Enhanced Release of Atrial Natriuretic Factor by Endothelin in Atria From Hypertensive Rats

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Intravenous (bolus) administration of endothelin results in a transient fall in blood pressure that is accentuated in spontaneously hypertensive rats (SHR) compared with Wistar-Kyoto normotensive rats (WKY). In attempting to discern possible mechanisms underlying this depressor response, we examined the ability of endothelin to release atrial natriuretic factor (ANF) from isolated, spontaneously contracting atria from SHR and WKY. Isolated right atria were suspended under 3.0 g of resting force in tissue baths with the amount of immunoreactive ANF (irANF) released after exposure to endothelin assessed by radioimmunoassay. Endothelin (10^{-8} and 10^{-7} M) caused a concentration-dependent increase (1.5–4.5-fold) in the release of irANF, which was significantly greater in atria of SHR compared with WKY. The greater release of irANF in atria of SHR versus WKY was not related to tissue weight or changes in contractile rate or force induced by endothelin. Therefore, endothelin appears to cause a direct release of irANF from rat right atria in vitro. As found for the depressor response in vivo, endothelin is more efficacious in the hypertensive compared with the normotensive atrial preparation. Release of ANF may be important in the hypotensive response to endothelin in vivo. (Hypertension 1989;14:111-114)

Endothelin, a 21-amino acid peptide produced by vascular endothelial cells, is a potent and efficacious contractile agent in isolated vascular preparations. Administration of endothelin in vivo (0.01–10 µg/kg i.v.) has been shown to result in sustained pressor responses in both anesthetized and conscious rats. However, the pressor response in vivo is preceded by an initial fall in blood pressure, the latter response being, paradoxically, the prominent effect of endothelin on blood pressure in spontaneously hypertensive rats (SHR). Although the initial, transient depressor response is evident in the blood pressure tracings published for intravenous injection of endothelin, the mechanism underlying this response has not yet been identified.

During our analysis of the depressor and pressor responses to endothelin in vivo, we became aware of a report demonstrating that endothelin can release atrial natriuretic factor (ANF) from cultured rat atrial myocytes. These data prompted us to assess the ability of endothelin to release ANF from isolated atria of SHR and Wistar-Kyoto rats (WKY). Release of ANF could explain, in part, the initial depressor response to endothelin.

Materials and Methods

Age-matched (77 days old at purchase; 12–13 weeks old at date of experimentation) SHR and WKY were purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts). The rats were housed in laminar flow units and had free access to food and water. Representative blood pressures (mean, conscious) were 156±4 mm Hg for SHR (n=4) and 115±6 mm Hg for WKY (n=4); representative weights were 290±10 g for SHR and 285±14 g for WKY. Rats were killed by cervical dislocation with the right atria rapidly removed and placed in a modified physiological salt solution (PSS) of the following composition (mM): NaCl 118, KCl 4.7, MgSO4 1.18, NaHCO3 23, KH2PO4 1.2, CaCl2 2.0, and dextrose 11, gassed with 95% O2 and 5% CO2, pH 7.3, at 37°C. Atria were aligned on punctate holders and connected with surgical thread to force-displacement transducers (Statham-Gould UC3 cells). Changes in isometric force were recorded on a Grass polygraph (Grass Instr. Co., Quincy, Massachusetts) whereas force and rate were cap-
tured by a Buxco Data Logger and Contractility Monitor (Buxco, Sharon, Connecticut). Atria were allowed to equilibrate under 3.0 g of resting force for 1 hour. This level of resting force, under these same experimental conditions, was found previously to be near-optimal for release of ANF from rat atria and to lack any nonspecific, deleterious effect on atrial tissue integrity.8

After the equilibration period, atria were washed and then incubated in 10 ml fresh PSS. At 10-minute intervals, 1 ml aliquots of the PSS were removed (with buffer replacement after each removal) for radioimmunoassay processing (see below). The first 10-minute period was designated as a control period (i.e., basal release) with tissues being incubated with increasing concentrations of endothelin (10^-8 and 10^-7 M) or vehicle (saline). After the final aliquot was removed, atria were recovered and dried at 50° C for 48 hours after being weighed.

For analysis of ANF levels by radioimmunoassay, aliquots were immediately heated at 95° C for 3 minutes followed by drying in vacuo. The dried samples were brought up directly in 1 ml radioimmunoassay buffer (supplied with the Peninsula Labs., Inc. kit, Belmont, California) and assayed for immunoreactive ANF (irANF) with the kit for rat ANF. The IC50 for rat ANF with this assay was 18 pg/tube.

The data generated in this study were analyzed by the Newman-Keuls multiple comparison test, or the Student's t test for unpaired samples where indicated, with p<0.05 taken as the level for statistical significance. Values are represented as mean±SEM. Endothelin was purchased commercially from Peninsula Labs., Inc. and, in rabbit aortic segments, was found to elicit contractile responses of similar potency to those reported in the literature.1-3

**Results**

Administration of endothelin caused a concentration-related increase in the release of irANF from isolated atria of both SHR and WKY (Figure 1). In atria of SHR, the release of irANF was statistically significant, compared with vehicle concentration (i.e., 10^-7 M) of endothelin caused a modest (16%) increase in rate, which failed to change further in the presence of the high concentration of endothelin. The baseline rate was 244±6 beats/min for atria of SHR and 283±13 beats/min for atria of WKY.

Endothelin caused a statistically significant positive inotropic response in atria of both WKY and SHR (Figure 2, right). The contractile response in atria of WKY was concentration-dependent with an approximate doubling (103±11% increase) of force occurring at 10^-7 M endothelin. In atria of SHR, 10^-8 M endothelin caused a similar (compared with WKY) increase in force (57±10% in SHR, 76±12% in WKY); however, 10^-7 M endothelin failed to cause any further inotropic response in the atria of SHR. The initial contractile force levels were 578±219 mg in SHR and 535±211 mg in WKY. There were no changes in the levels of diastolic force with the addition of endothelin.

The dry weights for the isolated atria were 56±5 µg for SHR and 79±4 µg for WKY (p<0.05, Student's t test). Endothelin (10^-7 M) alone (added to PSS in the absence of atria) showed no cross-reactivity in the radioimmunoassay.

**Discussion**

Intravenous endothelin elicits an initial depressor response that is prominent in SHR.5,6 The depressor response is most likely due to an indirect effect of endothelin because this peptide has direct contractile effects on isolated cardiac and vascular smooth muscle.1-3,9 The observation that endothelin can release ANF from isolated rat cardiac myocytes7 raised the possibility that the depressor response may be mediated indirectly, in part, by the actions of ANF.

We have found that endothelin is a potent secretagogue for ANF in isolated atria from SHR and WKY; thus, the initial observation of Fukuda et al7
which corroborates the recent study in guinea pig atria. Increases in the force of atrial contraction would be expected to evoke release of ANF from cardiac atria. However, in our study, the ability to release ANF was not related to the inotropic effects of endothelin. Increasing the concentration of endothelin (from 10^{-8} to 10^{-7} M) caused a marked increase in irANF but failed to cause any further change in contractile force in atria of SHR. It is unlikely that an initial increase in contractile force (as elicited by 10^{-8} M endothelin in SHR) would result in a delayed, further increase in the release of ANF because holding the force constant (i.e., maintaining the concentration of endothelin) for an additional 10-minute collection period did not lead to an increased release of irANF (data not shown). In addition, comparable increases in force were elicited by endothelin (10^{-8} M) in atria of SHR and WKY with only the tissue of SHR releasing significant levels of irANF. Endothelin had no effect on contractile rate, which is also an effect for ANF release from atria, in atria of either SHR or WKY (Figure 2, left). Therefore, the release of ANF by endothelin is not strictly (positively) correlated with any action on contractile rate or force. The endothelin-elicited release of ANF did not appear to be a consequence of damaged myocardial tissue because atrial rates were similar to those reported for rat right atria and diastolic levels of force remained stable.

Our results, therefore, demonstrate that endothelin is a more potent secretagogue for ANF in isolated atria of SHR versus WKY. Release of ANF may be important for the prominent depressor response to endothelin in the hypertensive rats.

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