonizing the renin-angiotensin-aldosterone axis (30). ANP exerts its biological effects by interacting with receptors in target tissues. Specific receptors that bind ANP with high affinity and generate second-messenger molecules or influence ion channels have been identified in a large number of organs, including the kidneys, blood vessels, adrenal glands, heart, lungs, central nervous system, and platelets (1). Two types of ANP receptors that signal through entirely different mechanisms have been defined. One type of receptor (ANPa and ANPb receptors) activates guanylate cyclase and signals most of the currently known biological effects of ANP. The more abundant ANPc receptors that comprise most of the total natriuretic peptide receptors in important target tissues such as the kidney clear the natriuretic peptide from the plasma and have recently been implicated in mediation of certain biological effects of the natriuretic peptides (1, 20, 22).

Most of the available information on the role of ANP in blood volume homeostasis is derived from studies on hypervolemia. In light of the well-characterized effects of high plasma ANP in hypervolemia, it was expected that hypovolemia induced by hemorrhage would decrease blood ANP levels. In this regard, most previous studies evaluated the effects of mild to moderate rapid blood withdrawal protocols of 10–30% of the total blood volume on plasma ANP (5, 6, 11, 12, 16, 21, 33). The results of these studies vary. In some studies, hemorrhage induced a decrease in plasma ANP (5, 6, 11, 12, 33). In others, bleeding of the same magnitude did not induce a significant change in plasma ANP (16, 21). In one study with more severe hemorrhage (40% of total blood volume) (31) and in hypovolemic hypotensive trauma patients (29), an increase in plasma ANP was reported that returned to control values after volume resuscitation. Furthermore, two studies in which hemorrhage induced an initial decrease in plasma ANP that was not enhanced by further bleeding (14, 33), as well as a report of decreased clearance of plasma ANP after hemorrhage (21), suggest that the plasma concentration of ANP at any given stage after hemorrhage may represent the net effect of a balance between changes in ANP secretion and clearance. Based on the previous reports it seems that the effect of severe slow-rate hemorrhage on plasma ANP is different from that of a short-term mild to moderate blood loss.

In an attempt to broaden the understanding of the time course and mechanisms of the divergent changes reported in ANP concentration after bleeding, we studied plasma ANP and its glomerular receptors (ANPa and ANPc) in a rat model of hemorrhagic shock induced by severe slow-rate hemorrhage.

MATERIALS AND METHODS

This study was carried out in adherence with the National Institutes of Health guidelines for the use of experimental animals.
Experiments were performed on male Sprague-Dawley rats weighing 240–320 g. The animals were maintained on regular rat chow and tap water ad libitum. The rats were anesthetized with pentobarbital sodium (50 mg/kg) administered intraperitoneally. All animals were subjected to the same surgical procedure. A polyethylene catheter (PE-50) was introduced into the right carotid artery for recordings of mean arterial blood pressure (MAP) and heart rate and for blood withdrawal during the induction of hemorrhagic shock. The trachea was cannulated to ensure an open airway. Rectal temperature was monitored continuously (Yellow Springs Instruments) and maintained at 36 ± 1°C throughout the experiment using a heating blanket. After completion of surgery, the animals were given heparin (700 U/kg ia) and were observed for a 30-min stabilization period before the start of the experiment.

A severe fixed volume bleeding protocol was employed in this study. In a previous study we determined the blood volume of adult male Sprague-Dawley rats using 51Cr-labeled red blood cells and found it to be 6.5 ± 0.1 ml/100 g body wt (4). Hemorrhagic shock was induced in this study by withdrawing blood from the carotid artery. Withdrawal rate was individually standardized to the rats’ weight to yield a constant rate bleeding of 50% of the total blood volume (total bleeding volume: 3.2 ml/100 g body wt) within 120 min. After the end of bleeding the rats were followed for an additional 60-min observation period and were then killed.

The animals were randomly assigned to one of the two experimental groups: 1) sham-shock rats (n = 17), and 2) hemorrhagic-shock rats (n = 17). Sham-shock rats underwent all surgical procedures but were not bled. MAP, heart rate, and rectal temperature were monitored continuously and recorded on a multichannel recorder (Gould 5000, Gould, Valley View, OH). The experimental information was displayed and analyzed on- or off-line using data acquisition and analysis hardware and software (WINDAQ; DATAQ Instruments).

In hemorrhaged rats, blood samples were withdrawn at the end of the stabilization period (time 0), at the end of the bleeding (time 120 min), and at the end of the experiment (time 180 min) for analysis of the hematocrit, plasma renin activity (PRA), and aldosterone, and for analysis of plasma ANP. In the sham-shock group of rats 1 ml of blood was taken for the same analyses at time 0 and was replaced by an equal volume of 0.9% NaCl solution. To avoid hemodynamic instability, only one more blood sample for the same determinations was taken in sham-shock rats at the end of the experimental protocol.

Kidneys were removed and immersed immediately after decapsulation in solution A (0.9% NaCl containing 10−7 M aprotinin, 10−6 M leupeptin, and 10−7 M pepstatin) at 4°C. Kidneys were then bisected, the white papilla was removed, and passed through stainless steel sieves with sequential slight modifications. The minceate was thoroughly washed and passed through stainless steel sieves with sequential pore sizes of 500, 190, and 140 μm. The filtrate was then centrifuged three times at 250 g for 5 min and the pellet was resuspended in solution A and washed over a 94-μm pore size sieve to retain the glomeruli. After centrifugation, the glomeruli were suspended in buffer B (10 mM NaHCO3, 1 mM EDTA, 10−6 M aprotinin, 10−6 M leupeptin, and 10−7 M pepstatin) at pH 7.4. The preparation thus obtained contained >95% glomeruli as evaluated by light microscopy. To exclude possible interference by prior receptor occupancy (29), glomerular preparations were centrifuged (250 g 10 min) and suspended in acid buffer (phosphate-buffered saline + acetic acid, pH 5.0) for 10 min. Suspensions were centrifuged and washed twice in buffer B. Preparations were frozen and stored overnight in liquid nitrogen.

Preparation of membranes. The glomerular preparations were thawed and then homogenized twice with a Polytron homogenizer (setting 6, for 10 s) followed by a Potter homogenization. The homogenates were diluted in buffer B and centrifuged (250 g for 10 min). The pellets were sonicated and homogenized with a Polytron homogenizer followed by an additional 10-min centrifugation at 250 g. The supernatants were then centrifuged at 30,000 g for 15 min, and the pellets were washed twice in buffer B and resuspended by gentle homogenization in buffer C (containing (in mM) 50 tris(hydroxymethyl)aminomethane (Tris)-HCl, 250 sucrose, 0.1 EDTA, and 1 MgCl2, pH 7.4). Aliquots were taken for protein determination, and the membranes were frozen in liquid nitrogen and stored at −70°C until assayed.

Receptor binding assays. Radioiodinated binding assays were carried out as described previously (9) by incubating glomerular membranes (10–20 μg protein/ml) with 6–8 pM of 125I-labeled ANP-(99–126) and varying concentrations (10−12–10−6 M) of unlabeled competing peptides. We used two parallel systems as follows: a homologous assay in which radiolabeled ANP was displaced by unlabeled rat ANP-(99–126) and a heterologous system in which 125I-ANP-(99–126) was displaced by increasing concentrations of the ANP analog des-[Gln18,bSer19,Gly20,Leu21,Gly22] ANP(4–23)-NH2 from rat (C-ANP; Peninsula Laboratories, Belmont, CA), which selectively binds only to clearance receptors. The incubation was carried out at 6°C, for 20 h, in 1 ml of 50 mM Tris-HCl buffer containing 1 mM EDTA, 5 mM MnCl2, and 0.5% heat-inactivated bovine serum albumin, pH 7.4, in the presence of 10−6 M phosphoramidon to prevent possible degradation of the hormone. In preliminary experiments, we showed that binding at 6°C reached equilibrium after 6 h and remained stable for 24 h (results not shown). Likewise, the linearity of binding in the range of membrane protein concentrations used was confirmed in this set of experiments. Membrane-bound 125I-ANP was separated from the non-bound ligand by filtration through 1% polyethyleneimine-treated GF/C filters (Whatman, Clifton, NY) followed by extensive washing with ice-cold buffer D (50 mM phosphate buffer, pH 7.4). Radioactive ANP retained on the filter was counted in a gamma counter (LKB, Minigamma, model 1275, Turku, Finland) with an efficiency of 80%.

Plasma analysis. Radioimmunoassay of ANP was performed on blood collected in polypropylene tubes containing EDTA (1 mg/ml). Plasma was separated by centrifugation at 4°C and acidified with 50% trifluoroacetic acid (TFA) to a final concentration of 1.100 TFA. Plasma samples were stored at −20°C until assay. Before radioimmunoassay, the acidified plasma was extracted by passage through Sep-Pak C18 cartridges (Waters, Milford, MA) (mean recovery 80%), eluted with ethanol, acidified to pH 4 with 4% acetic acid, and evaporated to dryness under air at 37°C. A nonequilibrium radioimmunoassay using a second antibody was used for separation of bound and free tracer. The tracer used was 125I-labeled rat ANP (Amersham IM-187). The antibody and rat ANP-(99–126), which was used as a standard, were both obtained from Peninsula Laboratories. The minimal detectable dose of ANP was 4 pg/tube, and intra- and interassay...
coefficients of variation were 10 and 15%, respectively. Samples of both groups of rats (hemorrhage and sham) were assayed in a single batch. PRA was measured by radioimmunoassay for angiotensin (ANG) I (New England Nuclear) and expressed as nanograms of ANG I formed per milliliter of plasma generated during a 1-h incubation period. Plasma aldosterone concentrations were measured on unextracted samples with a commercial radioimmunoassay kit (Coat-a-Count, Diagnostic Products, Los Angeles, CA). Protein concentration in membrane preparations was measured by bicinchoninic-protein assay reagent kit (Pierce, Rockford, IL).

Data analysis. The binding data was evaluated using the ALLFIT computer program (10) based on a four-parameter logistic equation to obtain estimates of the half-maximal effective dose and the corresponding slope factors of the curves. Then each competition curve was analyzed according to a model for the binding of competing ligands to one or several independent classes of binding sites based on the principle of law of mass action. A model involving two classes of sites was retained only when the level of the fit was statistically better than that for a single class of sites, as evaluated by a partial F test.

The results are expressed as means ± SE. Statistical differences were evaluated by the Wilcoxon rank test.

RESULTS

A severe constant volume bleeding model of hemorrhagic shock characterized by marked hypotension was employed in this study. The time course of MAP in the two experimental groups of hemorrhaged and sham-shock rats is illustrated in Fig. 1. Initial MAP in the two experimental groups was similar (147–149 mmHg). In hemorrhaged rats, MAP decreased gradually to 49 mmHg at the end of bleeding (time 120 min) and did not change significantly throughout the rest of the protocol, with a final value of 52 ± 7 mmHg. Sham-shock rats exhibited a minor reduction in MAP during the first 20 min with no further significant change throughout the study, indicating that the surgical procedure itself did not contribute significantly to the severity of the shock state. MAP was significantly lower in the hemorrhaged group of rats from time 20 min (P < 0.01) to the end of the study (P < 0.001).

Figure 2 illustrates the heart rates of the two experimental groups. The initial heart rate was similar in both groups (445 ± 7 and 433 ± 9 beats/min in hemorrhaged and sham rats, respectively). Heart rate did not change significantly in the sham-shock group. Slowing of the heart rate is a characteristic finding during early stages of hemorrhagic shock in the anesthetized rat. In the present study hemorrhaged rats exhibited a typical gradual decrease in heart rate that reached a minimal value of 269 ± 17 beats/min at 80 min. Later on the heart rate increased gradually, reaching a value of 333 ± 17 beats/min at the end of the study. It should be noted that the heart rate was significantly slower in hemorrhaged rats than in the sham-shock group until the end of the experiment.

The initial hematocrit was comparable in both groups (43 ± 0.8 and 42 ± 0.5% in the hemorrhaged and sham-shock groups, respectively). The hematocrit did not change significantly in the sham-shock group. Hemorrhage induced a gradual decrease of the hematocrit to 34 ± 1% at the end of bleeding and 32 ± 1.3% at the end of the experiment (P < 0.001 from sham rats at both times).

PRA is shown in Fig. 3. The initial values of PRA were not different among the groups (22.2 ± 6 and 15.4 ± 5.9 ng ANG I·ml⁻¹·h⁻¹ in hemorrhaged and sham rats, respectively). A significant increase in PRA at the end of the experimental protocol was found in sham rats (P < 0.01 from initial value). Hemorrhage augmented the increase in PRA significantly (P < 0.001 from sham rats at the end of the experiment).

Plasma aldosterone levels are shown in Fig. 4. Initial values in the two experimental groups were not significantly different. In sham-shock rats plasma aldoste-
rone at the end of the experiment was significantly higher than the initial value ($P < 0.05$). A significantly steeper increase in plasma aldosterone was observed in hemorrhaged rats, reaching markedly higher values at the end of bleeding with a further increase at the end of the experiment ($P < 0.01$ from initial values of this group and from sham rats at the end of the experiment).

Plasma ANP values are illustrated in Fig. 5. Initial values in the two groups were similar (39.3 ± 3.0 and 35.4 ± 5 pmol/l in hemorrhaged and sham rats, respectively). In the sham-shock group plasma ANP did not change significantly at the end of the experiment. In contrast, hemorrhaged rats exhibited a significant increase in plasma ANP, reaching a threefold higher value at the end of the experiment (114.7 ± 20.0 pmol/l) ($P < 0.001$ from initial values of this group and $P < 0.02$ from the final value of sham-shock rats).

Glomerular ANP receptor density is illustrated in Fig. 6 and Table 1. In sham-shock rats >80% of the receptors were of the ANP$_C$ type. Hemorrhage induced a significant decrease in total ANP receptor density.
Hemorrhage did not induce a significant change in the density of the ANP$_A$ receptors. The marked decrease in total ANP receptors is accounted for by the significant decrease in ANP receptor density without a significant change in the density of ANP$_C$ receptors in hemorrhaged and sham rats.

Table 1 summarizes the dissociation constants ($K_d$) for glomerular ANP binding sites. No significant differences were detected in the $K_d$ values of the two types of ANP receptors among hemorrhaged and sham rats.

**DISCUSSION**

The severe prolonged bleeding protocol employed in this study induced a reproducible shock state characterized by marked decreases in MAP, heart rate, and hematocrit and a characteristic increase in PRA and aldosterone. The slow-rate severe hemorrhage induced a significant threefold increase in plasma ANP concentration at the end of the 3-h experimental protocol. Most of the increase occurred in the hemorrhaged group during the last hour of the experimental protocol.

Our study demonstrates a significant decrease in the density of glomerular ANP receptors. Eighty-six percent of the total ANP binding sites in sham-shock rats were of the ANP$_C$ type. The severe bleeding protocol induced a significant decrease in the total ANP receptor density without a significant change in the density of ANP$_A$, glomerular receptors. The decrease in the total receptor density in the hemorrhage group may be accounted for primarily by a reduction in the number of ANP$_C$ receptors. No differences in the affinity of the glomerular receptors to ANP were detected between sham- and hemorrhagic-shock rats.

The effects of acute hemorrhage on plasma ANP have been evaluated in a number of previous studies in animals and humans (5, 6, 11–13, 16, 21, 29, 31, 33). Most were oriented toward an assessment of the physiological effect of an acute decrease in blood volume on the secretion of ANP. For this purpose, mild to moderate bleeding protocols were employed, in which 2–30% of the total blood volume was withdrawn within relatively short periods of time, with the plasma concentration of ANP being determined immediately thereafter. In the majority of these studies, hemorrhage induced a decrease in plasma ANP (5, 6, 11, 12, 33). It was suggested that the acute decrease in atrial pressure caused by hypovolemia reduces the secretion of ANP. However, in some studies hemorrhage of the same magnitude did not induce a significant change in plasma ANP in animals (12) or in man (16). Moreover, a study of a more severe hemorrhage of 40% of the total blood volume reported an increase in plasma ANP (31). Increased plasma ANP was also reported in severely hypotensive hypovolemic trauma patients (29). Some of the differences between the reported effects of hemorrhage may be related to the different animal species, types of anesthesia, and fluid resuscitation, as well as the presence of tachycardia and changes in the blood levels of other hormones that alter the secretion of ANP. However, it seems that the effect of severe slow-rate hemorrhage on plasma ANP is different from that of a short-term mild to moderate blood loss.

Hemorrhage induces an acute decrease in blood volume, which decreases atrial dimensions and pressure and may reduce ANP secretion. It is obvious that this mechanism alone does not account for the variable effects of hemorrhage on plasma ANP. Other mechanisms that affect the release of ANP or its clearance from the blood should be taken into consideration. In one report, it was suggested that tachycardia after hemorrhage increases ANP secretion and opposes the effects of decreased blood volume (5). However, in our study, heart rate in the hemorrhagic-shock group was significantly lower than that of the sham-shock group throughout most of the experimental protocol. Therefore, tachycardia cannot explain our finding of increased plasma ANP in hemorrhaged rats.

Elevated plasma and tissue levels of other hormones and mediators that enhance the production and secretion of ANP may offset the effect of reduced atrial pressure and may underlie the increase in plasma ANP after hemorrhage. In this regard, glucocorticoids, catecholamines, and endothelin-1 seem to be the most relevant, because they all increase after hemorrhage and all have been shown to enhance the transcription of the ANP gene and the biosynthesis and secretion of ANP within a few hours (13, 18, 30). Hence, rapid augmentation of ANP secretion by some of the “stress” hormones and mediators may be relevant to our observation of increased plasma ANP after hemorrhage. ANG II, arginine vasopressin, and prostaglandin F$_{2x}$ also increase after hemorrhage and have the capacity to increase plasma ANP. However, their relevance to our model of hemorrhagic shock is questionable, both because they affect ANP after longer periods of time and because their effects on ANP secretion in physiological plasma concentrations are debatable (30).

Changes in the clearance of ANP may be of the utmost importance to an explanation of the divergent effects of rapid mild to moderate bleeding and severe prolonged hemorrhage. In this regard, Geer et al. (14) observed in dogs a slight decrease in the plasma ANP after bleeding of 10 ml/kg (~15% of the total blood volume), with no further decline after bleeding of 30% of the total blood volume. Plasma ANP increased slightly when bleeding was continued to ~45% of the total blood volume; however, all changes were not statistically significant. In a model of stepped hemorrhage in rats, Yoshida et al. (33) found a significant decrease in...
plasma ANP after nonhypotensive bleeding of 0.8% of body weight (~12% of the total blood volume). Plasma ANP did not decrease significantly further after bleeding 30% of the total blood volume. These observations are consistent with the suggestion of a biphasic effect of hemorrhage on ANP, in which mild bleeding induces a rapid decrease in ANP secretion, with no further decline after continued, more severe hemorrhage. Diminished clearance of ANP from the blood may explain the arrested decline in its plasma levels during continued hemorrhage. In this regard, a recent study by Leskinen et al. (21) reported a shift of the ANP disappearance curve to the right in hemorrhaged rats. This study lends further support to the suggestion that decreased ANP clearance occurs after severe bleeding and may even account for our observation of increased plasma ANP after prolonged severe hemorrhage.

ANP is eliminated from the blood by almost every tissue. The lungs, kidneys, and the liver/splanchnic area are the most important organs in ANP clearance and degradation. The three known mechanisms involved in the clearance of ANP are 1) receptor-mediated binding, uptake, and metabolism by target tissues; 2) degradation by enzymes and other processes at plasma membranes; and 3) excretion into nonplasma fluids such as urine. The first mechanism of receptor-mediated degradation of ANP is quantitatively the most important. Two types of ANP receptors have been identified in target tissues (1). The first type are guanylate cyclase-linked receptors (ANP$_A$, which is most abundant in the kidney and adrenal glands, and ANP$_B$, which is most abundant in the brain), which mediate most of the biological actions of ANP. Another receptor (ANP$_C$) was originally considered to possess only a clearance function but has recently been shown to elicit some of the physiological actions of ANP by inhibiting adenylate cyclase and augmenting phospholipase C activity (1, 25). The ANP$_C$ receptor is the most abundant and most widely distributed in many tissues and cells, including the renal cortex, vascular endothelium, and smooth muscle cells (25, 30). In these tissues, the largest population of receptors are ANP$_C$ receptors, which may constitute >90% of the total ANP receptor population (20, 22). The majority of ANP clearance involves its binding to the ANP$_C$ receptor at the cell membrane, rapid internalization, and subsequent intralysosomal degradation of the ligand to amino acids. ANP$_C$ receptors are then recycled to the cell surface membrane. This process is the major determinant of the mean clearance rate of ANP.

Various experimental conditions have been claimed to regulate receptor-mediated degradation of ANP (30). For example, it has been shown that water deprivation (19) and ligature of bile ducts (15) increase ANP$_C$ receptor density on glomerular membranes. These two examples support the role of changes in ANP clearance in the control of plasma ANP activity. However, it is still debated whether the data presented in some studies of the physiological regulation of ANP receptors truly represent changes in receptor density or whether they might rather reflect prior changes in occupancy of the receptor by ANP. To overcome this difficulty in interpretation, we undertook the necessary precautions to remove excessive ANP binding to receptors and assessed receptor density after acid washing, a procedure designed to remove ANP already bound to the receptor. Furthermore, we did not find a significant change in the $K_d$ of both ANP$_A$ and ANP$_C$ receptors for ANP after hemorrhage. It may therefore be concluded that our data represent a true decrease in ANP$_C$ receptor density in hemorrhaged rats and not decreased binding due to high prior occupancy by ANP or changes in the affinity of the receptor for its ligand. To the best of our knowledge, no previous published data are available on changes in specific ANP receptors after severe hemorrhage and shock. On the basis of our findings, it is suggested that severe prolonged hemorrhage of 50% of the total blood volume induces a decrease in the density of renal ANP$_C$ receptors. The mechanism of the suggested downregulation of renal ANP receptors after hemorrhage is unclear. Increased levels of the ligand can downregulate the receptor by inhibition of new receptor production. However, the time frame of our experiment (3 h) is incompatible with this explanation. It has been shown that every hour the total population of surface ANP$_C$ receptors is internalized and recycled (28). It is possible that severe hemorrhage either enhances internalization or diminishes recycling of the ANP$_C$ receptors and thus decreases their density on the outer cell surface. It seems rational to suggest that in our model the primary event is downregulation of ANP$_C$ receptors. Because receptor-mediated clearance of ANP is the most important determinant of the elimination of ANP from the blood, we also suggest that the decrease in receptor-mediated clearance increases plasma ANP after severe hemorrhage and hemorrhagic shock.

Compensatory responses after volume loss due to bleeding include a disproportionate decrease in blood flow to the kidneys and the splanchic vascular bed that might reduce the local metabolic clearance of ANP by neutral endopeptidase and other enzyme systems. Because the kidney has a great capacity for enzymatic degradation of ANP, it is possible that decreased metabolic clearance of ANP also contributes to the increase in plasma ANP after major blood loss. However, we are unaware of data substantiating this hypothesis. Hypovolemia also decreases urine output, but urinary excretion does not play a major role in the elimination of ANP.

The physiological significance of the increase in plasma ANP after severe hemorrhage and hemorrhagic shock is unclear. A marked decrease in renal blood flow and the glomerular filtration rate (GFR) and ischemic damage characterize the renal response to severe hemorrhage. The resulting impairment in renal function is an important determinant of outcome in circulatory shock. The exogenous administration of high doses of ANP has been shown to be beneficial to the preservation of GFR both in acute and established phases of ischemic renal injury (7, 24, 32). ANP also exerts direct and indirect effects distal to the glomerulus on the renal tubules, and at a lower dose ANP causes natriuresis without detectable changes in GFR (2, 3, 17). For
example, in a hypovolemic model of acute pancreatitis, ANP raised urinary volume and natriuresis with no change in GFR (23). It may therefore be suggested that high plasma ANP improves renal function and attenuates renal damage in low-flow states such as hemorrhagic shock. However, the exact role of ANP in body fluid regulation and its alleged role in the preservation of renal function in various pathological states has yet to be established.

In summary, the present study, severe hemorrhage of 50% of total blood volume in rats induced a marked increase in plasma ANP. Hemorrhage also induced a significant decrease in the density of glomerular ANP receptors due to a decrease in glomerular ANPC receptors. It is suggested that downregulation of renal ANPC receptors leads to reduced clearance of ANP and contributes to elevation of its plasma level after severe hemorrhage. Our data indicate that changes in plasma ANP after hemorrhage reflect the net effect of factors that increase or decrease ANP secretion and changes in its clearance. Also, in contrast to small and moderate acute changes in the volume status, the net effect of ANP after hemorrhage reflects the net effect of factors that increase or decrease ANP secretion and changes in its clearance, or increase in plasma ANP. Hemorrhage also induced a significant decrease in the density of glomerular ANP receptors due to a decrease in glomerular ANPC receptors leading to reduced clearance of ANP and contributes to elevation of its plasma level after severe hemorrhage. Our data indicate that changes in plasma ANP after hemorrhage reflect the net effect of factors that increase or decrease ANP secretion and changes in its clearance. Also, in contrast to small and moderate acute changes in the volume status, the net effect of hemorrhage induces a significant increase in plasma ANP.

Perspectives

Despite 15 years of research, the importance of ANPs is still debated. It has been established that the most abundant natriuretic peptide, ANP, has a role in blood volume and pressure homeostasis. It is also clear that it exerts different effects in normal and pathological conditions and that some of its effects are different in acute and chronic conditions. The data on ANP in hypovolemia is limited and controversial. Previous studies of ANP after acute hemorrhage yielded contradictory findings, with some showing a decrease, no change, or increase in its plasma levels. Careful analysis of the available data in acute hemorrhagic hypovolemia suggests that differences in plasma ANP concentration may be related to the severity of the hypovolemia and the rapidity of its induction. Because the severe stress associated with hemorrhage provokes numerous regulatory mechanisms, it is expected that plasma ANP level at any given moment represents the net result of mechanisms that either increase or decrease it. Our data showing a marked delayed increase in plasma ANP after a severe and relatively prolonged acute hemorrhage may help reconcile some of the controversies. It is suggested that, although a mild to moderate rapid loss of blood may initially be associated with a decrease or no change in plasma ANP, a more prolonged and severe bleeding increases it. In addition, our data on renal ANP receptors are consistent with a suggestion that a decrease in ANPC receptors and the resultant decrease in renal ANP clearance may be an important mechanism of increased plasma ANP in severe hypovolemia.

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REFERENCES


19. Kollenda, M. C., A. M. Vollmar, G. A. McEnroe, and A. L. Gerbes. Dehydration increases the density of C receptors for...


