An Atrial Peptide is a Potent Renal Vasodilator Substance

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SUMMARY. Renal intra-arterial administration of rat atrial extracts elicits a concentration dependent renal vasodilation (independent of prostaglandin or dopamine release) in anesthetized rats. The atrial extracts do not alter skeletal muscle (hindlimb) vascular resistance or systemic arterial blood pressure. The high molecular weight peptide fraction of atrial extracts obtained by gel filtration, reduces renal resistance intra-arterially only after proteolytic activation (in vitro) or following intravenous (i.e., systemic in vivo activation) administration. The low molecular weight peptide fraction of the atrial extract which is active intra-arterially as a renal vasodilator has been further purified to yield two major peptides. The 21 amino acid peptide, designated atriopeptin I, was previously demonstrated to be natriuretic and to relax intestinal but not vascular smooth muscle strips. This peptide exerted little or no intra-arterial effect on renal resistance. The 23 amino acid peptide (the phenylalanine-arginine C terminal extension of atriopeptin I), designated atriopeptin II, was natriuretic and spasmolytic (in vitro) on both intestinal and vascular strips and was a potent renal vasodilator in vivo. Thus, the renal vasodilator activity present in cardiac atrial extracts appears to derive from a proteolytic process which selectively generates the 23 amino acid peptide, atriopeptin II. Further cleavage with the loss of phenylalanine-arginine C-terminal, as occurs with atriopeptin I, markedly suppresses the renal vasodilation. (Circ Res 54: 612-616, 1984)

EXTRACTS of mammalian atria cause a marked natriuresis and diuresis when administered to rats (deBold et al., 1981; Keeler, 1982; Trippodo et al., 1982). This biological activity is heat and acid stable, but is abolished by trypsin treatment (deBold et al., 1981; Keeler, 1982; Trippodo et al., 1982, 1983; Thibault et al., 1983). The effect of atrial extract on arterial blood pressure is unclear. Some investigators report a mild hypotension (deBold et al., 1981); others find no difference in response between atrial or ventricular extracts (Keeler, 1982; Trippodo et al., 1982, 1983). The same investigators report that the atrial extracts do not effect renal blood flow (Keeler, 1982; Borenstein et al., 1983), and others describe a redistribution of renal blood flow from the outer cortex into the inner cortex (Borenstein et al., 1983).

We discovered that rat atrial extracts possessed potent smooth muscle relaxant activity on isolated vascular and intestinal smooth muscle strips (Currie et al., 1983). The observation that bioactive substances present in the cardiac atria have the potential to alter sodium, volume, and vascular resistance provides a candidate endocrine system that could regulate volume homeostasis. We have fractionated rat atrial extracts by gel filtration chromatography into low molecular weight (<10,000 daltons) and high molecular weight (20,000-30,000 daltons) fractions (Currie et al., 1983). The high molecular weight fraction was relatively inactive as a smooth muscle relaxant (in vitro) on the intestinal strips (Currie et al., 1983), yet was potent as a natriuretic-diuretic when administered intravenously to rats. In contrast, the low molecular weight fraction exhibited potent biological activity in vitro (as a spasmolytic) and in vivo (natriuretic) (Currie et al., 1983). We did not observe a fall in systemic blood pressure at doses of atrial extract adequate to produce a 50-fold increase in natriuresis. The similarity of the biological activity of the high and low molecular weight peptides led us to test the possibility of a precursor-product relationship. We found that mild proteolytic treatment of the high molecular weight peptide fraction markedly enhanced the smooth muscle relaxant activity (Currie et al., 1984a). Subsequent chromatographic analysis of the digested high molecular weight preparation revealed that the newly generated peptide now co-migrated with the low molecular weight fraction derived from the original atrial extract. The crude high molecular weight fraction has previously been shown to be natriuretic-diuretic when administered to rats in vivo (deBold and Flynn, 1983; Trippodo et al., 1982; Thibault et al., 1983; Currie et al., 1983). However, such experiments don’t preclude the possibility of proteolytic
processing of the high molecular weight fraction to a low molecular weight peptide which could be the active agent affecting the kidneys. In preliminary reports, the high molecular weight peptide was found to be inactive, and cleavage was thought to be necessary for activity (Throckmorton and Gilmore, 1983; Hathaway and Solomon, 1983).

The low molecular weight fraction from the Sephadex G-75 column was further purified by ion exchange and high pressure liquid chromatography to yield several purified peptides (Currie et al., 1984b). Two such peptides were separated from each other on the basis of differences in charge, hydrophobicity, and biological profile. The first peptide, designated atriopeptin I, exhibits natriuretic and diuretic activity and relaxes intestinal (chick rectum) smooth muscle (in vitro) but not vascular (rabbit aorta) smooth muscle strips. Sequence analysis of atriopeptin I indicates that it is composed of 21 amino acids (Currie et al., 1984b). The second peptide, atriopeptin II, is a potent natriuretic-diuretic that relaxes both intestinal and vascular strips. The sequence of atriopeptin II is the same as that of atriopeptin I, but with the addition of a Phe-Arg extension at the carboxy terminus. deBold and Flynn (1983) described the purification of a low molecular weight peptide that was designated as "cardionatin I" which contained 49 amino acid residues but was not sequenced. Flynn et al. (1983) has very recently reported the structure of a low molecular weight atrial peptide which possesses the Ser-Leu-Arg-Arg-N-terminal extension and a Tyr-C-terminal extension of the peptide we designated atriopeptin II (Currie et al., 1984b). Similarly, Kangawa and Matsuo (1984) has described the structure of a human low molecular weight peptide (designated "α-human atrial natriuretic polypeptide") that differs from the peptide described by Flynn et al. (1983) by a methionine for isoleucine substitution at position 12.

In the current investigation, we studied the apparent paradox that rat atrial extract which did not cause a hypotensive effect in anesthetized rats was readily capable of relaxing precontracted blood vessel segments in vitro. We therefore analyzed the effect of atrial extracts by intra-arterial administration into autopерфused regional vascular beds of the rat. An inactive precursor (e.g., a high molecular weight peptide) administered intravenously could rapidly undergo in vivo hydrolysis to an active product. Thus, intra-arterial injection of the high and low molecular weight peptide fractions may provide an opportunity to distinguish which species is directly vasoactive in that vascular bed. Furthermore, a striking biological difference in renal vaso dilator potency of the low molecular weight peptides appears with the addition of Phe-Arg at the C-terminus present in atriopeptin II to the 21 amino acid sequence atriopeptin I.

**Methods**

**Atrial Peptide Preparation**

Boiled rat atrial homogenates were prepared as previously described and were desalted by elution from a Sephadex G-15 column (Currie et al., 1983). High (20,000–30,000 daltons) and low molecular weight (<10,000 daltons) fractions, assayed for natriuretic and smooth muscle relaxant activity, were obtained by gel filtration (Sephadex G-75) separation of atrial extracts obtained from the G-15 column as previously described (Currie et al., 1983). In vitro activation experiments, the high molecular weight peptide fraction (1–300 μg protein) was incubated with one unit of trypsin (bovine pancreas, type III, Sigma no. T-8253) per milliliter at room temperature for 1 hour. The reaction was terminated with a 10-fold excess of soybean trypsin inhibitor (Sigma, no. T-9003) (Currie et al., 1984a). The subsequent purification and structural analysis of the low molecular weight peptides resulting in the characterization of atriopeptins I and II was performed as previously described (Currie et al., 1984b).

**Perfused Vascular Bed Preparation**

The method for perfusion of rat peripheral organs was performed according to a modification of the method described by Sakai et al. (1980). Male Sprague-Dawley rats (~500 g) were anesthetized with urethane (1 g/kg iv). The right jugular vein was cannulated with PE-50 tubing (Clay Adams) for intravenous drug administration; the brachial or the femoral artery was cannulated with PE-90 tubing for recording systemic arterial blood pressure. Heparin sodium (1000 U/kg) was injected iv. The carotid artery was cannulated (PE-90) and connected via Silastic tubing to a tapered cannula (PE-90) inserted into either the femoral or renal artery via a Harvard peristaltic pump. The renal artery was exposed by a retroperitoneal flank or midabdominal incision. The pump was adjusted so that the pressure in the autoperfused vascular bed matched mean systemic arterial blood pressure (recorded with Statham transducers on a Beckman recorder); in the kidney, a constant flow of approximately 2.8 ml/min, and in the hindlimb approximately 2.5 ml/min, were required. The dead space in the Silastic tubing was 1.3 ml. With the pump set for a constant flow infusion, measurement of pressure (distal to the pump) provides an index of regional vascular resistance. The preparations were stable for 3–4 hours. Drugs were administered by intra-arterial injection into a rubber tube connected to the Silastic tubing entering the perfused arterial bed in volumes of 30 μl or less with a Hamilton micro-syringe.

**Results**

**Selectivity of Atrial Extracts on Regional Vascular Resistance**

Either the right hindlimb or the kidney of the anesthetized rat was blood perfused so that we might measure the effect of the atrial extracts on regional vascular resistance. Single bolus intra-arterial injections of either crude rat atrial or ventricular extracts produce a slight transient fall in hindlimb resistance. Extensive trypsin treatment (100 U/ml, 37°C, 60 minutes) did not abolish the hindlimb
vasodilation of these extracts. Such trypsin treatment completely abolishes the in vivo natriuretic and diuretic effect, as well as destroying the smooth muscle relaxant activity of the rat atrial extracts (Currie et al., 1983). The intra-arterial injections of the atrial extract did not alter the systemic arterial blood pressure.

In sharp contrast to the lack of systemic arterial or skeletal muscle vascular resistance, the renal vascular bed was very sensitive to the vasodilator effects of the atrial extracts. Rat atrial extracts produced a pronounced and prolonged renal vasodilation that was abolished when the peptide was pretreated with large concentrations of trypsin (100 U/ml, 37°C, 60 minutes). The response of the renal vascular bed to the atrial extract (Sephadex G-15 fraction) was concentration dependent, whereas the femoral vascular bed was inactive at the doses tested (Fig. 1). Crude ventricular extract exerted no effect on renal resistance.

The renal vasodilation produced by the atrial extract (10 μl of Sephadex G-75 fraction low molecular weight fraction) was not inhibited by pretreatment (n = 3) with: the dopamine antagonist, haloperidol (0.5 mg, ia); the inhibitor of prostaglandin biosynthesis, indomethacin (10 mg/kg, iv); by the α-adrenergic blocker, phenoxybenzamine (0.1 mg, ia); by the β-adrenergic blocker, propranolol (0.1 mg, ia); by the acetylcholine muscarinic inhibitor, atropine (0.3 mg, ia); or by the histamine antagonists, pyrilamine (0.1 mg, ia) or metiamide (0.1 mg, ia).

Comparative Effectiveness of the High and Low Molecular Weight Atrial Peptides as Renal Vasodilators

The high molecular weight peptide fraction obtained by gel filtration chromatography on a Sephadex G-75 column was largely ineffective when injected directly into the renal artery. However, gentle (in vitro) pretreatment of the high molecular weight peptide fraction with trypsin (1 U/ml, room temperature, 60 minutes) resulted in a pronounced renal vasodilation when injected (ia) (Fig. 2, left panel). We previously demonstrated that (in vitro) gentle proteolytic conversion of the high molecular weight fraction to a smaller peptide markedly enhanced the intestinal smooth muscle relaxant activity, as well as the vascular smooth muscle activity (Currie et al., 1984a). Quantitative comparison of the renal vasodilation indicates that the low molecular weight peptide fraction is not affected or is slightly inactivated by the gentle trypsin treatment (Fig. 2, right panel), but the high molecular weight peptide fraction is markedly activated at all doses tested.

Intravenous injection into anesthetized rats of the high molecular weight fraction results in a natriuresis and diuresis. The autoperfused (in situ) rat
Renal Vasodilation with Purified Atrial Peptides

Our ability to isolate, purify, and characterize two major low molecular weight peptides (atriopeptins) (Currie et al., 1984b) provided us with unique substances for our study of the renal responsiveness to atrial peptides. A comparative dose-response curve of the purified peptides is shown in Figure 4. The 23 amino acid peptide, atriopeptin II, produced a concentration-dependent decrease in renal resistance, with a clearly discernible response being produced by a bolus injection of 10 pmol of peptide. On the other hand, the 21 amino acid peptide, atriopeptin I, was relatively inactive as a renal vasodilator at the concentrations tested (Fig. 4). A slight reduction of renal resistance was detected at 100 pmol of peptide.

Discussion

Rat atrial extracts contain a low molecular weight peptide which is a potent renal vasodilator having no effect upon systemic or skeletal muscle vascular resistance. The preferential renal vasodilation produced by atriopeptin II is reminiscent of the selective renal effect of dopamine. Hemorrhagic shock elicits the endogenous release of potent vasoconstrictor substances which produce a profound renal vasoconstriction, which, if untreated, results in acute tubular necrosis. Dopamine is widely employed in the treatment of shock because of its selective renal vasodilation at low doses but higher concentrations stimulate adrenergic receptors and could cause a vasoconstriction. Although the renal vasodilator effect of dopamine is blocked by haloperidol (Yeh et al., 1969), this antagonist did not abolish the effectiveness of the atrial peptide. Thus, administration of atriopeptin II may provide a useful, readily manage-
able strategy to manipulate pathological situations involving marked reductions in renal blood flow, without the prospect of inducing a vasoconstriction.

The high molecular weight atrial peptide fraction was natriuretic when administered intravenously to rats (deBold and Flynn, 1983; Currie et al., 1983; Thibault et al., 1983; and Trippodo et al., 1982), but was relatively inactive compared to the low molecular weight fraction when tested in vitro on smooth muscle preparations (Currie et al., 1984a). In the current investigation, the high molecular weight peptide was impotent as a renal vasodilator following close-arterial injection, suggesting that there was no proteolytic cleavage of the larger peptide at renal sites proximal to the resistance determinant (i.e., the afferent or efferent arteriole). However, renal vasodilation was induced when the high molecular weight peptide fraction was subjected to partial proteolysis in vitro (Fig. 2) or was systemically activated by intravenous administration (Fig. 3), thereby indicating the existence of nonrenal sites of cleavage. These experiments do not establish whether the intact high molecular weight fraction acts directly on the kidney to produce natriuresis-diuresis.

We had previously purified atriopeptin I and II and found both materials to be natriuretic and diuretic (Currie et al., 1984b). The Phe-Arg extension in the 23 amino acid atriopeptin II results in a peptide that relaxes isolated thoracic aorta strips (in vitro), reduces renal resistance (in vivo), and exhibits potent natriuretic-diuretic activity. Indeed, our data show that the Phe-Arg containing atriopeptin is the active principle from the atrial extracts which causes the renal vasodilation. The 21 amino acid, atriopeptin I, did not reduce vascular resistance in vitro or in vivo at the doses tested, although it is a natriuretic agent having about one-fourth the effect of atriopeptin II (Currie et al., 1984b). We had inadequate material to determine whether atriopeptin I at very high doses is a renal vasodilator. The current data also indicate that the natriuretic and diuretic effect induced by the atrial peptides can be dissociated from their effect upon renal vasodilation. Finally, renal vascular recognition appears to exhibit stringent structural and conformational requirements, as evidenced by the observations that the high molecular weight precursor (which contains the active core peptide) is inactive as a vasodilator. Furthermore, shortening of the active low molecular weight peptide with the loss of the C-terminal Phe-Arg (as in atriopeptin I) markedly reduces its renal vasodilatory activity. These findings suggest that the selectivity of the proteolytic processing of the high molecular peptide appears to be a critical determinant for the renal vascular responsiveness.

References


INDEX TERMS: Atrium • Vasodilators • Natriuretic and atriopeptin
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