Neural control of the endocrine rat heart

(atrial peptide/scorpion venom/sodium channels)

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ABSTRACT Although atrial stretch is the accepted stimulus for atrial natriuretic factor (ANF), *in vivo* studies suggest a stretch-independent, neurally induced ANF release mechanism. Thus the hypothesis that cardiac nerves can stimulate ANF secretion was tested in the Langendorff-perfused, paced rat heart. Venom from the scorpion *Centruroides sculpturatus* was used to activate neuronal sodium channels, veratridine was added to activate sodium channels (predominantly in myocytes), and electrical stimulation was applied to the right atrial appendage. The efficacy of nerve stimulation was verified by measurements of increased neuropeptide Y in the effluent. ANF levels in the effluent increased by 120% over baseline with 0.5 μ M scorpion venom and by 88% with 0.5 μ M veratridine ($P < 0.01$). Cardiac mechanics did not explain the large, concentration-dependent ANF response to the scorpion venom, since changes in the left ventricular developed pressure were small, opposite to those induced by veratridine, and unaffected by sympathectomy or adrenergic receptor blockade. Prior chemical sympathectomy and adrenergic receptor blockade almost abolished the ANF response to scorpion venom but hardly affected the ANF response to veratridine. Addition of 1 μ M tetrodotoxin abolished all ANF responses. Electrical stimulation of the atrial appendage increased the ANF secretion by 60.2% ($P < 0.02$), in conjunction with neuropeptide Y, whereas control stimulations were ineffective. These studies unequivocally demonstrate that stimulation of cardiac sympathetic nerves potently stimulates ANF secretion.

Atrial natriuretic factor (ANF), a potent natriuretic, diuretic, and vasorelaxant peptide (1), is thought to play an important role in the regulation of blood volume (2, 3). Atrial stretch (4) and blood volume expansion (5) stimulate ANF release, and ANF reduces the blood volume through increased renal excretions, inhibition of fluid-retaining hormones, and loss of fluid to the interstitial space (2, 3). Quenching of circulating ANF with anti-ANF antibody reduces the renal response (diuresis) to acute volume expansion (6). However, doubts have been raised about this appealing concept in recent years. Chronic ANF infusions in man and dog have transient effects on renal excretions of salt and water (7, 8), challenging the concept that ANF is a blood volume regulatory hormone. ANF is released in the absence of atrial stretch in hypoxic hearts (9), hypoxic animals (10) and humans (11), and in pulmonary hypertension (12, 13). Both endothelium-derived compounds (14) and neural factors (13) may be involved in this ANF release.

The evidence for a neural regulation of ANF is intriguing, but it has remained indirect and inconclusive. The ANF response to an unphysiological (20 ml/kg of body weight) volume expansion is blunted in pithed rats (15), but pithing inevitably alters baseline parameters (e.g., baseline ANF). Others found that the ANF response to a similar blood volume expansion is unaltered by autonomic denervation or

blockade (16, 17), but other stimuli (e.g., hypoxia) were not tested. Both α - and β -adrenergic agonists stimulate ANF secretion in isolated atria (18–20), isolated hearts (21, 22), cultured atrial myocytes (23), and *in vivo* (24), but it is not known whether endogenously released catecholamines can stimulate ANF secretion. Although hypoxia-induced ANF secretion from isolated hearts is reduced by adrenergic receptor blockade (9), hypoxia may cause a nonexocytotic neurotransmitter release in glucose-deprived hearts (25). Electrical and chemical stimulation of the rostral ventrolateral medulla significantly enhances ANF secretion (26). However, stimulation of the right stellate ganglion fails to increase ANF secretion in rabbits (27).

It was essential, therefore, to directly test the hypothesis regarding a neural control of the endocrine heart. The isolated, perfused heart is an ideal experimental model for these studies, because hemodynamic and hormonal influences existing *in vivo* are absent, and in contrast to atrial myocytes in culture, the anatomical relationships between cardiac nerves, myocytes, and endothelial cells is preserved. Furthermore, the atria are perfused and not superfused, and although the ventricles are present, the atria contribute at least 90% of the ANF secretion (28). We now report that the activation of sympathetic cardiac nerves potently stimulates ANF secretion, providing conclusive evidence for a neural control of ANF.

MATERIALS AND METHODS

Isolated Heart Preparation. The study was carried out on 74 isolated hearts from male Wistar rats (250–350 g) using a modified Langendorff perfusion system as described (9). The hearts were suspended in a water-jacketed organ bath (35 ml) and perfused at 10 ml/min by a modified Krebs–Henseleit buffer equilibrated with 95% O₂/5% CO₂ (pH 7.4) at 37°C, and exiting buffer was pumped onto preconditioned Sep-Pak C₁₈ columns (Waters). Mean perfusion pressure and mean left ventricular developed pressure (LVP) were measured with Statham (Hato Rey, PR) (P23 ID) transducers and plotted on a Grass Instruments (Quincy, MA) polygraph. Changes in LVP were monitored as indicators of potential mechanical effects on the atria, since neurotransmitters and neuropeptides exert similar effects on ventricles and atria (29). The hearts were paced (see below) at a rate of 5.5–9.1% over the intrinsic heart rate (249 ± 15 beats per min) from 20 min prior to and throughout the experiment, in order to eliminate ANF responses induced by changes in heart rate.

Protocols. After an 80-min equilibration (time –80 to 0 min), samples were collected continuously throughout a 12-min control period (time 0–12 min), a 10-min administration (time 12–22 min) of buffer containing 0.5 μ M venom of the American scorpion *Centruroides sculpturatus* (Sigma;

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Abbreviations: ANF, atrial natriuretic factor; NPY, neuropeptide Y; 6-OHDA, 6-hydroxydopamine; TTX, tetrodotoxin; LVP, left ventricular developed pressure.

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no. V-9752, lot no. 90H0754) or 0.5 μM veratridine (Sigma; no. V-5754), and a 30-min recovery period (time 22–52 min) with return to normal buffer. The 0.5 μM concentration of venom was chosen from preliminary studies. This venom stimulates neuronal sodium channels (30) and has minor effects on cardiac myocytes (31). The 0.5 μM concentration of veratridine was selected to minimize stimulation of sympathetic nerves (32, 33). Samples were taken over 4 min during control and recovery periods and over 2 min during toxin administration. To study the role of the sympathetic cardiac nerves, the experiments were repeated in chemically sympathectomized rat hearts ($n = 10$). Rats were injected via tail vein with 60–100 mg of 6-hydroxydopamine (6-OHDA; Sigma; no. H4381) diluted in saline (containing 0.1% ascorbic acid) per kg of body weight 24 hr before the experiments. To test the effects of adrenergic receptor blockade on toxin-induced ANF responses, in two groups the hearts were stimulated with toxins while the perfusate contained either 15 μM atropine sulfate (Sigma; $n = 11$) or a combination of 15 μM atropine sulfate, 0.1 μM propranolol (Sigma), and 1 μM phentolamine (Regitine; CIBA Pharmaceutical) ($n = 11$). These antagonist concentrations were found to be effective in a previous study (9). In two experimental groups, the specificity of the toxins was tested by addition of 1 μM tetrodotoxin (TTX) ($n = 11$), a sodium channel blocker (34). To verify that noradrenaline stimulates ANF secretion, five isolated hearts were infused during 10 min with 1 μM noradrenaline (Sigma). Time controls ($n = 6$) were performed, and hearts ($n = 10$) were also stimulated electrically (see below) during time 10–20 min.

To verify whether scorpion venom elicits a dose-dependent ANF secretion and correspondent exocytotic release of neurotransmitter, dose-response studies were performed in five isolated paced hearts. After the 80-min equilibration, the hearts were perfused for successive 10-min periods at increasing concentrations of scorpion venom of 0, 0.01, 0.1, 0.5, and 2.5 μM . Samples were taken over 2 min during venom infusion and stored at -80°C for ANF and neuropeptide Y (NPY) assay. Venom concentrations, as estimated from a molecular weight of 7000 for the active compounds (30), could not be raised beyond 2.5 μM because of the appearance of extrasystoles.

Electrical Stimulation of the Right Atrial Appendage. A bipolar silver electrode was placed at the root of the right atrial appendage, and electrical pulse trains (three constant current pulses per beat, 0.8–1 mA, 0.5-msec pulse width, interval of 25 msec) were applied in synchrony with ventricular pacing. The first impulse coincided with the ventricular pacing stimulus, whereas the following two impulses fell into the refractory period. To further minimize the possibility that atrial stimulation would interfere with the ventricular pacing, the strength of the atrial stimulation was adjusted, in 8 of 10 hearts, to 15–20% below the threshold of cardiac pacing. Since nerves are activated at a much lower threshold than heart muscle, it was possible to stimulate ANF secretion at currents that were adjusted to be 20% below the threshold of cardiac pacing. Controls were performed as described in the legend to Fig. 4.

Peptide Assays. ANF extraction and the RIAs were as described (26), except that rabbit anti-rat α -ANF-(1–28) antiserum (Amersham, IM 1861) was used. Recovery of 4 ng of unlabeled rat α -ANF-(1–28) (Peninsula Laboratories) added to 20 ml of perfusion buffer was $91.3\% \pm 2.5\%$ (101 controls, 15 assay series). All ANF values were corrected for recovery. The sensitivity as defined by the 5% and 50% displacement of radiolabeled ANF was 0.39 ± 0.04 pg per tube and 7.3 ± 0.4 pg per tube, respectively (15 assays, rat standards). The correlation coefficient for the log-logit regressions of standard curves was 0.97 ± 0.01 . The mean intraassay coefficient of variation was $3.3\% \pm 0.3\%$, the

interassay coefficient of variation with 20 pg per tube was 14.8%, and the buffer blanks were 12.4 ± 2.1 pg/ml of perfusion buffer ($n = 45$).

NPY, and not noradrenaline, was measured as a marker of neurotransmitter secretion (35), since noradrenaline may be released by nonexocytotic mechanisms as well (25). NPY was extracted from the buffer effluent together with ANF. Recovery of 200 pg of unlabeled NPY added to 20 ml of perfusion buffer was $96.1\% \pm 2.3\%$ ($n = 9$, three assay series). NPY was assayed by RIA using ^{125}I -labeled NPY (NEX-222; NEN), NPY antiserum (no. 14158V; Peptides International), and rat NPY standard (no. 7180, Peninsula Laboratories) in a nonequilibrium assay. Twenty-five microliters of antiserum and 50 μl of standard or sample (up to 1:4 dilution in assay buffer) were incubated for 3 days, and 25 μl of tracer (2000–2200 cpm) was added on the second day. Separation of bound from free labeled NPY was as described for the ANF assay (26). The sensitivity of the NPY assay as defined by the 5% and 50% displacement of radiolabeled NPY was 2.2 ± 0.3 and 37.1 ± 0.6 pg per tube, respectively. The correlation coefficient for the log-logit regression of standard curves was 0.96 ± 0.01 . The mean intraassay coefficient of variation was $3.7\% \pm 0.1\%$, the interassay coefficient of variation with 50 pg per tube was 10.3%, and the buffer blank was 0.04 ± 0.02 pg/ml of perfusion buffer ($n = 20$). Neither veratridine nor scorpion venom interfered with the ANF or NPY assays. All assay data were calculated in picograms per milliliter of perfusion buffer.

Statistical Analysis. Data were analyzed by two-way ANOVA for repeated measures using the computer programs of the SAS Institute (Cary, NC). Significant differences from baseline within groups and between groups at each time point were determined by $P < 0.05$. The ANF data were normalized relative to baseline, because the ANF response (in pg/ml) was positively correlated with the baseline for both venom ($P < 0.05$) and veratridine ($P < 0.001$). The baseline was calculated as the mean of the three samples (taken during time 0–12 min). Since ANF responses, expressed as a percentage of the baseline, are not normally distributed, the percent values were transformed by the arcsin (square root of X) function (36) before entering them into the SAS program.

RESULTS

Effects of Toxin Administration on ANF Release. The 10-min infusion of either 0.5 μM scorpion venom or 0.5 μM veratridine significantly increased ANF release from isolated hearts (Figs. 1 and 2). Although baseline ANF levels varied between groups (legend to Fig. 1), they did not contribute to the venom-induced ANF release. There was no correlation between baseline ANF and either peak percent increase ($r = 0.52$, $P > 0.05$) or mean percent increase ($r = 0.08$, $P > 0.05$) of ANF over baseline.

Toxin infusions induced significant but contrasting changes in LVP (Table 1 and Fig. 2). Veratridine elicited a pronounced, sustained increase in LVP, whereas scorpion venom induced a transient, small increase followed by a sustained reduction. Although the baseline LVP differed between groups (Table 1), there was no correlation between baseline LVP and peak percent change ($r = 0.237$, $P > 0.05$) or mean percent change ($r = 0.17$, $P > 0.05$) of ANF from baseline. Changes in perfusion pressure and heart rate were minor.

Role of Sympathetic System in Toxin-Induced ANF Release. 6-OHDA was used to cause an effective chemical sympathectomy (37). This was checked by tyramine administration (0.2 mg in a 0.5-ml bolus), which elicited only a minor heart rate increase (3.0 ± 0.8 beats per min; $n = 10$) in hearts from 6-OHDA-treated rats as compared to controls (32.9 ± 1.2 beats per min; $n = 6$). The sympathectomy did not alter the

FIG. 1. Time course of ANF concentration in effluent buffer for $0.5 \mu\text{M}$ scorpion venom- and $0.5 \mu\text{M}$ veratridine-perfused rat hearts. Each step represents the perfusate concentration of ANF (mean $\pm$ SEM) over 4 min in terms of the percent change from baseline (scorpion venom, 202 ± 50 pg/ml; veratridine, 446 ± 68 pg/ml; time control, 361 ± 35 pg/ml). Significant differences relative to baseline at each time point are designated by * ($P < 0.05$) and ** ($P < 0.01$). Single-sided T-shaped error bars denote significant differences between toxin groups and time control. Double-sided T-shaped error bars denote significant differences between the venom and veratridine groups.

perfusion pressure, LVP, and the baseline heart rate and insignificantly reduced the release of ANF to veratridine by 36% (Fig. 2). However, sympathectomy almost abolished the response to scorpion venom (mean percent change in ANF was reduced by 88%; $n = 6$, $P < 0.01$) (Fig. 2). The reduction of the ANF response was not due to altered cardiac mechanics, since toxin infusions caused similar changes in LVP as in intact hearts (Table 1). Thus, sympathetic nerves, and also activation of sodium channels (see TTX, Fig. 2), mediate increased ANF secretion in response to scorpion venom infusion.

Additional support for the role of sympathetic nerves in the ANF response is provided by the noradrenaline infusion and neurotransmitter receptor blockade studies. Infusion of $1 \mu\text{M}$ noradrenaline over 10 min caused a $75.6\% \pm 9.7\%$ peak increase ($P < 0.01$) and $36.0\% \pm 10.4\%$ mean increase ($P < 0.05$) in ANF secretion over baseline ($n = 5$ hearts). ANF secretion was still significantly elevated 5 min after cessation of infusion, and mean levels then declined to baseline with a time constant of 12 min. The simultaneous administration of

FIG. 2. Mean percent increase in ANF (ΔANF) over baseline in buffer effluent and mean change in LVP (ΔLVP). Results are shown for $0.5 \mu\text{M}$ veratridine (V) and $0.5 \mu\text{M}$ scorpion venom (S). Mean changes were calculated for each heart over the 12–32 min interval and averaged over each group. Baseline ANF values are as follows: V alone, 446 ± 68 pg/ml; V + atropine, 320 ± 35 pg/ml; V + 6-OHDA, 392 ± 95 pg/ml; V + block, 241 ± 31 pg/ml; V + TTX, 403 ± 41 pg/ml; S, 202 ± 50 pg/ml; S + atropine, 201 ± 27 pg/ml; S + 6-OHDA, 452 ± 32 pg/ml; S + block, 332 ± 45 pg/ml; S + TTX, 260 ± 39 pg/ml; time control, 361 ± 35 pg/ml. For other baselines, see Table 1. TTX, $1 \mu\text{M}$ TTX was added to the perfusate. Block, combination of atropine sulfate ($15 \mu\text{M}$), propranolol ($0.1 \mu\text{M}$), and phentolamine ($1.3 \mu\text{M}$) (added to the perfusate). Significant differences relative to baseline are indicated by * ($P < 0.05$) and ** ($P < 0.01$); P levels over brackets are relative to toxin alone.

atropine, phentolamine, and propranolol greatly reduced the venom-induced ANF release by up to 90% and only slightly reduced the ANF response to veratridine (Fig. 2). Atropine alone enhanced the veratridine-induced response (Fig. 2), suggesting that acetylcholine or vagal efferents may inhibit ANF secretion under certain experimental conditions (see

Table 1. Perfusion pressure, paced heart rate, and LVP in veratridine- and scorpion venom-perfused hearts

Group	n	Perfusion pressure mmHg		Paced heart rate, beats per min	LVP, mmHg				
		Baseline	13–14*		Baseline	13–14*	14–22*	22–32*	32–52*
V	5	36 ± 1	38 ± 2	286 ± 5	63 ± 5	$84 \pm 8^\dagger$	$75 \pm 8^\dagger$	66 ± 5	64 ± 5
V + atropine	5	35 ± 1	36 ± 1	264 ± 7	59 ± 3	$82 \pm 8^\dagger$	$70 \pm 5^\dagger$	62 ± 4	65 ± 4
V + 6-OHDA	4	39 ± 1	42 ± 2	286 ± 9	61 ± 4	$83 \pm 5^\dagger$	$71 \pm 5^\dagger$	61 ± 4	62 ± 4
V + blockers	5	39 ± 1	41 ± 1	274 ± 4	62 ± 1	$83 \pm 4^\dagger$	$72 \pm 2^\dagger$	67 ± 4	64 ± 3
V + TTX	5	$40 \pm 1^\ddagger$	41 ± 2	268 ± 2	58 ± 5	$70 \pm 6^\dagger$	61 ± 5	54 ± 4	54 ± 4
S	5	39 ± 2	40 ± 1	278 ± 7	$71 \pm 6^\ddagger$	$82 \pm 6^\dagger$	66 ± 5	60 ± 5	64 ± 6
S + atropine	6	39 ± 2	40 ± 1	278 ± 5	65 ± 5	$76 \pm 5^\dagger$	57 ± 4	55 ± 3	58 ± 3
S + 6-OHDA	6	$41 \pm 2^\ddagger$	40 ± 1	292 ± 11	$73 \pm 5^\ddagger$	$82 \pm 5^\dagger$	67 ± 6	68 ± 5	70 ± 5
S + blockers	6	$41 \pm 1^\ddagger$	42 ± 1	257 ± 3	$72 \pm 3^\ddagger$	$84 \pm 5^\dagger$	71 ± 3	69 ± 3	72 ± 4
S + TTX	6	$40 \pm 2^\ddagger$	39 ± 1	270 ± 4	66 ± 3	$72 \pm 3^\dagger$	63 ± 3	65 ± 3	68 ± 3
Control	6	36 ± 2	37 ± 1	268 ± 10	60 ± 4	60 ± 4	61 ± 4	58 ± 4	58 ± 3

V, veratridine; S, scorpion venom; blockers, combination of atropine, propranolol, and phentolamine.

*Minutes in protocol.

$^\dagger P < 0.05$ relative to baseline.

$^\ddagger P < 0.05$ relative to control baselines.

also refs. 14 and 38). Thus, besides sympathetic nerves and sodium channels, adrenergic receptor activation is essential in the venom-induced ANF release.

Release of NPY and ANF in Response to Scorpion Venom Infusion. Increasing concentrations of scorpion venom caused a dose-related increase in both ANF and NPY levels in the exiting buffer (Fig. 3). The ANF level returned to 173 ± 25 pg/ml and NPY level returned to 5.4 ± 1.5 pg/ml, 20 min after cessation of venom infusion. The lack of dose-related changes in left ventricular pressure and heart rate further demonstrate a dissociation between ANF secretion and mechanical responses of the heart.

Effect of Nerve Stimulation on ANF and NPY Release. Electrical stimulation of the nerve supply to the right atrial appendage induced a significant, reversible increase of ANF and NPY levels in the effluent buffer (Fig. 4). In contrast, electrical stimulation applied outside of the right atrial appendage failed to induce either ANF or NPY release. The ANF response to nerve stimulation was not related to cardiac mechanics; since the stimulation was applied only to the right atrial appendage, perfusion pressure, left ventricular pressure, and heart rate changed by <1%.

DISCUSSION

The major finding of this study is that activation of sympathetic neural sodium channels potentially stimulates ANF secretion. Several arguments support this contention. First, the venom of the American scorpion *C. Sculpturatus* activates neural sodium channels (30), potentially stimulates ANF secretion

from the isolated rat heart (Figs. 1–3), and has minor effects on the contractility of cardiac myocytes (ref. 31; Figs. 2 and 3). Second, prior chemical sympathectomy almost abolishes the stimulatory effect of the scorpion venom on ANF release (Fig. 2). Third, the scorpion venom acts mainly on sodium channels, since TTX also abolishes the ANF response (Fig. 2). Fourth, noradrenaline infusion strongly stimulates ANF secretion in isolated hearts (as described above) as well as other preparations (see Introduction). Fifth, tyramine (which elicits endogenous noradrenaline release) also stimulates ANF secretion in isolated hearts (9). And sixth, adrenoceptor blockade inhibits the venom-induced ANF release (Fig. 2). In contrast, veratridine has potent effects on the contractility of cardiac myocytes (ref. 39; Fig. 2) and stimulates ANF secretion even after chemical sympathectomy (Fig. 2). The ANF stimulatory effect of veratridine is totally TTX-reversible, indicating that veratridine specifically affected the myocyte sodium channels. Whether the target of veratridine is the ventricular or atrial myocyte remains to be determined.

Cardiac mechanics appear to play no role in the ANF response to the scorpion venom. First, the atria were cut open by the dissection and could therefore not be stretched by the perfusate. Second, scorpion venom and veratridine had opposite mechanical effects on the ventricular myocytes (Fig. 2), yet both caused ANF secretion. Since mechanical

FIG. 3. Dose-dependent increase of ANF and NPY in buffer effluent and corresponding changes of LVP in response to scorpion venom ($n = 5$ hearts). The hearts were perfused for successive 10-min periods at increasing concentrations of scorpion venom. ANF levels were averaged over each 10-min period for each heart and expressed as a change over baseline (186 ± 19 pg/ml; mean $\pm$ SEM). NPY levels were not detectable in 6-OHDA-treated hearts during venom infusion and rose to 1.05 ± 0.12 pg/ml in intact hearts in response to veratridine. Other baselines (LVP = 73 ± 2 mmHg; heart rate (HR) = 270 ± 7 beats per min; perfusion pressure = 41.6 ± 0.6 mmHg) were within the ranges shown in Table 1. The pacing rate was increased by <5% in some of the hearts to minimize the number of extra systoles. Significant differences from baseline for ANF and NPY are indicated by * ($P < 0.05$) and ** ($P < 0.01$).

FIG. 4. (A) Percent change in ANF from baseline (mean $\pm$ SEM over 2 min) for electrically stimulated hearts. (B) Corresponding changes in NPY (in pg/ml). In the "on appendage" group, the electrode tips were placed 3 mm apart at the root of the right atrial appendage. In the "off appendage" group, one or both electrode tips were placed outside the right appendage, with a tip separation of 4–5 mm. Significant differences relative to the average value of baselines are indicated by * ($P < 0.05$) and ** ($P < 0.01$). † indicates significant difference between groups. There were no significant differences between groups for baseline ANF, heart rate (HR), perfusion pressure (PP) and LVP: on appendage group, baseline ANF = 258 ± 30 pg/ml, HR = 308 ± 5.3 beats per min, PP = 36.8 ± 1.3 mmHg, LVP = 53.2 ± 2.1 mmHg; off appendage group, ANF = 330 ± 81 pg/ml, HR = 294 ± 8.3 beats per min, PP = 34.2 ± 2.2 mmHg, LVP = 54.4 ± 3.5 mmHg.

responses to sympathetic neurotransmitters and neuropeptides are similar in atria and ventricles (29), the measurements of LVP (Figs. 2 and 3) indicate that atrial mechanical change cannot be responsible for the ANF response to scorpion venom. Third, heart rate was kept constant by pacing, and perfusion pressure remained stable (Table 1); thus, these factors could not have been involved in ANF release (40). Finally, there was, overall, a striking dissociation between hormonal and mechanical responses of the heart.

This study thus shows that in addition to atrial stretch (2, 3), neural activation is a potent stimulus for ANF secretion in isolated hearts. The receptors mediating this release mechanism require further study, although both α - and β -adrenoreceptors (9) and possibly calcitonin gene-related peptide (41) or NPY may play a significant role. Previous negative evidence regarding a neural control of ANF (27) may be explained by a lack of effective cardiac sympathetic activation (only the right stellate nerve was stimulated) or a preexisting sympathetic tone due to the anesthesia. Previous positive evidence from hypoxia studies (9) was not compelling either, because hypoxia may cause a nonexocytotic catecholamine release (ref. 25; see also Introduction). For this reason, NPY, a cotransmitter in cardiac sympathetic nerves (35), was measured as a marker of exocytotic (35) noradrenaline release (Figs. 3 and 4). To our knowledge, the present work provides the first direct evidence that chemical and electrical activation of sympathetic cardiac nerves and exocytotic neurotransmitter release stimulates ANF secretion. This alternative mechanism would explain the *in vivo* release of ANF in absence of atrial stretch (10, 13, 26) and may offer an increased understanding of ANF stimulus-secretion coupling in exercise and respiratory distress syndromes (citations in ref. 26).

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