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The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties

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Summary. A 28 amino acid peptide with diuretic and natriuretic activity has been purified from rat atrial muscle. The primary structure of this atrial peptide is H-Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-(Arg)-Tyr-OH. The biological activity of this peptide is identical to that of atrial natriuretic factor and cardionatrin I isolated from rat atria.

There is now considerable evidence to support the proposition (1) that mammalian atria have the potential to regulate fluid volume. Thus, it has been observed that rat atrial homogenates or purified atrial granules cause significant natriuresis and diuresis when injected into rats (2-5) while homogenates of rat ventricular muscle have no such effects. The atrial muscle of mammals, in contrast to the muscle of the ventricle, contains secretory-like storage granules called specific atrial granules (6,7). These granules have morphological and histochemical properties in common with storage granules present in cells which store and synthesize polypeptide hormones (8). Significant changes occur in the number of atrial granules in the rat following alterations in electrolyte and water balance (9,10) and this has led to the view that these granules play a role in fluid volume regulation.

Chemical characterization studies suggest that the substance present in specific atrial granules responsible for the diuretic and natriuretic effect is a peptide referred to as atrial natriuretic factor (11,12). In recent work it has been shown that rat atrial muscle extracts when subjected to reverse phase high pressure liquid chromatography (RP-HPLC) could be resolved into

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four distinct chromatographic areas, each exhibiting natriuretic and diuretic activity (13). A 5,500 dalton peptide, named cardionatrin I, was purified from one of these regions and its spectral properties and chemical composition determined. These data suggested that cardionatrin I was a peptide 49 residues long containing a disulfide bond and no tryptophan.

We now report the purification and amino acid sequence of a 28 residue peptide with diuretic and natriuretic properties. This peptide appears to be part of the 49 residue peptide previously isolated.

MATERIALS AND METHODS

Atria were obtained from adult male Sprague-Dawley rats and homogenized and extracted as described previously (13). Eluates obtained after processing through Sep-Pak cartridges (Waters) were lyophilized, resuspended in 6 ml of 1.0 M acetic acid containing 1% NaCl and centrifuged at 15,000 x g for 30 min. The supernatant was applied to a Bio-Gel P10 column (26 x 40 mm), previously equilibrated with 1.0 M acetic acid and 1% NaCl. Fractions with natriuretic and diuretic activity, assayed in the bio-assay rat (11), were pooled and chromatographed by RP-HPLC as described in "Results".

Amino acid analysis was performed as described previously (13). Amino acid sequencing was carried out either with a Beckman 890C automatic sequencer and a 0.33 M Quadrol program or an Applied Biosystems Gas-Phase model 470A sequencer. Amino acid thiazolinones were converted to phenylthiohydantoins (PTH) by automatic conversion and then analyzed by RP-HPLC using an IBM-cyanopropyl column and a modification of the method of Johnson et al. (14). For sequencing in the Beckman spinning cup sequencer 10-20 nmol of peptide was used. Prior to sequencing four pre-cycles of gly-gly (100 nmol) in Polybrene (3 mg) were run. For sequencing in the gas-phase sequencer 400-600 pmol of peptide was used. The positions of the two cysteine residues were determined after reduction of the peptide with 2-mercaptoethanol and carboxymethylation with [^{14}C] iodo acetic acid (Amersham). Approximately 2 nmol of reduced, carboxymethylated peptide was sequenced in the presence of 50 nmol of apomyoglobin. Radioactivity was determined in each cycle of the sequencer by removing an aliquot of the final butyl chloride extract, mixing with scintillation fluid and counting in a Beckman LS-250 liquid scintillation system.

RESULTS AND DISCUSSION

Purification of peptide. Fractions with diuretic and natriuretic activity which eluted from the Bio-Gel P10 column in the 2,000-5,000 molecular weight range were pooled, pumped into the HPLC column and eluted with a linear gradient of 20-40% CH_3CN in 0.1% trifluoracetic acid (TFA) as described in the legend to Fig. 1. The column effluent was collected and fractions with biological activity (Fig. 1) were pooled, diluted 1:1 with aqueous 0.13% heptafluorobutyric acid (HFBA) and rechromatographed by RP-HPLC using a linear

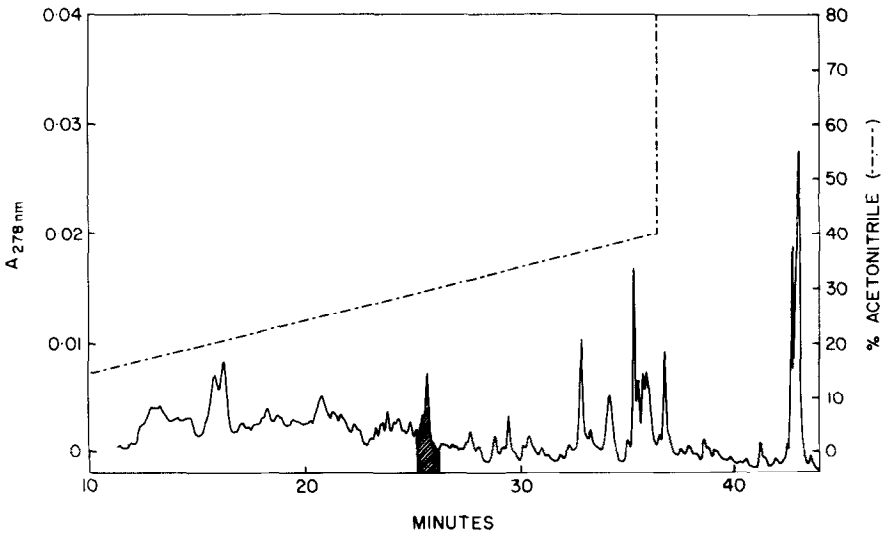

Fig. 1. Initial purification of peptide with diuretic/natriuretic activity by RP-HPLC. The column (Vydac C_{18}, 26 x 0.4 cm, 5 μ particle size), equilibrated with a solution of 15 % CH_3CN in 0.1% TFA, was eluted with a linear gradient of 15-40% CH_3CN in 0.1% TFA at a flow rate of 3.0 ml/min. Fractions were collected every 2 min. and assayed for natriuretic and diuretic activity as previously described (11). Active fractions (hatched area) were collected and pooled.

gradient of 30-50% CH_3CN in 0.13% TFA (Fig. 2a). Biologically active fractions recovered after this chromatography were diluted 1:1 with aqueous 0.1% TFA and rechromatographed with a linear gradient of 20-40% CH_3CN in 0.1% TFA (Fig. 2b). The major difference in the procedures described here and those used previously (13) for the purification of cardionatrin I is the introduction of the Bio-Gel chromatographic step prior to the RP-HPLC procedures and the use of Vydac ODS columns. The result of the purification procedure is a single well defined peak of protein, aliquots of which (approx. 0.5 nmol) induced the same characteristic diuretic and natriuretic response in the non-diuretic bio-assay rat as obtained previously for cardionatrin I (13). However, this modified procedure produced a peptide which compositionally and sequentially corresponds to a portion, rather than the whole, of cardionatrin I (see below).

Amino acid composition and amino acid sequence. The amino acid composition of the purified atrial peptide is shown in Table 1. It differs significantly from that obtained previously for cardionatrin I (13) in that it contains no

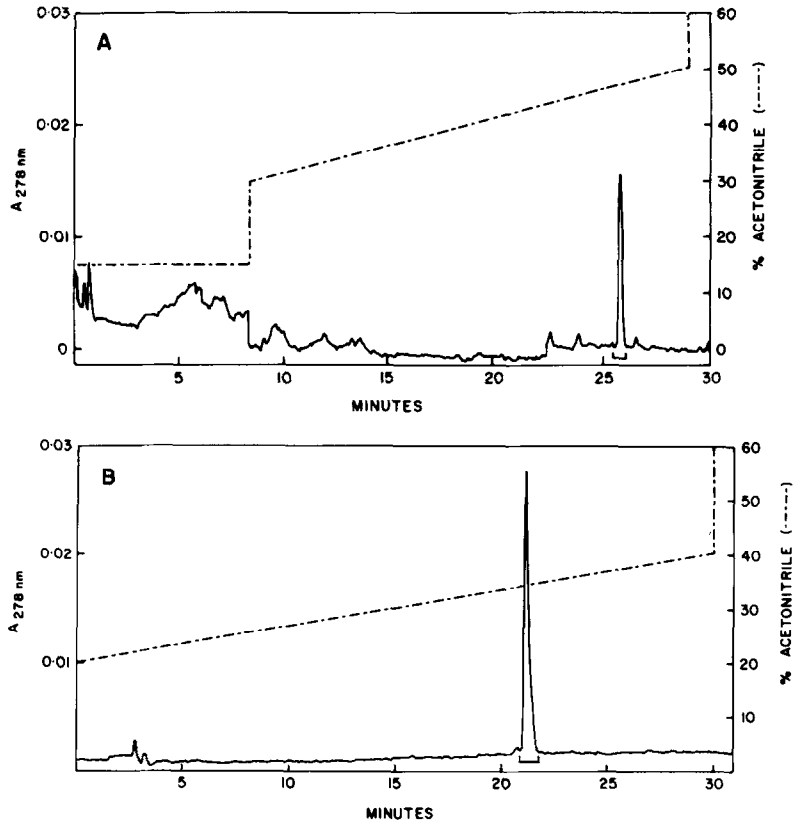

Fig. 2. Final purification of diuretic/natriuretic peptide by RP-HPLC. a) The column (see legend to Fig. 1) previously equilibrated with 15% CH_3CN in 0.13% HFBA was eluted with a linear gradient (30-50% CH_3CN in 0.13% TFA) over 40 min. at a flow rate of 1.5 ml/min. Biologically active fractions (bar) were pooled. b) Pooled fractions from the previous chromatography (Fig. 2a) rechromatographed on the same column with a linear gradient of 20-40% CH_3CN in 0.1% TFA. Biologically active fractions are indicated by the bar.

methionine, threonine, valine, isoleucine, proline or lysine. It does however contain the cystine residue present in cardionatrin I. The cystine residue was clearly evident in the hydrolysate of non-oxidized sample, eluting after alanine and before valine in the single column analysis used. After performic acid oxidation of the peptide, hydrolysates showed a quantitative conversion of cystine to two mol of cysteic acid. From the mol ratios of amino acids present a minimum molecular weight of about 3,500 was calculated for this peptide.

The amino acid sequence of the peptide is shown in Fig. 3. The peptide was sequenced from the NH_2-terminus in one continuous run. The sequence

Table 1
Amino Acid Composition of Rat Atrial Peptide

Amino Acid	Residues/mol
Asx	2.19 (2)
Ser	4.72 (5)
Glx	1.39 (1)
Gly	5.03 (5)
Ala	1.32 (1)
1/2 Cys[1]	2.40 (2)
Ile	2.37 (2)
Leu	2.16 (2)
Tyr	1.37 (1)
Phe	2.28 (2)
Arg	4.73 (5)

[1]Calculated after performic acid oxidation. Non-oxidized peptide had 1 cystine residue/mol.
Values in parenthesis indicate residues found in sequence.

analysis revealed no PTH-amino acid in cycles 7 and 23. This would occur if Cys 7 and Cys 23 were joined by a disulfide bond. Confirmation of this was obtained by sequencing reduced, [^{14}C] carboxymethylated peptide. Significant radioactivity was found only in fractions corresponding to cycles 7 and 23 indicating that the positions of the Cys residues are 7 and 23.

This sequence was also obtained when cardionatrin I (purified as described previously (13)) was subjected to automatic sequencing. The biological activity of cardionatrin I may then be attributable to this peptide sequence. In three separate sequencing runs of cardionatrin I no sequence was obtained beyond residue 28 indicating possibly that internal cleavage of cardionatrin I had occurred. During the sequencing of cardionatrin I no other sequence was evident suggesting that the remainder of cardionatrin I, i.e. the 20 unsequenced residues with the composition, Asx_2 Thr Ser Glx_4 Pro_2 Gly_2 Ala_2

H-Ser-Leu-Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-
└S-S┘
Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-(Arg)[1]-Tyr-OH

Figure 3 Amino Acid Sequence of Rat Atrial Peptide

[1]Assumed from amino acid composition. Only a small (nonquantifiable) peak of PTH-Arg was evident in the aqueous extract of cycle 27. No PTH amino acid was present in the organic extract.

NOTE: This sequence has no homology with any protein of known structure when searched through the latest Protein Sequence Database (Version 7, August 15, 1983) by the National Biomedical Research Foundation.

Val Met Leu Lys_3 His Arg, was refractory to sequencing. Reasons for this are not clear but it might mean that the NH_2 terminal of this peptide is blocked. Whether this unsequenced portion is part of the covalent structure of cardionatrin I is not known, but if it is it may be the NH_2 terminal portion of the whole cardionatrin I molecule. What is certain, however, is that the peptide, whose primary structure is shown in Fig. 3 and which apparently is derived from cardionatrin I, has potent diuretic and natriuretic activity and represents either a portion, or the whole, of the active component of the much sought after atrial natriuretic factor.

The C-terminal tyrosine in the sequence shown in Fig. 3 suggests that this peptide has been arrived at through processing by a chymotryptic-like proteolytic cleavage. Other peptides with diuretic and natriuretic activity are present in extracts of rat atria (13) some of which have molecular weights of the order of 12,000 (A.J. de Bold, unpublished work). It is tempting to speculate that these peptides are precursors of the peptide described here.

The presence of a disulfide bond in the peptide described in this paper is reminiscent of the structure of several protein hormones e.g. insulin and consistent with the structures of secreted proteins. If this peptide is secreted by the heart to exert a diuretic and natriuretic effect on the kidney then this would suggest the existence of a control mechanism in which the heart participates in the maintenance of water and electrolyte balance and thus plays an endocrine role. This speculation would be substantiated greatly by the demonstration of the presence of this or structurally similar peptides in the blood. Currently, efforts in this, and in other laboratories, are directed towards the development of a radioimmunoassay for the detection in the blood of atrial peptides with diuretic and natriuretic properties.

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