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HYPOXIA-INDUCED RELEASE OF ATRIAL NATRIURETIC FACTOR (ANF) FROM THE ISOLATED RAT AND RABBIT HEART

Alex J. Baertschi, Claudia Hausmaninger, Robert S. Walsh, Robert M. Mentzer Jr., David A. Wyatt, and Richard A. Pence

Departments of Physiology and Surgery,
University of Virginia School of Medicine,
Charlottesville, VA 22908

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The effect of hypoxia on the release of atrial natriuretic factor (ANF) was studied in isolated, constant-flow perfused hearts of rats and rabbits. Effluent samples were frozen pending extraction and radioimmunoassay of ANF. Hypoxia (10 min) caused a 3.9-fold (rats) and 4.6-fold (rabbits) increase of ANF release over control values. ANF release returned to control levels within 8-11 min of reoxygenation. Prolonged (20 min) hypoxia evoked further ANF release. The increase in ANF release and decrease in ventricular pressure, heart rate and coronary perfusion pressure were fully reversible, suggesting that tissues were not damaged. These results demonstrate that hypoxia induces a massive release of ANF by an as yet unexplained mechanism.

The studies of deBold et al. (1-3) have led to the isolation of a 28-amino acid peptide - atrial natriuretic factor (ANF) - with powerful natriuretic and diuretic actions. ANF is released from the heart atria in response to atrial stretch (4) and is thought to play an important role in blood volume homeostasis (see e.g. 5-7 for review). Moreover, ANF has recently been shown to cause bronchodilatation (8), and large amounts of ANF (nanograms per g tissue) have been detected in the lung (9). These findings raise the question if ANF is released during hypoxia to facilitate the pulmonary gas exchange. The present study demonstrates, for the first time, that hypoxia is a potent stimulus for ANF secretion from the isolated heart.

MATERIALS AND METHODS

Isolated heart preparation. A total of 14 male Wistar rats (250-300g) were anaesthetized with halothane and injected intra-peritoneally with 500 U heparin. A combination of Xylazine (2mg) and ketamine (20mg) were injected intra-muscularly for further anaesthesia and muscle relaxation. The hearts were rapidly excised, arrested in ice-cold buffer, and immediately perfused retrogradely through the cannulated aorta on a modified Langendorff apparatus (10). The coronary arteries were thus perfused at a constant flow of 20-21 ml/min with Krebs-Henseleit buffer (117 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 3 mM CaCl2, 0.5mM EDTA, 23.7 mM NaHCO3, 11.5 mM glucose and 1 mM pyru-

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vate). The perfusate was gassed with 95% O2/5% CO2. A water-filled latex balloon was inserted via the left atrium into the left ventricle and inflated to an end-diastolic pressure of 0-5 mm Hg. The left ventricular pressure ("Developed Pressure") was measured with a Hewlett-Packard pressure transducer. In separate experiments, coronary perfusion pressure was monitored through a side-arm of the aortic canula. The spontaneously, isovolumetrically contracting hearts were allowed to equilibrate for 17 min before initiation of the experiment (see Protocols).

The excised hearts of 4 male New Zealand rabbits (1.8-2.2 kg) were perfused at a constant flow of 42 ml/min. The equilibration period, buffers, protocols and assays were as described for the rat experiments. Minor differences are indicated in text.

Protocols. Following equilibration, the hearts were perfused for 8-10 min with the oxygenated buffer (PO2 = 563 ± 7 mm Hg), while effluent samples of 15ml (rats) or 30 ml (rabbits) were collected every other minute. Hypoxia was induced by perfusing the hearts for 10 min with the same solution but gassed with 95% N2/5% CO2 (PO2 = 21 ± 4 mm Hg). The hearts were reoxygenated with the 95% O2/5% CO2-gassed buffer for 30 min (rats) or 20 min (rabbits). During the final 10 min of reoxygenation the potassium concentration was raised from 4.7 mM to 8 mM to test whether ANF secretion could be stimulated, since a high potassium environment is known to cause the release of peptide hormones and neurotransmitters. This moderately elevated potassium concentration was chosen, because higher potassium levels would have led to profound bradycardia or cardiac arrest.

Control experiments were performed by perfusing 4 rat hearts with oxygenated buffer for 48 min, and by collecting effluent samples as above. In addition, separate experiments were conducted on 5 rat hearts to investigate whether a long-term hypoxia (20 min) was capable of inducing further ANF secretion. In all experiments, the effluent samples were collected on ice and frozen pending assay.

ANF extraction and radioimmunoassay. ANF was extracted from the effluent samples by Sep-Pak columns, as described in detail for the extraction of ACTH (11). Sep-Pak columns (Waters) were prewashed with 5 ml methanol and 10 ml distilled water before the samples were applied. The columns were then washed with 5 ml of 1% TFA and eluted with 5 ml of MeOH/distilled water/TFA (80/19/1; vol/vol/vol). The eluted samples were dried at 60°C under a jet of pure filtered nitrogen, reconstituted in ANF assay buffer (0.25-0.5ml) and stored at -70°C until radioimmunoassay. The samples were diluted 4-12 times (rats) and 50 times (rabbits) before assay. Recovery of rat α-ANF (28 amino acid) (Peninsula, code 9103) added to perfusion buffer was 77% ± 4% (mean of means ± SE from 8 separate assay series).

Radioimmunoassays were performed with antibody and radioactive iodine-labelled ANF supplied by Amersham (kit IM.1871). The recommended procedures were considerably modified to increase the number of assay tubes from 300 to 800 per kit. Total counts were 2200 cpm instead of 3000 cpm; antibody dilutions were 1:20 instead of 1:15, and volumes 25 µl instead of 50 µl; assay volumes were 175 µl instead of 350 µl; and the separation of bound from free tracer was performed by a second antibody method (12). The minimal detectable amount of ANF was 3.3 ± 0.5 pg/tube (mean of means ± SE from 10 assay series); maximal amounts detectable were 535 ± 18 pg/tube. Correlation coefficients (r squared) for the dose-response curves (log-logit) were 0.980 ± 0.004. Perfusion buffer blanks were 13.7 ± 4.6 pg/ml, far below the basal values (200-220 pg/ml) we and others (13) have measured in effluent samples from isolated rat hearts. ANF was not detectable in effluent from 2 rat hearts where atria had been surgically removed.

Data Analysis. For each group of animals, pressures and heart rates were measured and averaged every 3.3-10.0 min and expressed as mean ± SE. ANF data were corrected for recovery and expressed in pg per ml of perfusate.

ANF values were normalized relative to the mean of the control samples, and averaged for each group of animals. Absolute values (pg/ml) are indicated in the figure legends.

The statistical significance of changes in pressures, heart rates and normalized ANF values relative to the initial control levels were determined by Analysis of Variance (ANOVA) (14), as indicated by star symbols in the figures. Differences between means of test (hypoxia) and control (normoxia) groups at each time interval were also analyzed by ANOVA.

RESULTS

Hypoxia induced a steep rise in ANF release from isolated rat hearts (Fig. 1, closed circles). ANF release was maximal 8-12 min following initiation of hypoxia, and returned to control values within 8 min of reoxygenation. The baseline of ANF secretion in the control group (normoxia) slightly decreased throughout the 48 min experiment (Fig. 1), while heart rate (Fig. 2) and developed pressure (Fig. 3) were stable. ANF secretions in hypoxia and normoxia groups were indistinguishable over the final 24 min. The increase of potassium concentration to 8 mM in the oxygenated buffer had no effect on ANF release or developed pressure (Fig. 3), and caused a significant decrease in heart rate (Fig. 2).

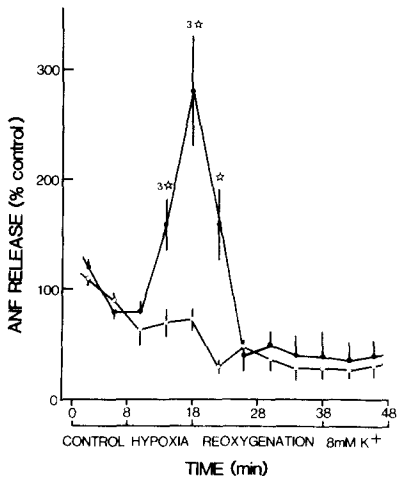

Fig. 1. Release of ANF from isolated rat hearts during hypoxia (●). In a control group (O), no hypoxia was applied. Individual ANF data were normalized relative to the mean of the 2 control samples and averaged (mean ± SE) for each group and each 4 min period. ANF release during control was 217 ±36 pg/ml perfusate (hypoxia) and 224 ± 42 pg/ml (normoxia). Peak ANF release at 18 min was 611 ± 155 pg/ml (hypoxia), significantly different ($p<0.05$) from controls. Star symbols indicate significant changes in normalized ANF levels relative to the 2nd control sample (☆: $p<0.05$; 3☆: $p<0.01$). Significant ($p<0.05$) differences between groups are indicated by two-sided standard error bars.

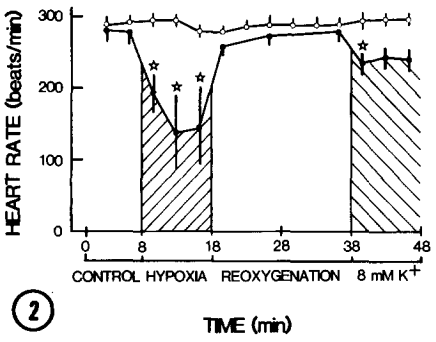

Fig. 2. Heart rates (mean ± SE) for groups of rats shown in Fig. 1 (● = hypoxia; ○ = normoxia). Symbols are as in Fig. 1.

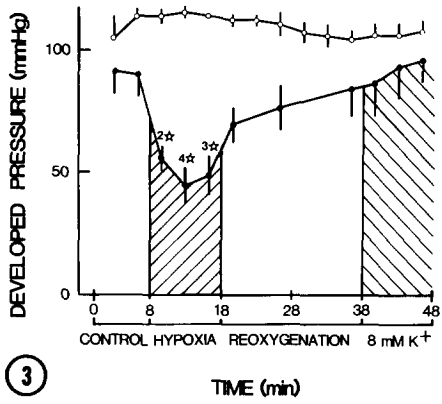

Fig. 3. Developed ventricular pressure (mean ± SE) for groups of rats shown in Fig. 1 (● = hypoxia; ○ = normoxia). Symbols are as in Fig. 1 (2☆ : $p<0.025$; 4☆ : $p<0.005$).

Hypoxia induced a pronounced decrease in both heart rate (Fig. 2) and developed pressure (Fig. 3). These changes preceded by 4-6 min the increase in ANF release (Fig. 1). Within 2 min of reoxygenation, heart rate and developed pressure were not significantly different from the initial control values.

To investigate whether the atria could still secrete more ANF beyond the 10 min of hypoxia, a prolonged hypoxia (20 min) was applied in a group of 5 rats. ANF secretion stayed elevated throughout the hypoxia, and was 4.01 times higher ($p<0.05$; ANOVA; $F=6.36$) than in the normoxia group at 18-22 min following initiation of hypoxia. ANF release followed a similar time course as for the short-term hypoxia; reoxygenation did not evoke further ANF secretion (results not shown). The decrease in heart rate and developed pressure was comparable to that shown in Figures 2 and 3. Coronary perfusion pressure decreased by 15-18% during hypoxia. None of the parameters were significantly different from control within 3 min (heart rate) to 12 min (pressures) of reoxygenation.

To determine whether another species also responded to hypoxia with a massive release of ANF, 4 isolated rabbit hearts were exposed for 10 min to hypoxia (Fig. 4). ANF release increased 2.7- to 7.1-fold over the initial

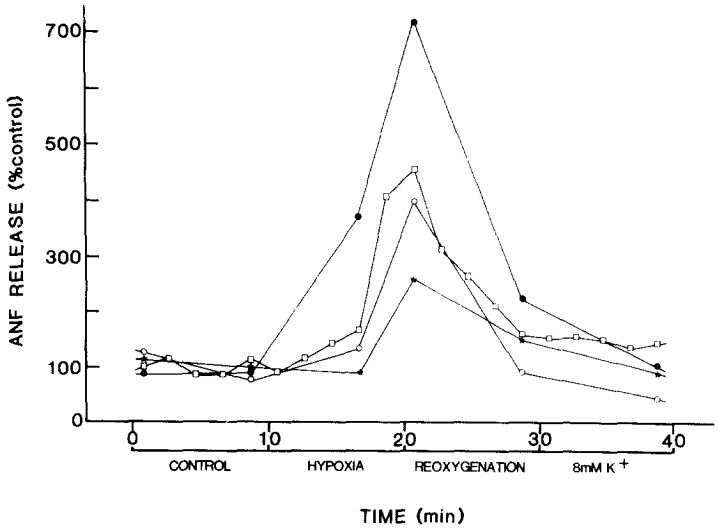

Fig. 4. Release of ANF from isolated rabbit hearts. Symbols refer to 4 individual experiments. Detailed time course of release at 2 min intervals is shown for one experiment (□). ANF values were normalized relative to individual mean control levels. ANF release was 439 ± 114 pg/ml during control. Peak ANF release was 1940 ± 577 pg, significantly different from control.

control levels at 11 min following initiation of hypoxia (mean ± SE = 457% ± 96%; $p<0.01$ relative to control). ANF secretions returned to control levels within 10-15 min of reoxygenation. Increased potassium concentration in the oxygenated buffer had no effect, presumably because the potassium concentration was not sufficiently elevated to cause release. Heart rate, developed pressure and coronary perfusion pressure significantly decreased during hypoxia (results not shown). These parameters returned to control levels within 2-4 min of reoxygenation.

DISCUSSION

The results clearly indicate that hypoxia is a powerful stimulus for ANF secretion from the isolated heart of both rats (Fig. 1) and rabbits (Fig. 4). The decrease in heart rate (Fig. 2), developed pressure (Fig. 3) and coronary perfusion pressure (see text) was fully reversible by reoxygenation, suggesting that tissue damage was not responsible for ANF release. The persistent and reversible release of ANF during prolonged hypoxia further indicates that ANF was not lost from damaged tissues during hypoxia. The time course of ANF release during hypoxia was clearly distinct from the rapid adenosine

response reported by many authors. Perfusate could not have accumulated in the atria and caused atrial distension, since it freely escaped through the holes left from the surgical preparation. The pulsatile stretch of the atria - a potent stimulus for ANF secretion (15) - was presumably decreased during hypoxia, because both heart rate and developed ventricular pressure were reduced. The possibility that atrial stretch did not mediate the hypoxia-induced ANF secretion remains to be verified in other in vitro systems.

The ANF released during hypoxia may act on the lung (8,9) to facilitate the pulmonary gas exchange. One single in vivo study has been performed, showing elevated levels of plasma ANF during chronic hypoxia (16). This finding was attributed to an elevated left atrial pressure. A direct effect of hypoxia on ANF release was not considered (16). Alternatively, the heart may be a target for atrial peptides. Although a 23-amino acid ANF-like fragment was shown to cause coronary vasoconstriction (17), atrial peptides co-released with ANF should be investigated as potential vasodilators in the coronary arteries. ANF may also excite cardiac sensory nerves to evoke a reflex-mediated protective action on the heart (18). Clearly, further studies are required to establish that hypoxia-induced release of atrial peptides is a physiologically important mechanism.

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