Inhibition of Sympathoadrenal Activity by Atrial Natriuretic Factor in Dogs

JÜRGEN HOLTZ, OLAF SOMMER, AND EBERHARD BASSENGE

SUMMARY In six conscious, trained dogs, maintained on a normal sodium intake of 2 to 4 mEq/kg/day, sympathetic activity was assessed as the release rate of norepinephrine and epinephrine during 15-minute i.v. infusions of human α-atrial natriuretic factor. Mean arterial pressure (as a percentage of control ± SEM) during randomized infusions of 0.03, 0.1, 0.3, or 1.0 μg/kg/min was 99 ± 1, 95 ± 1 (p < 0.05), 93 ± 1 (p < 0.01), or 79 ± 6% (p < 0.001), respectively, but no tachycardia and no augmentation of the norepinephrine release rate (up to 0.3 μg/kg/min) were observed, which is in contrast to comparable hypotension induced by hydralazine or nitroglycerin. The release rate of epinephrine (control, 6.7 ± 0.6 ng/kg/min) declined immediately during infusions of atrial natriuretic factor to a minimum of 49 ± 5% of control (p < 0.001) during 0.1 μg/kg/min and to 63 ± 5% (0.1 > p > 0.05) or 95 ± 13% (not significant) during 0.3 or 1.0 μg/kg/min. Steady state arterial plasma concentrations of atrial natriuretic factor were 39 ± 10 pg/ml (n = 6) during infusions of saline and 284 ± 24 pg/ml (n = 6) and 1520 ± 300 pg/ml (n = 9) during 0.03 and 0.1 μg/kg/min infusions of the factor. In these 21 trials with steady state plasma atrial natriuretic factor below 3000 pg/ml, the relative epinephrine release rate during infusion (as a percentage of control release rate) correlated inversely (r = -0.63, p < 0.01) with log(plasma atrial natriuretic factor), indicating a 24 ± 2% decline in epinephrine release per 10-fold augmentation of plasma atrial natriuretic factor and no change in epinephrine release at an atrial natriuretic factor concentration of 23 pg/ml, which was not significantly different from the preinfusion level (40 ± 6 pg/ml). It is concluded that endogenous atrial natriuretic factor under physiological conditions substantially modulates sympathetic activity by inhibiting epinephrine release and baroreceptor reflexes. (Hypertension 9: 350-354, 1987)

Key Words • epinephrine release rate • norepinephrine release rate • sympathetic activity • adrenal gland • atrial natriuretic factor

The cardiac hormone atrial natriuretic factor (ANF)1,2 seems to be involved in the regulation of blood pressure and fluid homeostasis.3-5 Among its multiple actions, an inhibition of sympathoadrenal activity by ANF has been tentatively suggested6,7 as contributing to its hypotensive effect. Therefore, we assessed sympathoadrenal activity during ANF infusions in conscious dogs by measuring the release rate of norepinephrine (NE) and epinephrine (EPI) into the plasma.8 Synthetic human α-ANF was used, which is identical to endogenous canine α-ANF.9

Materials and Methods

Animals

Six mongrel dogs of either sex, weighing 30 ± 1 kg and with the carotid artery translocated into a cutaneous loop, were used in this study. They were trained to lie quietly on the experimental table with the carotid artery in the loop punctured transcutaneously for recording of arterial pressure and blood sampling and two veins punctured for infusions. Each dog was used five to six times randomly in the protocols, with 3 to 6 days between consecutive trials. Following an experiment, the dogs were kept in metabolic cages for 32 hours to collect urine containing the infused 3H-activity (see Protocols). The dogs were kept on a standard diet containing sodium, 2 to 4 mEq/kg, with free access to tap water. Care of the animals was in accordance to the guidelines of the American Physiological Society and was supervised by an independent veterinarian.
Protocols

Following puncture of the vessels, I-[7-3H(N)]-NE (23 Ci/mmol; NEN, Dreieich, West Germany), 0.02 μCi/kg/min, in 12 trials or I-[N-methyl-3H]-EPI (55 Ci/mmol; NEN), 0.02 μCi/kg/min, in 21 trials was infused intravenously throughout the protocol.

Following a 60-minute equilibration period, human α-ANF ([Ser'-Tyr²⁸] human atriopeptin-2⁸; Sigma, Taufkirchen, West Germany) was infused intravenously for 15 minutes. Infusion rates (in μg/kg/min) were 0.03 (n = 6: 3 trials with [³H]NE and 3 trials with [³H]EPI as tracer); 0.1 (n = 9: 3 trials with [³H]NE, 6 trials with [³H]EPI); 0.3 (n = 9: 3 trials with [³H]NE, 6 trials with [³H]EPI); and 1.0 (n = 3: all trials with [³H]EPI). Arterial blood samples for catecholamine determination (see Measurements) were withdrawn repeatedly, as shown in Figure 1, and arterial samples for determination of plasma ANF immunoreactivity were taken at the end of both the equilibration period and the infusion period. In 10 trials, an additional sample was obtained between the 8th and 10th minute of the infusion. During each trial arterial blood, 2.2 to 2.7 ml/kg, was obtained for analyses.

In an additional series in four of these dogs, 12 trials were performed as already described, but hydralazine (60 μg/kg/min; n = 4) or nitroglycerin (1.5 and 5.0 μg/kg/min; n = 4 each) was infused instead of α-ANF, and [³H]NE was used as tracer. In this series, plasma ANF immunoreactivity was not determined.

Measurements

Readings of mean arterial pressure and heart rate (obtained by a cardiotachometer from the arterial pressure signal) were averaged over 1 minute immediately before withdrawing a blood sample. The rate of catecholamine release into plasma (sometimes also called "spillover rate") was determined according to the method of Esler et al.⁴ as a parameter for overall sympathetic activity. Under steady state (which was obtained at the end of the equilibration period; see Figure 1), the catecholamine clearance is (rate of [³H]-catecholamine infusion)/(plasma [³H]-catecholamine concentration) and the release rate is (catecholamine clearance) × (plasma catecholamine concentration).

Concentrations of labeled and unlabeled catecholamines were determined radioenzymatically following separation as previously described.⁶ Plasma ANF immunoreactivity was determined using a rabbit antiseraum (Amersham Buchler; Braunschweig, West Germany) against synthetic human α-ANF, following extraction according to the method of Lang et al.²⁰ and was expressed as picograms per milliliter of human α-ANF. Cross-reactivity of the antiseraum against atriopeptin I and II was below 2%. The limit of detection in our assay was 12 pg/ml. One assay (containing the samples of the trials with the highest ANF infusion) had to be discarded because of a technical error.

Statistical Analysis

Values are given as means ± SEM. Significance of differences between values within a protocol was obtained by performing a two-way analysis of variance (ANOVA). Since variances of catecholamine release rates varied, the log transformation of these values was used for ANOVA, but the absolute values are presented in the results. If ANOVA indicated significance, Bonferroni’s correction was used to compare each of the five values during infusion with the last control value (Time 0, see Figure 1). Furthermore, the Tukey procedure⁹ was used to compare the group of control values (Time: —15, —10, —5, and 0 minutes; see Figure 1) with the group of steady state values during infusion (Time: 6, 9, and 12 minutes). The average of these steady state values, expressed as a percentage of the average of the group of control values, was used as quantification of the effect of infusions and for regression analysis by the method of least squares.

Results

Following the equilibration period of 60 minutes with the dogs resting on the experimental table, preinfusion heart rate was 76 ± 3 beats/min, mean arterial pressure was 93 ± 1 mm Hg, and arterial plasma levels of NE and EPI were 108 ± 7 and 83 ± 6 pg/ml, respectively (33 trials in 6 dogs). In 21 of the 33 trials, the catecholamine clearance was determined by using [³H]EPI as tracer and amounted to 74.7 ± 3.0 ml/kg/min; in 12 trials with [³H]NE as tracer, a similar value (76.6 ± 4.8 ml/kg/min) was observed. Therefore, release rates for both catecholamines were calculated for each trial by using the same clearance value, regardless of the applied tracer. These release rates were 8.3 ± 0.7 ng/kg/min for NE and 6.7 ± 0.6 ng/kg/min for EPI (n = 33 each).

The ANF immunoreactivity in arterial plasma before the onset of infusion was 40 ± 6 pg/ml (n = 30) and reached 39 ± 10 (n = 6), 284 ± 24 (n = 6), 1520 ± 30 (n = 9), and 7080 ± 1290 pg/ml (n = 9) at the end of 15-minute infusions of saline or ANF (0.03, 0.1, or 0.3 μg/kg/min, respectively). In 10 trials (4 with ANF, 0.1 μg/kg/min; and 6 with ANF, 0.3 μg/kg/min), ANF immunoreactivity in arterial samples withdrawn between the 8th and 10th minute of infusion was 101 ± 7% of the value in the 15th minute of infusion. The threshold dosage of ANF for a significant reduction in mean arterial pressure was 0.1 μg/kg/min. With this dosage, significant reductions in plasma EPI and in EPI release rate were observed with a reduction in heart rate of borderline significance (Figure 1). These parameters maintained a steady state level from the 6th to the 12th minute of infusion (see Figure 1). The average of these steady state values during ANF infusion for mean arterial pressure amounted to 95 ± 1% (p < 0.05, by ANOVA and Tukey’s test) of the average of the control readings (at —15, —10, —5, and 0 minutes). The respective average was 58 ± 7% (0.1 > p > 0.05) for plasma EPI and 49 ± 5% (p < 0.001) for EPI release rate (49 ± 4%, if only trials with [³H]EPI as tracer are regarded). The average reduction in heart rate to 94 ± 3% did not reach the level of significance. Catecholamine parameters returned to
control levels immediately with the end of infusion, while arterial pressure during the last minute before blood sampling was still low (see Figure 1).

With higher dosages of ANF (0.3 and 1.0 µg/kg/min), mean arterial pressure decreased further to steady state levels of 93 ± 1 and 79 ± 6% of the respective control average, but heart rate did not change (Figure 2). The catecholamine parameters demonstrated a bimodal pattern with increasing ANF dosages: reductions (for EPI) or no changes (for NE) were observed with infusion rates up to 0.3 µg/kg/min, while the highest dosage induced a tendency toward elevation for NE with no change for EPI (see Figure 2). Sham treatment and ANF, 0.03 µg/kg/min, did not modify steady state levels significantly, but transient reductions of plasma EPI to 64 ± 17% of the last control value at the 6th min of infusion (0.1 >p>0.05) and of EPI release rate to 64 ± 20% (6th minute; 0.1>p>0.05) occurred during the ANF infusion of 0.03 µg/kg/min.

For analysis of the bimodal pattern in catecholamine responses, we separately analyzed the trials with plasma levels of ANF immunoreactivity below 3000 pg/ml (arbitrary cutoff, separating trials with infusion rates of 0.1 µg/kg/min or lower from those with 0.3 µg/kg/min or higher). In these trials (six with saline; six with ANF, 0.03 µg/kg/min; and nine with ANF, 0.1 µg/kg/min), log(plasma ANF) correlated inversely with steady state EPI release rate during infusion (as a percentage of control release), indicating a 24 ± 2% decline in EPI release per 10-fold augmentation of plasma ANF and no change in EPI release at an ANF level of 23 pg/ml. Similarly, log(ANF) during infusion correlated (r = −0.59, p<0.01) with EPI plasma concentration (as a percentage of control), with a 24 ± 2% decline per 10-fold increase in plasma ANF and no change at an ANF level of 38 pg/ml. If this analysis was restricted to those trials with [3H]EPI as tracer (n = 12), a similar correlation (r = −0.71, p<0.01) was obtained: 29 ± 3% decline in EPI release rate per 10-fold increase in plasma ANF, and no change at an ANF level of 38 pg/ml. The catecholamine clearance was not significantly affected by ANF infusions. The respective steady state values as a percentage of control clearance during ANF infusions of 0.03, 0.1, 0.3, and 1.0 µg/kg/min were 98 ± 6, 86 ± 5, 84 ± 4, and 100 ± 5%. Similar values of 98 ± 6, 86 ± 5, 84 ± 4, and 100 ± 6% were observed, if only the trials with [3H]EPI as tracer were taken into account.

In the experiments with other vasodilators (12 trials in 4 dogs), hemodynamics and catecholamine parameters following the 60-minute equilibration period did not differ from the respective preinfusion values of the experiments with ANF infusion. Relative changes during infusions of the vasodilators are summarized in Table 1 and indicate sympathetic activation during hypotension induced by hydralazine or nitroglycerin.
TABLE 1. Effects of Vasodilators (15-Minute i.v. Infusions) in Conscious, Resting Dogs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hydralazine*</th>
<th>Nitroglycerin†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 µg/kg/min</td>
<td>1.5 µg/kg/min</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>91 ± 11</td>
<td>94 ± 19</td>
</tr>
<tr>
<td>Heart rate</td>
<td>193 ± 19</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>Plasma norepinephrine</td>
<td>148 ± 24</td>
<td>184 ± 24</td>
</tr>
<tr>
<td>Norepinephrine release rate</td>
<td>181 ± 41</td>
<td>190 ± 18</td>
</tr>
<tr>
<td>Plasma epinephrine</td>
<td>105 ± 10</td>
<td>195 ± 44</td>
</tr>
</tbody>
</table>

Values are means ± SEM and are presented as percentage of the preinfusion control values. Values represent four experiments in four dogs each.

*Values obtained at the end of 15-minute infusion; thereafter, mean arterial pressure slowly dropped further with ongoing tachycardia.
†Steady state values during minutes 6 through 15 of infusion; thereafter, hemodynamics returned to control within minutes.
‡p < 0.001, §p < 0.01, ‖p < 0.05, || p > 0.05, compared with preinfusion control value.

Discussion

These experiments demonstrate a substantial attenuation of basal EPI release by low, probably physiological, dosages of exogenous ANF in the intact animal. It is unlikely that this reduction of EPI release was secondary to some ANF action on systemic hemodynamics or secondary to natriuretic or diuretic actions of ANF, since arterial pressure was not augmented by the ANF infusion, ANF does not affect the venous system of the dog at these dosages,12 and the threshold dose for measurable diuretic action of ANF in conscious dogs13 is 0.15 µg/kg/min, arguing against diuresis as the primary cause for the immediate and strong inhibition of EPI release with an ANF dose of 0.1 µg/kg/min (see Figure 1).

The inhibition of EPI release probably is an important element in the physiology of endogenous ANF under normal conditions. The observed negative correlations between log(plasma ANF) and relative release rate (or concentration) of EPI indicate that the threshold for this inhibitory ANF action might be close to the physiological plasma level of ANF in the healthy organism. The absence of tachycardia and any augmented NE release during ANF-induced hypotension (up to infusions of 0.3 µg/kg/min) indirectly suggests an inhibitory action of ANF on sympathetic activation by baroreceptor unloading, as is indicated by the comparison with the hypotension induced by hydralazine or nitroglycerin.

High affinity membrane receptors for ANF have been shown in cardiovascular regulatory areas of the central nervous system outside of the blood-brain barrier,14-18 and they may exist on adrenal medullary cells as well.17-18 ANF augments intracellular cyclic guanosine 3',5'-monophosphate (cGMP), probably by a receptor-mediated activation of particulate guanylate cyclase,4 in all cell types with ANF receptors in which this process has been studied. In bovine adrenal medullary cells, a mediator role of elevated intracellular cGMP has been proposed in the muscarinic inhibition of nicotine-stimulated catecholamine secretion.19 At high concentrations (10 nM), ANF inhibited nicotine stimulation of catecholamine production in primary culture of bovine adrenal medullary cells.20 Therefore, central nervous as well as peripheral adrenal actions of the circulating ANF might have contributed to the observed inhibition of sympathoadrenal activity in our dogs.

The involvement of sympathoadrenal inhibition in the ANF-induced hypotension has been proposed to explain the lower hypotensive activity of ANF in sympathomotized rats,6 the ANF-induced suppression of urinary catecholamine excretion in renal hypertensive rats5 and in adrenalectomized rats,21 and the transient reduction in plasma EPI by bolus injections of ANF in normotensive22 and hypertensive humans.23 ANF in a pharmacological dose lowered renal sympathetic nerve activity in rats with sinoaortic denervation,24 and in vitro, it inhibited stimulation-induced NE release in isolated perfused rat arteries.25 By measuring the release of EPI in vivo, this study documents and partially quantifies this proposed sympathoadrenal inhibition by ANF, indicating that this inhibition is relevant at physiological rates of ANF release. Thus, hormonal ANF may act synergistically to the inhibitory reflexes arising from volume receptors in the vasomotor low pressure system.26

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References

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