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EVIDENCE FOR α-1 ADRENERGIC RECEPTOR REGULATION OF ATRIOPEPTIN RELEASE FROM THE ISOLATED RAT HEART

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Summary. Atrial myocardium is the source of a recently described peptide hormone termed atriopeptin. Atriopeptin is thought to have a role in the regulation of systemic arterial pressure, fluid balance and plasma electrolyte homeostasis. Isolated rat hearts release atriopeptin into the coronary effluent, and we have found that this release is stimulated by the administration of norepinephrine, a compound with α and β adrenergic properties. Infusion of the pure β-receptor agonist, isoproterenol, failed to stimulate the release; however, the α-1 receptor agonist phenylephrine induced the release in a dose-dependent manner. The stimulation of atriopeptin release by norepinephrine and phenylephrine was inhibited by α-blockade with phentolamine. Administration of BHT-920, a selective α-2 agonist, had no effect on atriopeptin release. We conclude that atriopeptin secretion by the atrial myocyte is stimulated by activation of the α-1 adrenergic receptor. This finding suggests an involvement of the sympathetic nervous system in the physiologic regulation of the secretion of this hormone.

During the past several years study of the heart has revealed that this organ has an endocrine function (1,2). In mammals, this function is predominantly localized in the atria where myocytes possess granules typical of protein secretory cells. Within these granules is the atrial peptide hormone, termed atriopeptin. Atriopeptin when released into the circulation is a potential hormonal modulator of plasma electrolytes, extracellular volume, and systemic blood pressure (1,2).

Atriopeptin immunoreactivity has been demonstrated to be present in plasma of rat (3,4) and human (5) where the predominant circulating form in the rat is a 28 amino acid peptide (6). Increased plasma concentration of immunoreactive atriopeptin (iAP) results from acute volume expansion and saline loading in the rat (7). Furthermore, pharmacological doses of pressor agents including vasopressin, phenylephrine, angiotensin II, and oxytocin induce the release of iAP

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into the circulation (8). As yet, the physiological significance of the induced changes in plasma levels remains to be clearly defined.

In previous studies, efforts were focused on developing an in vitro model for study of the regulation of the release of atriopeptin. The isolated perfused heart was shown to release atriopeptin as measured by smooth muscle bioassays (9). Despite the presence of atriopeptigen, the proposed prohormone of atriopeptin, in atrial tissue this precursor was not found to be released into the coronary effluent. Thus, it was proposed that atriopeptigen is processed in the atria to the more biologically active atriopeptin. Subsequently, this finding was confirmed and extended with the observation that atrial stretch tends to enhance the release of immunoreactive atriopeptin (7). Efforts to study atriopeptin release from isolated atria by an in vivo natriuretic bioassay suggest that muscarinic and adrenergic agonists are stimulants of this response but these studies lacked quantitative measurement of hormone release (10,11). We report in this present study the stimulated release of atriopeptin from isolated rat heart through an apparent α-1 adrenergic receptor mediated mechanism.

MATERIALS AND METHODS

Isolated Perfused Rat Heart: A modified Krebs-Henseleit solution (KHS) was equilibrated with 95% O_2 - 5% CO_2 and perfused Langendorf style into hearts isolated from 200-300 g male rats (Sprague-Dawley). The composition of the KHS in mM was as follows: NaCl, 127.2; KCl, 4.7; $CaCl_2$, 2.5 · KH_2PO_4, 1.2; $MgSO_4$, 1.1, $NaHCO_3$, 24.9, Na pyruvate, 2.0; glucose, 5.5. A roller pump was used to maintain perfusion flow constant at 12 ml/min. Left ventricular pressure (LVP) was recorded from a latex balloon inserted into the left ventricle through the mitral valve and connected via a fluid filled tube to a Statham P23db pressure transducer. Left ventricular dP/dt was obtained by RC differentiation of the pressure signal and is used here as an index of contractility. All drugs were infused into the perfusion stream 1 cm above the aortic valve with a Harvard infusion pump delivering 0.12 ml/min. All reported drug doses are final concentrations in the perfusion stream. Radioimmunoassay of atriopeptin: Atriopeptin immunoreactivity of coronary effluent and synthetic AP III was measured directly with polyclonal rabbit antiserum (APG 11; final dilution 1:5000) raised against the 92 amino acid cyanogen bromide fragment of atriopeptigen (12). Synthetic atriopeptin III (supplied by Monsanto Co.) was iodinated by the chloramine-T method and purified by HPLC. The incubations for RIA were in a total volume of 0.3 ml and contained: 0.1M sodium phosphate buffer, pH 7.4 containing 0.25% BSA, 3% PEG, primary antiserum (1:5000), ^{125}I-AP III (10,000 CPM) and either 1-1000 pg of AP III or unknowns. The incubations were for 18 hours at 4°C with goat anti-rabbit IgG (1:750) added at the beginning of the incubation. At the end of the incubation period, the tubes were centrifuged for 30 min at 10,000 rpm. The supernatant was decanted and the pellet was analyzed for radioactivity with an automatic gamma counter.

Statistical Analysis: Data were analyzed by analysis of variance with repeated measures (13). Levels of $P < 0.05$ were accepted as significant.

RESULTS AND DISCUSSION

In order to study the release of atriopeptin, isolated rat hearts were perfused by the Langendorf method at a constant flow of 12 ml/min (8). The coronary effluent of isolated perfused rat heart was analyzed for atriopeptin concentration by radioimmunoassay. Ten minutes following the initiation of the perfusion of the heart the concentration of iAP was high (3820 $\pm$ 750 pg/ml; n = 6), declined with time, and approached a steady state after a 60 minute equilibration period. Following this equilibration period, the concentration of iAP released from the heart remained in a steady state (240 $\pm$ 60 pg/ml; n = 5) for the next 120 minute period. Surgical removal of the atria from the isolated perfused heart with ventricular perfusion remaining intact reduced the level of measurable iAP to below that of the limit of detection (< 4.0 pg). Analysis of the iAP found in the coronary effluent by reverse-phase high pressure liquid chromatography indicated the presence of one major peak of immunoreactivity. The retention time for this peak was consistent with the form of the peptide being the 28-amino acid form of atriopeptin rather than the prohormone form of this peptide. Finally, the cardiac effluent caused displacement of radioligand binding parallel to that produced by synthetic atriopeptin III.

The effect of norepinephrine on atriopeptin immunoreactivity (iAP) of the coronary effluent and maximal rate of rise of left ventricular pressure (dP/dt) is shown in Figure 1A. Norepinephrine (1 μM) infusion for 30 minutes produced a significant increase of iAP as well as the expected increase in myocardial contractility. Similar results were obtained with epinephrine (1 μM) (data not shown). In contrast, isoproterenol (1 μM) despite increasing cardiac contractility to a similar extent had no effect on iAP levels in the coronary effluent (Fig. 1B). Besides affecting dP/dt in a similar manner these three adrenergic agents changed heart rate, left ventricular pressure, and perfusion pressure to a comparable extent (data not shown). These results indicate that the stimulated release of iAP is not due to the physical or mechanical action of norepinephrine and epinephrine since isoproterenol increased heart rate, dP/dt, and left ventricular

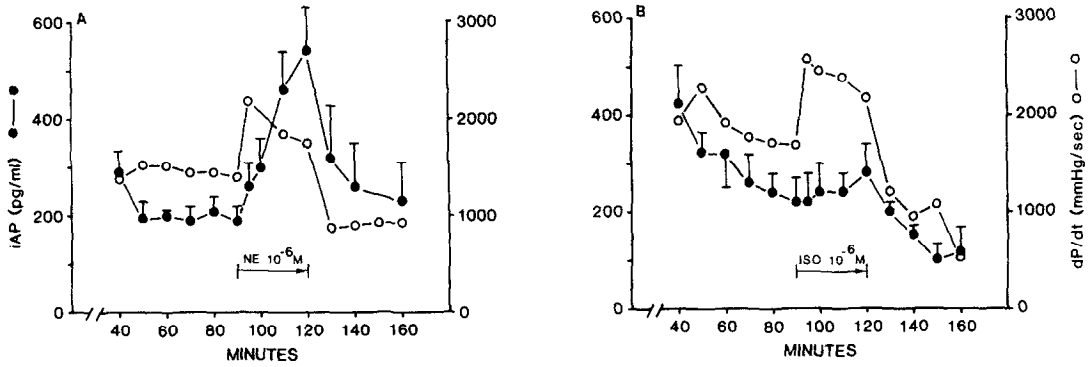

Figure 1A: Stimulation of iAP release into the coronary effluent induced by norepinephrine (NE). Isolated rat hearts were perfused for a basal period of 90 minutes during which time steady state values of iAP release were collected at 40,50,60,70,80, and 90 minutes. Immediately after this basal period, a norepinephrine infusion (1×10^{-6} M for 30 minutes) was begun and collections for iAP measurement were made at 5,10,20, and 30 minutes after the initiation of the infusion. This was followed by a 40 minute recovery period. Values are means ± standard errors with n=4. Standard error bars are omitted on mean dP/dt values for clarity.

Figure 1B: Treatment of isolated perfused rat hearts with isoproterenol (ISO). Conditions are as described for Fig. 1A.

pressure in an equivalent way, but did not cause an increase in the release of atriopeptin as seen with epinephrine and norepinephrine.

Since stimulation of iAP release was caused by norepinephrine and epinephrine, agonists with both α and β adrenergic receptor activity, but not by isoproterenol, a pure β adrenergic agonist, we studied the action of α-adrenergic receptor agonists. Administration of graded doses of phenylephrine, an α-1 receptor selective agonist, (5×10^{-7} M to 5×10^{-4} M) produced a dose-dependent increase of iAP that increased with time during the 30 min infusion of the drug and decreased toward basal during the following 40 min post-infusion period (Fig. 2). We observed no significant changes in heart rate, left ventricular pressure, or dP/dt due to phenylephrine. BHT-920 (1 μM), an α-2 receptor selective agonist (14), lacked an effect on iAP release from the heart and had no effect on the other physiological variables (data not shown).

These results suggested that norepinephrine stimulates iAP release through an α-1 receptor mediated mechanism. In an attempt to further define the adrenergic stimulation of atriopeptin release into the coronary effluent, we examined the effect of the α antagonist phentolamine on norepinephrine and phenylephrine-

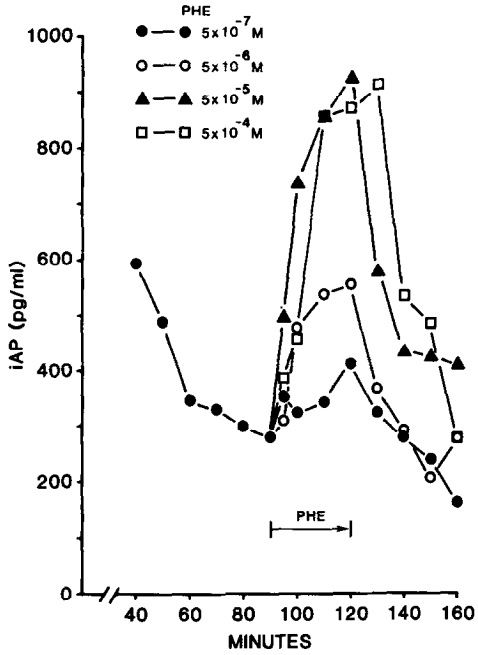

Figure 2: Dose-dependent stimulation of iAP release into the coronary effluent by phenylephrine (PHE). Collections for iAP measurement were made as described in the legend of Fig. 1A. The values are the means with n=4. Standard error was omitted for clarity.

induced release. The time-dependent release of iAP induced by norepinephrine was significantly inhibited by α-receptor blockade (Fig. 3). Similarly, the dose-dependent stimulation of iAP release by phenylephrine was shifted significantly to the right in the presence of phentolamine (Fig. 4).

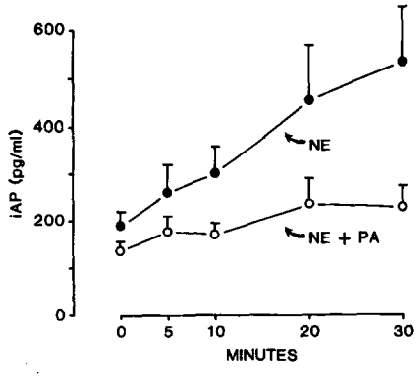

Figure 3: Inhibition of norepinephrine-induced release of iAP by phentolamine (PA). After a 90-minute equilibration period, a basal sample was collected just prior to, as well as 5,10,20, and 30 minutes after either the infusion of norepinephrine (NE, 1×10^{-6} M) or an infusion of both norepinephrine and phentolamine (PA, 1×10^{-5} M). Values are means ± standard errors with n=4. Curves are significantly different ($P < 0.05$) by analysis of variance with repeated measure.

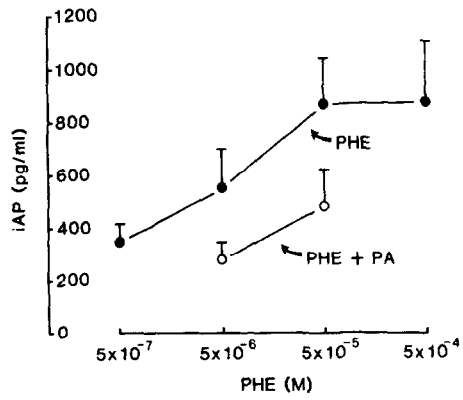

Figure 4: Shift of the dose-response curve for phenylephrine to the right by phentolamine. After a 90-minute equilibration period, a basal sample was collected just prior to, as well as 20 minutes after either the infusion of various doses of phenylephrine (PHE) or these doses of PHE with phentolamine (PA, 1 x 10^{-6} M). Values are means ± standard errors with n=4. Curves are significantly different ($P < 0.05$) by analysis of variance with repeated measure.

The cellular mechanism of the adrenergic induced release of atriopeptin by the atrial myocardium remains to be determined. However, the effects of α-1 receptor activation in other tissues is apparently mediated by stimulation of phosphotidylinositol turnover and subsequent activation of protein kinase C (15). This is a plausible scenario for the mediation of α-1 stimulation of atriopeptin secretion and is supported by the finding that phorbol ester stimulates an increase in iAP from the isolated rat heart (16).

Atrial tissue is richly innervated by adrenergic nerve fibers and physiological and biochemical consequences of adrenergic stimulation of cardiac tissue is well documented (17). Our finding that the release of iAP from the isolated heart is stimulated by adrenergic agonists apparently through an α-1 receptor activation suggests that the secretion of atriopeptin from the atrial myocyte is modulated by the activity of the sympathetic nervous system. In situations of relatively prolonged general activation of the sympathetic nervous system, such as in immobilization stress, the plasma level of atriopeptin is increased in the rat (18). Atriopeptin may indeed be an ideal modulator of many of the events that occur during stress. Studies with atriopeptin indicate that this peptide inhibits vasopressin secretion (19), renin secretion (20), aldosterone secretion (21,22) and vasoconstriction induced by norepinephrine and angiotensin (23,24). Possibly atriopeptin functions as a hormonal modulator to dampen the physiological reactions

to stress and thereby serves to protect the organism from the deleterious results of this reaction.

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