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ATRIAL NATRIURETIC FACTOR: ATRIAL CONVERSION OF HIGH TO LOW MOLECULAR WEIGHT FORMS

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The atrial natriuretic factor elutes by gel filtration in high and low molecular weight fractions. Extraction and elution of rat atria in 1.0 M acetic acid yielded a predominance of the high molecular weight form(s); whereas when these procedures were carried out in 0.1 M acetic acid, there was a predominance of the low molecular weight forms. When partially purified high molecular weight natriuretic activity was eluted in 0.1 M acetic acid, the high molecular weight form(s) remained intact. When partially purified high molecular weight natriuretic activity was mixed with crude atrial extract in 0.1 M acetic acid, there was an apparent conversion to the low molecular weight forms. Extraction of rat atria in boiling 0.1 M acetic acid blocked this conversion. It is concluded that rat atria contain a heat labile factor that converts high molecular weight natriuretic activity to the low molecular weight forms.

Mammalian atrial tissue contains two or more peptides that possess both natriuretic and smooth muscle relaxant activities (1-3). Gel filtration studies indicated that activity eluted in both high (10-40 K) and low (10 K) molecular weight fractions (3-5). Purified active low molecular weight peptides of about 5,500 daltons (6) and 3,800 daltons (7) have been reported. It is not known whether the high molecular weight natriuretic activity is comprised of multiple forms, nor is the relationship between the large and small molecular forms known. Our previous studies showed that extraction and gel filtration with 1.0 M acetic acid yielded mostly high molecular weight natriuretic activity (2,5). However, Ghai et al (abstract submitted to the 68th Annual Meeting of the Federation of American Societies for Experimental Biology) observed that when atria were extracted and fractionated by gel filtration in 0.1 M acetic acid, the yield of natriuretic/relaxant activity was mostly in the low molecular weight range. In the present study, we tested the hypothesis that the natriuretically active high molecular weight substance(s) was a

Abbreviations: K, $X10^3$

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precursor of the smaller atrial natriuretic factors and that extraction and gel filtration in 0.1 M acetic acid favors the conversion of high to low molecular weight activity.

METHODS

Extractions Atria from Sprague-Dawley rats were extracted and eluted by gel filtration with either 1.0 or 0.1 M acetic acid. In some experiments the same buffer was used for both extraction and elution, while in others the buffers were switched. For each experiment atria from 20 rats were homogenized as described previously (5) using 10 ml/g and rehomogenizing the pellet after a 40 K x g centrifugation in 5 ml/g. The lyophilized combined supernatant was homogenized in 5 ml buffer and centrifuged. This supernatant was fractionated on a column (1.5 x 100 cm) of Sephadex G-75. Pooled fractions were divided into three aliquots and lyophilized. Each aliquot was dissolved in 2 ml phosphate-buffered saline (pH 7.3), and 4 ml/kg assay weight (about 0.8 ml) was injected into anesthetized rats for assay of natriuretic activity.

Bioassay Male Sprague-Dawley rats, weighing 150-200 g were anesthetized with Inactin (1 mg/kg) and prepared with catheters as previously described (2). Thirty minutes after completion of surgery, urine was collected during a 30-min control period. The test sample was injected intravenously in 1 min and the change in urinary sodium excretion was determined during the 10 min following the start of injection. Based on previous findings of the dose-response relationship of the assay using similarly prepared partially purified rat atrial extract (5), the following procedures were used for estimating natriuretic activity. Since the minimum detectable response was 14 ± 3 μEq Na/kg·10 min (± SE), samples eliciting a response less than 15 μEq Na/kg·10 min were considered negative (below the sensitivity of the assay). Likewise, since on the average, the maximum response of the assay was about 400 μEq Na/kg·10 min, samples eliciting a response greater than this value were considered as having maximum natriuretic activity. Dilutions were made on all samples having maximum natriuretic activity until the responses of the assays were between 15 and 400 μEq Na/kg·10 min. Since responses of the assay in this range were found to be linearly related to log dose, it was necessary to estimate natriuretic activity by converting response to "dose" using a log function. In 40 assay rats, the average slope (±SE) of the log dose-response line was 510 ± 47 μEq Na/kg·10 min per log (mg/kg), where dose was given as mg protein/kg body weight. Dose in terms of mg protein would be meaningless since specific activity varies among extracts. Therefore, one unit of natriuretic activity was defined as that amount giving a response of 20 μEq Na/kg·10 min. This definition was chosen since 20 is near the minimum detectable response but well within the linear range of the log dose-response line. Furthermore, as a reference, this was the average response of the assay to 0.1 mg/kg furosemide. Based on this definition, response could be converted to log dose with the following equation: log dose (log natriuretic unit) = $(R_u - 20)/510$, where R_u is the response of the assay in μEq Na/kg·10 min to the unknown sample, 20 is the response to one defined unit, and 510 is the slope of the log dose-response line in μEq Na/kg·10 min per log unit. The units of natriuretic activity contained in each sample were obtained by taking the antilog and correcting for the proportion of sample actually injected and for any dilutions that were made. All negative samples were assayed in a minimum of two assay rats. The results reported for all active samples are the average of two to five assays that had responses on the linear part of the log dose-response line.

RESULTS AND DISCUSSION

All data have been corrected for differences in tissue weight and are reported as natriuretic units per g tissue. Atria extracted and gel filtered in 1.0 M acetic acid yielded a total of 151 units/g with the greatest peak of activity eluting at 70-90 ml (10-25 K dalton range), which was referred to as peak I (Fig. 1A). Approximately 25% of the total recovered activity eluted in the <10 K dalton range. Atria extracted and gel filtered in

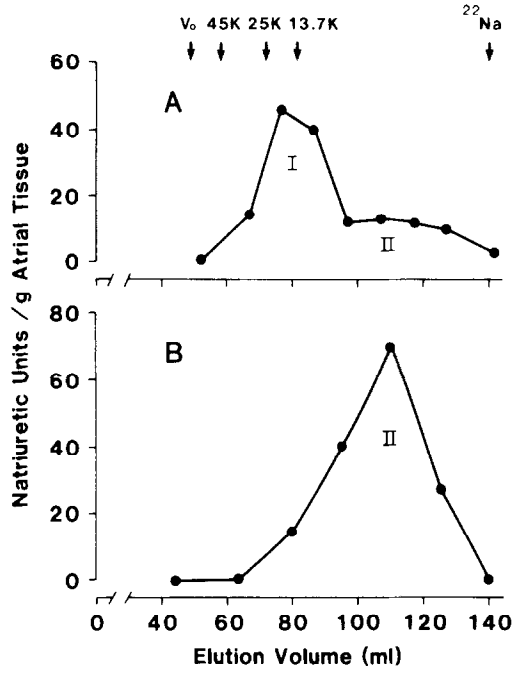

Figure 1. Elution of natriuretic activity from rat atrial extracts on Sephadex G-75. Natriuretic units were determined from the change in urinary sodium excretion in anesthetized assay rats using the empirical log dose-response relationship: log unit = $(R_u-20)/510$, where R_u was the response (change in urinary sodium excretion) during the 10 min following injection of the unknown sample, 20 was the response to one defined natriuretic unit and 510 was the slope of the log dose-response line. Arrows at the top indicate elution volumes of the void volume (V_o), ovalbumin (45K), chymotrypsinogen (25K), ribonuclease (13.7K), and ^{22}Na. Natriuretic activity eluting between 70 and 90 ml (25-10K daltons) is designated peak I, between 100 and 120 ml (<10K daltons) peak II.

A. Extraction and elution in 1.0 M acetic acid.
B. Extraction and elution in 0.1 M acetic acid.

0.1 M acetic acid also yielded a total of 151 units/g; however, most natriuretic activity was found at elution volume 100-120 ml (<10 K daltons), which was referred to as peak II (Fig. 1B).

These results indicated that extraction and fractionation of rat atria in 1.0 M acetic acid yielded a predominance of high molecular weight natriuretic activity as previously reported from this laboratory (2,5); whereas extraction and fractionation of rat atria in 0.1 M acetic acid yielded a predominance of low molecular weight natriuretic activity. There are at least three possible interpretations of these results.

Extraction hypothesis. It was possible that both the high and low molecular weight forms of natriuretic activity were present in the tissue and that the 1.0 M buffer extracted mostly the high molecular weight form(s) leaving the major portion of the low

molecular weight forms in the pellet; whereas the 0.1 M buffer extracted mostly the low molecular weight forms leaving the high molecular weight form(s) in the pellet. To test this hypothesis, the pellets from each of the two types of extracts were reextracted in opposite buffers. That is, the pellet from a 1.0 M extraction was homogenized in 0.1 M acetic acid, lyophilized and eluted in 0.1 M acetic acid; whereas the pellet of a 0.1 M extraction was homogenized in 1.0 M acetic acid, lyophilized and eluted in 1.0 M acetic acid. If there were natriuretic activity remaining in the pellets because of a lack of extraction in a preferential buffer, it would have been solubilized during this second extraction. However, no additional natriuretic activity could be detected from either pellet (data not shown), indicating that the extraction hypothesis was an unlikely explanation of the findings.

Aggregate hypothesis. It was possible that the native type of natriuretic activity in the tissue was the low molecular weight material and that aggregates formed under the conditions of 1.0 M but not 0.1 M acetic acid. To test this hypothesis, the pooled high molecular weight fractions of a 1.0 M acetic acid extract eluted in 1.0 M acetic acid (peak I, Fig. 1A) was lyophilized and eluted in 0.1 M acetic acid. If the natriuretic activity was in the form of a high molecular weight simply due to the formation of aggregates in 1.0 M acetic acid, elution of this material in 0.1 M acetic acid would have caused disaggregation and the elution of natriuretic activity in peak II. However, this was not the case. Natriuretic activity still eluted predominantly in peak I (Table I, Experiment C) indicating that partially purified high molecular weight natriuretic activity could not be converted to low molecular weight simply by dissolving and eluting it in 0.1 M acetic acid.

Conversion hypothesis. It was possible that the native type of natriuretic activity in the tissue was mostly of the high molecular weight form(s) and that under the conditions of 1.0 M acetic acid extraction, this form(s) was extracted largely intact; whereas under the conditions of 0.1 M acetic acid extraction, there was a rapid conversion (enzymatic or otherwise) to the low molecular weight forms. Since the extraction and aggregate explanations seemed unlikely, we adopted the conversion concept as a working hypothesis. It was found in the previous experiments that the high molecular weight natriuretic activity did not convert to the low molecular weight forms when it was partially purified

TABLE I
Natriuretic activity of rat atrial extract eluted on Sephadex G-75

Experiment	Comments[a]	Natriuretic Activity[b] (units/g) Peak I	Peak II	Peak Height Ratio Peak I/Peak II
A	Atrial tissue extracted and eluted in 1.0 M	86	25	3.4
B	Atrial tissue extracted and eluted in 0.1 M	15	70	0.2
C	Peak I from A eluted in 0.1 M	51	6	8.5
D	Atrial tissue extracted in 1.0 M, eluted in 0.1 M	17	38	0.4
E	Peak I from A plus atrial tissue extracted in 1.0 M, eluted in 0.1 M	25	66	0.4
F	Atrial tissue extracted in boiling 0.1 M, eluted in 0.1 M	65	20	3.3

[a]All extractions and elutions were carried out in either 1.0 M or 0.1 M acetic acid. Extraction buffer was removed by lyophilization before extracts were gel filtered in elution buffer.

[b]All natriuretic activity peaked at elution volume 70-90 ml (10-25K daltons), designated peak I or at 100-120 ml (<10K daltons), designated peak II. Units of natriuretic activity are defined in Methods and in Figure 1 legend.

and then eluted in 0.1 M acetic acid (Table I, Experiment C), yet when atria were extracted and eluted in 0.1 M acetic acid, an apparent conversion took place (Fig. 1B). It was reasoned that besides the conditions provided by the 0.1 M acetic acid, some unidentified substance in the crude 0.1 M extract must also be present to facilitate this conversion.

To determine if this putative converting factor was extractable in 1.0 M acetic acid, the supernatant of a 1.0 M acetic acid extract (which was previously found to contain largely high molecular weight activity) was lyophilized to remove the acetic acid and then fractionated in 0.1 M acetic acid. Under these conditions, the predominance of natriuretic activity eluted in peak II, suggesting that conversion of high to low molecular weight activity took place (Table I, Experiment D). These results suggested that the putative converting factor was extractable in 1.0 M acetic acid.

It was reasoned that if a converting factor were present in the 1.0 M acetic-acid-extracted material and that it is activated in 0.1 M acetic acid, the mixing of partially

purified high molecular weight natriuretic activity with lyophilized crude 1.0 M extract in 0.1 M acetic acid would lead to the conversion of high to low molecular weight natriuretic activity. To test this notion, partially purified high molecular weight natriuretic activity (peak I) was mixed in 0.1 M acetic acid with the lyophilized material from a crude 1.0 M acetic acid extract and eluted in 0.1 M acetic acid. Natriuretic activity eluted predominantly as peak II with no apparent shift toward the high molecular weight range (Table I, Experiment E). If no conversion had occurred, the ratio of peak I/peak II natriuretic activity would have been greater than that found in Experiment D (Table I). However, this ratio was the same in these two experiments. Thus, in contrast to the results obtained when partially purified high molecular weight natriuretic activity was eluted alone in 0.1 M acetic acid, when peak I material was mixed with crude extract in 0.1 M acetic acid, an apparent conversion of high to low molecular weight natriuretic activity occurred. These results lend further support to the conversion hypothesis.

To determine if the apparent "0.1 M acetic-acid-activated" conversion of high to low molecular weight natriuretic activity in crude atrial extract could be prevented by heat, rat atria were added to boiling 0.1 M acetic acid and homogenized while boiling. The supernatant from the 40 K x g centrifugation was lyophilized, homogenized in 0.1 M acetic acid and centrifuged. This supernatant was fractionated in 0.1 M acetic acid. Under these conditions, natriuretic activity eluted predominantly in the high molecular weight range (Table I, Experiment F), demonstrating that heat prevented the conversion of high to low molecular weight natriuretic activity. These results indicate that the putative converting factor was destroyed by heat.

Others have used boiling during the extraction process (3,7), but only after homogenization, when the conversion from high to low molecular weight natriuretic activity could have already taken place. Therefore, since it appears that the conversion occurs very rapidly even at room temperature, it is not surprising that a fair amount of natriuretic activity occurred as the low molecular weight forms in these earlier studies. Furthermore, judging from the data in Fig. 1A and Table I, Experiment F, some low molecular weight natriuretic activity may be present in the tissue before 0.1 M acetic-acid-activated conversion takes place.

These results indicate that rat atria contain a heat labile factor, which under conditions of 0.1 M acetic acid extraction is capable of rapidly converting high molecular weight natriuretic activity to the low molecular weight forms. The atrial converting factor does not appear to be active in 1.0 M acetic acid nor does it coelute with high molecular weight natriuretic activity.

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