Endothelin Antiserum Decreases Volume-Stimulated and Basal Plasma Concentration of Atrial Natriuretic Peptide

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Background. Endothelin-1 (ET-1) is the most powerful factor known to release atrial natriuretic peptide (ANP) in vivo and in cultured cardiac myocytes or preparations of atrium. We tested the role of endogenous ET-1 in the regulation of ANP release by passive immunization in anesthetized rats.

Methods and Results. Intravenous injection of antiserum against ET-1 was shown to decrease basal and volume-stimulated plasma concentrations of ANP, whereas control serum was without effect. Antiserum generated in rabbits cross-reacted 100% with endothelin-2 and -3. In pentobarbital-anesthetized Wistar rats treated with ET-1 antiserum, plasma ANP concentration measured by radioimmunoassay was reduced by 37% from starting level after 10 minutes and by 30% after 60 minutes. Control rat serum had no effect on plasma ANP. Rapid intravenous infusion of 8 mL of 0.9% NaCl caused a sixfold increase of plasma ANP concentration in control rats but only twofold in rats pretreated with ET-1 antiserum (P<.01). This effect of ET-1 antiserum was dose dependent. ET-1 antiserum changed neither blood pressure nor heart rate significantly in anesthetized rats. Pretreatment with ET-1 antiserum did not affect the initial hypotensive response to intravenous ET-1 0.5 nmol/kg but significantly attenuated the subsequent hypertensive response to endothelin.

Conclusions. Endothelin may be a physiological modulator of both basal and stimulated ANP release. (Circulation. 1993;88:1172-1176.)

KEY WORDS • atrial natriuretic factor • atrial natriuretic peptide • endothelin

Atrial natriuretic peptide (ANP) is released from atrial myocytes in response to increased atrial filling pressure and atrial wall distension.1-3 The mechanism whereby atrial wall stretch distension causes ANP release is unknown. Several vasoactive hormones, including epinephrine,4 norepinephrine,5 calcitonin gene-related peptide (CGRP),6 endothelin-1 (ET-1),7,8 and vasopressin,9 reportedly stimulate ANP release in vitro or in vivo. Whether these hormones are physiological regulators of ANP release is currently unknown. Cellular influx of calcium appears to play a role, since calcium ionophores stimulate and calcium entry blockers decrease ANP release.2

ET-1, a 21-amino acid peptide produced by endothelial cells,10 is a potent secretagogue for ANP release in cultured atrial myocytes, in the isolated rat heart preparation, and in the intact animal. ET-1 production in endothelial cells is increased by fluid dynamic shear stress,11 transforming growth factor-β,12 thrombin,10 angiotensin II,13 vasopressin,13 and insulin.14 The effect of ET-1 on ANP release has been studied only by application of synthetic polypeptide. To test whether endogenous endothelin modulates ANP release in vivo, we treated Wistar rats with rabbit antiserum to ET-1. We observed that such treatment attenuated volume-stimulated ANP release and lowered basal plasma ANP levels.

Methods

Experimental Animals

Male Wistar rats weighing 265 to 400 g were used. Rats were fed standard rat food and tap water ad libitum. Study design was accepted by the institutional Ethics Committee. Rats were anesthetized by administration of pentobarbital, 40 mg/kg body weight IP.

Basal State and Acute Volume Expansion

Indwelling cannulas were inserted into femoral veins and femoral arteries of anesthetized rats. To study the effect on basal ANP levels, rabbit ET-1 antiserum (experimental animals; n=10) or control rabbit serum, 0.1 mL (control animals; n=9), was given intravenously. Arterial blood samples were drawn before and 10 and 60 minutes after administration of antiserum or control serum. To study effects on volume-stimulated plasma ANP (n=36; 8 to 10 per group), 1 minute after pretreat-
ment with rabbit antiserum to ET-1 or nonimmune rabbit serum (controls), 0.1 mL IV (diluted 1:1, 1:5, and 1:25, respectively), 8.0 mL of 0.9% saline was rapidly infused, in 1 minute, via femoral venous catheter. To avoid biases, rats were allocated randomly to one of these treatment procedures. Each rat received only one antiserum concentration and was used for one experiment only. Blood samples were drawn via femoral artery catheter before and 1 minute after injection of ET-1 antiserum and 1, 5, and 10 minutes after intravenous saline infusion into ice-chilled tubes containing EDTA 15 mmol/L (final concentration) and trasyloil 500 U. Plasma was separated by centrifugation and stored at -20°C until assayed for ANP by radioimmunoassay as described.15

Measurement of Blood Pressure

For recording of blood pressure and heart rate, male Wistar rats (210 to 280 g) were anesthetized with urethane (1.5 g/kg IP), and the left femoral arteries and veins were cannulated for the cardiovascular recording and intravenous injections, respectively. For technical details, see Reference 16. Intravenous injections were administered as boluses. Initially, the effects of ET-1 antiserum bolus injection alone were compared with those of control serum. In the second experiment, the cardiovascular effects of a bolus of ET-1 were modified by a preceding bolus injection (1 minute earlier) of ET-1 antiserum or control rabbit serum. The areas under the curves from 8 to 48 minutes were compared.

Endothelin Antiserum

Antiserum to ET-1 was generated in rabbits by immunization as described elsewhere.17 The antiserum used showed <0.1% cross-reaction with big ET-1 1-38 and 22-38 fragments, with the 20-50, the 74-91, and the 171-201 sequences of preproendothelin (Peptide Institute, London, UK), human ANP (Peninsula, London, UK), angiotensin II (Schwarz-Mann, St Louis, Mo), and arginine vasopressin (Ferring, Malmö, Sweden). It cross-reacted 100% with endothelin-2 and endothelin-3 (Peptide Institute). This antiserum bound 20% of 125I-labeled ET-1 when diluted 1:24 000 in a radioimmunoassay for ET-1.17

Statistical Evaluation

Statistical analysis was done by paired and unpaired t test, as appropriate, combined with correction for multiple comparisons by Bonferroni technique.18

Results

Basal Plasma ANP Levels

After a 0.1-mL IV bolus injection of endothelin antiserum, plasma ANP was reduced (Fig 1) from starting level at 10 minutes by 37% (P<.01) and at 60 minutes by 30% (P<.05). The starting levels of plasma ANP, 25.6±7 pg/mL (mean±SEM) for rats receiving antiserum and 33.1±9 pg/mL in controls, were not significantly different. Bolus injection of 0.1 mL of control serum did not affect plasma ANP levels.

Plasma ANP After Acute Volume Load

Rats pretreated with 0.1 mL IV of endothelin antiserum showed a markedly attenuated response to acute volume load achieved by rapid infusion of 8.0 mL of 0.9% NaCl (Fig 2) compared with rats treated with control serum (P<.01). Results were corrected for dilution according to determination of serum concentration of albumin. This effect of endothelin antiserum was gradually decreased with dilution 1:5 (P<.01) and 1:25 (P<.05 at 1 minute; other time points not significant) of the antiserum bolus injected, indicative of a dose-response relation. The starting plasma ANP concentration of rats receiving antiserum, 29.4±10 pg/mL, was not significantly different from that of control rats (23.0±6 pg/mL).
Antagonism of the Cardiovascular Effect of ET-1 by Antiserum

Intravenous bolus injection of endothelin antiserum, 0.1 mL, exerted no significant effect on mean arterial pressure or heart rate (Fig 3). After a brief hypotensive response, ET-1, 0.5 nmol/kg IV, increased mean arterial pressure by about 10 mm Hg (maximum, 14±4 mm Hg) for the entire observation period of 50 minutes while, concomitantly, the heart rate was decreased (maximum, 34±4 beats per minute; Fig 4, A and B). Pretreatment with ET-1 antiserum did not modify the initial hypotensive response to ET-1 but significantly decreased the magnitude and duration of the hypertensive response. Bradycardia induced by the ET-1 bolus was not significantly modified by the antiserum pretreatment.

Discussion

Immunological inhibition of endogenous endothelin was shown here to reduce basal plasma ANP levels in anesthetized rats. Moreover, endothelin antiserum clearly attenuated the increase of plasma ANP originally shown to occur in response to acute volume load. These observations strongly suggest that endothelin plays a modulatory role in the regulation of both basal and stimulated ANP secretion.

Our findings accord with reported stimulation by synthetic ET-1 of ANP release from superfused rat atria. Both basal and stretch-induced ANP release was stimulated by ET-1 in perfused rat heart preparation. Moreover, endothelin-induced release of ANP was shown in a coculture of bovine endothelial cells and rat atrial myocytes. ET-1 infusion or intravenous bolus injection caused increase of plasma ANP concentrations in rats. However, these observations do not justify definite conclusions regarding the possible role of endogenous endothelin in ANP regulation.

Endothelin causes rapid influx of extracellular calcium into myocytes. This may mediate ANP release from storage granules. In fact, calcium ionophores increase ANP release from myocytes, and calcium channel blockers do the opposite. Accordingly, endothelin-induced ANP release from rat atria was inhibited in part by verapamil, a calcium channel blocker.

In comparison with other hormones causing ANP release, ET-1 appears to be the most powerful one on a molar basis. CGRP, which is present in the atrial wall, is almost as potent as ET-1 in this regard. Less potent but also capable of releasing ANP are angiotensin II, epinephrine, and norepinephrine. Vasopressin was recently shown to restore volume-stimulated ANP release in pituitary rats. Thus, ANP release may be modulated by permissive or facilitating effects of several vasoactive hormones, including ET-1, CGRP, and vasopressin.

In view of the time course within which endothelin antiserum was shown here to decrease basal and stim-
ulated ANP release, already after 1 (stimulated) to 10 (basal) minutes, de novo synthesized endothelin could not be responsible for ANP release. Moreover, it is unlikely that endothelin would be released from cellular stores, because such storage sites have not been described. We propose that endothelin plays a permissive role, facilitating the release of ANP from cardiac myocytes. This role may encompass a “tonic” regulation of intracellular calcium, which, if interrupted by endothelin antibody, may render ANP secreting myocytes less sensitive to stretch.

In tests of the role of endothelin in ANP release, passive immunization with endothelin antiserum offers some advantages over the administration of the polypeptide itself. First, under physiological conditions, endothelin probably acts locally in a paracrine way and not as a circulating hormone. Thus, application of exogenous endothelin may not mimic local secretion closely enough. Second, when infused in pharmacological doses, endothelin may cause release of ANP simply by increasing atrial pressure. Third, infused ET-1 is effectively cleared by the lungs, which may play a detoxifying role to protect from harmful systemic effects.25 A weakness of using ET-1 antiserum is that blockade of ET-1 is incomplete, since only about 90% or even less of the administered ET-1 bolus dose may be bound by the antibodies administered (unpublished data from our laboratory). This may explain why endothelin antiserum only partly inhibited stimulated ANP release and the blood pressure–raising effect of synthetic endothelin. The initial lowering of blood pressure after intravenous ET-1, thought to be caused by rapid release of nitric oxide from vascular endothelial cells,26 was not inhibited by endothelin antiserum. This may be explained by a difference between endothelin receptors in endothelial cells and those in myocytes. In fact, endothelial cells reportedly carry nonspecific endothelin receptors of endothelin B-type, which bind endothelins 1 through 3,27 whereas myocytes have endothelin A-type receptors specific for ET-1. It is of note that inhibition of ET-1 pressor responses by the endothelin A-type receptor antagonist FR 139317 in the rat was nearly complete, but the initial blood pressure reduction was not affected.28 Moreover, blood ET-1 concentrations after administration of endothelin (5 nmol/kg body weight) may have been too high to allow effective immunological blockade at the level of the endothelium.

Endothelin antiserum may also block ANP release recently shown to be mediated by carotid-aortic baroreceptors.29 These investigators29 pointed out that ET-1 is found in the hypothalamus and neuronal terminals of the neurohypophysis30 and may, after its release into the circulation, stimulate ANP release.7,8

Because endothelin antiserum affected neither blood pressure nor heart rate, the decrease of basal and stimulated plasma ANP concentration could not have been brought about by hemodynamic effects of the antiserum bolus injection.

In conclusion, we have shown that passive immunization with endothelin antiserum decreases basal plasma ANP concentrations and volume-stimulated increase of plasma ANP, suggesting an important modulatory role for endothelin in the regulation of ANP release.

Acknowledgments

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