processing of CRP RNA and quantification of mature mRNA. These studies should facilitate a detailed description of the molecular mechanisms controlling CRP biosynthesis during the acute phase response.

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Bioactive Cardiac Substances: Potent Vasorelaxant Activity in Mammalian Atria

Abstract. *Mammalian atrial extracts possess natriuretic and diuretic activity. In experiments reported here it was found that atrial, but not ventricular, extract also causes relaxation of isolated vascular and nonvascular smooth muscle preparations. The smooth muscle relaxant activity of atrial extract was heat-stable and concentration-dependent and could be destroyed with protease. Rabbit aortic and chick rectum strips were used for the detection of atrial biological activity. The atrial activity was separated by column chromatography into two peaks having apparent molecular weights of 20,000 to 30,000 and less than 10,000. The atrial substance that copurified with the smooth muscle relaxant activity in both peaks caused natriuresis when injected into conscious rats. It appears that atria possess at least two peptides that elicit smooth muscle relaxation and natriuresis, suggesting an endogenous system of fluid volume regulation.*

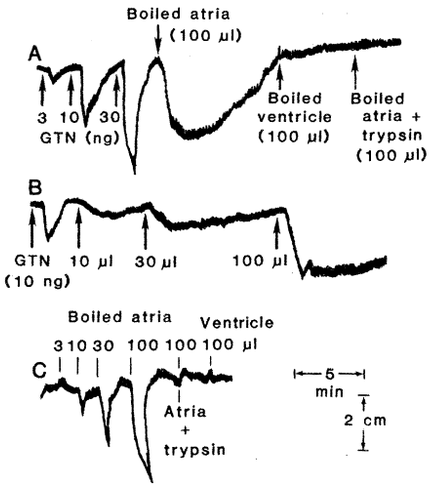
Muscle fibers of the atria but not of the ventricles possess granules typical of protein secretory cells (*1*). The content and function of these granules remain unresolved; however, atrial granularity is enhanced by water deprivation and sodium deficiency (*2*). Since the atria are a site of fluid volume receptors (*3*), they would be an ideal site for the synthesis and release of substances that participate in the regulation of fluid volume.

The possibility that the atrial granule content regulates extracellular fluid volume and ionic concentration is supported by the observation that rat atrial homogenates or purified atrial granules cause natriuresis and diuresis when injected into rats (*4*). Rat ventricular homogenates have no such effect on kidney function. Initial efforts to characterize the atrial substance that causes natriuresis and diuresis have shown that the material is heat-stable and sensitive to protease digestion (*4*). Partial purification of the activity was achieved by fractionation on a Sephadex G-75 column (*5*). The activity eluted in fractions of low molecular weight (3600 to 5000 daltons) and high molecular weight (36,000 to 44,000 daltons). Efforts at purification have been hampered by the necessity to assay each fraction in an intact rat. Such preparations exhibit considerable variability, are time-consuming, and cannot readily be used to test multiple samples.

To determine whether rat atrial extracts facilitate sodium and fluid excretion by inducing renal prostaglandin synthesis, we injected the material into perfused hydronephrotic rabbit kidneys in vitro. Although prostaglandin was not released, renal resistance in the perfused kidneys was reduced. The ventricular extract was inactive. This renal vasodilation was not expected, since others have reported that the atrial extract does not alter renal blood flow or glomerular filtration rate (*4, 5*). We therefore initiated studies to ascertain the presence of a vasodilator in the atrial extracts, reasoning that an atrial substance that could alter sodium content, fluid volume, and vascular tone would be an ideal regulator of extracellular volume. Furthermore, we hoped to develop an in vitro smooth muscle assay that would enable the rapid, quantitative assessment of atrial biological activity to facilitate efforts at purification.

After screening numerous vascular segments, we found that the rabbit aorta strip maintained in tone by a continuous infusion of norepinephrine was a most reliable and sensitive assay tissue. Addition of rat atrial homogenate caused a rapid, transient (2 to 5 minutes) contraction of rabbit aorta strips, immediately followed by a prolonged relaxation (10 to 30 minutes). The ventricular extract caused the rapid contractile response but no vasorelaxation. The contractile activity in the atrial and ventricular homogenates was destroyed by boiling the extracts for 10 minutes. Atrial vasorelaxant activity was not affected by boiling for 3 to 10 minutes, but was abolished by trypsin (Fig. 1A). The atrial vasorelaxant activity was dose-dependent, with as little as 10 μl of extract (~ 2 percent of the total extract from a single rat atrium) capable of eliciting a response (Fig. 1B). Boiled atrial, but not ventricular, extract relaxed isolated chick rectum strips maintained in tone by a continuous infusion of carbachol (Fig. 1C). The intestinal smooth muscle activity of the atrial extract was heat-stable, trypsin-sensitive, and dose-dependent (Fig. 1C). The response of the chick rectum to the atrial extract lasted just 2 to 3 minutes, compared to 10 to 30 minutes for the rabbit aorta. Both preparations exhibited no sign of refractoriness after repeated injections of extract and were stable for up to 6 hours. The chick rectum preparation, therefore, provides a rapid and simple bioassay that allows for the testing of a large number of samples.

Atrial granules occur in several mammalian species, including man (*1*). We found that boiled human atrial extract,



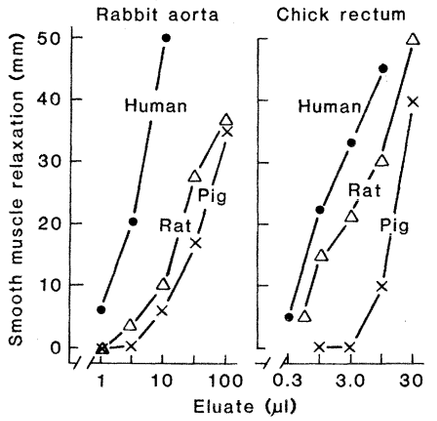


Fig. 1 (left). Bioassay of rabbit aorta (A and B) and chick rectum (C) relaxant activity derived from rat atria. Strips of rabbit thoracic aorta and chick rectum (under 2 g of tension) were continuously perfused at the rate of 15 ml/min with Krebs-Henseleit solution (95 percent O_2 and 5 percent CO_2) at 37°C. Muscle tone (measured with a Harvard myograph) was induced in the aorta strips by the continuous infusion of norepinephrine ($2 \times 10^{-8}M$) and in the rectums by carbachol ($2 \times 10^{-8}M$). Rat atrial and ventricular extracts were prepared by homogenizing the tissues in phosphate-buffered saline (PBS) (1:5, weight to volume) in the presence of the proteolysis inhibitors phenylmethylsulfonyl fluoride (1 μg/ml) and pepstatin (1 μg/ml). The homogenate was centrifuged at 2500*g* for 10 minutes at 4°C. The resulting supernatant was boiled for 10 minutes and then centrifuged at 10,000*g* for 10 minutes at 4°C. The effect of trypsin was tested by incubating the extract with trypsin (100 U/ml) at 37°C for 10 minutes and then adding a tenfold excess of trypsin inhibitor. Abbreviation: *GTN*, glyceryl trinitrate (for standardization of the aorta). Fig. 2 (right). Dose dependence and potency of atrial extracts as smooth muscle relaxants. Boiled atrial extracts from humans, pigs, and rats were eluted from a Sephadex G-15 column in 0.5*M* acetic acid, lyophilized, and dissolved in one-tenth of one volume of PBS for testing on smooth muscle preparations. Glyceryl trinitrate (10 ng) caused a 15-mm relaxation of the rabbit aorta. Extracts from human atria (autopsy material obtained 8 hours after death) and pig atria (obtained fresh from a local slaughterhouse) were boiled and centrifuged as described in the legend to Fig. 1. This experiment was repeated three times, each with similar results.

like the boiled rat extract, elicited a prolonged vasorelaxation and a transient relaxation of intestinal smooth muscle. However, boiled pig atrial extract still elicited vasoconstriction of short duration (2 to 3 minutes), followed by prolonged vasorelaxation (30 to 45 minutes). Separation of the contaminating vasoconstrictor activity from the vasorelaxant activity was accomplished by passing the rat atrial extract through a Sephadex G-15 column. The relaxant activity eluted in the void volume while the vasoconstrictor activity was retained by the column. After partial purification by the Sephadex G-15 column, the atrial extracts of rat, pig, and man were capable of eliciting a dose-dependent relaxation in both assay tissues (Fig. 2).

Further purification and fractionation of the atrial extract by Sephadex G-75 column chromatography resulted in two distinct peaks of smooth muscle relaxant activity. Figure 3A shows that the atrial natriuretic activity is found together with the smooth muscle relaxant effect (peak 1 at 60 ml and peak 2 between 70 and 90 ml). Peaks 1 and 2 have apparent molecular weights of 20,000 to 30,000 and less than 10,000, respectively. The natriuresis produced by atrial extracts was complete in 20 minutes. Comparison of dose-response curves of fractions from peak 1 and peak 2 suggests that the chick rectum activity is associated with rabbit aorta relaxant activity in peak 2 but dissociated in peak 1 (Fig. 3B). Further purification is necessary to determine whether the high-molecular-weight and low-molecular-weight factors are structurally related.

The atrial natriuretic and smooth muscle relaxant activities appear to be closely related. They are heat-stable, trypsin-sensitive, and copurify in column

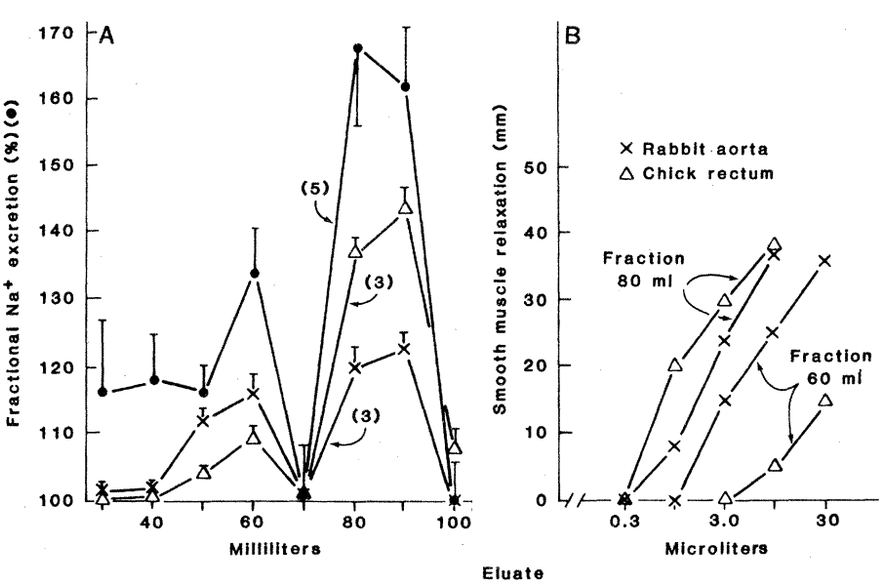


Fig. 3. (A) Biological activity of Sephadex G-75 fractions of atrial extracts. Boiled rat atrial extract (100 ml) was chromatographed and lyophilized as described in the legend to Fig. 2. The residue, dissolved in 10 ml of 0.5*M* acetic acid and centrifuged at 12,000*g* for 10 minutes, was chromatographed in a Sephadex G-75 column (1.5 by 51 cm) in 0.5*M* acetic acid at 12 ml/hour. Column fractions (10 ml) were lyophilized and then dissolved in PBS (1 ml). The column void volume was 30 ml and molecular weight markers were at 46 ml (ovalbumin, molecular weight 43,000), 60 ml (soybean trypsin inhibitor, 21,000), 68 ml (horse heart cytochrome, 13,000), and 85 ml (NaCl). Ten microliters of the G-75 column fractions was tested on rabbit aorta and chick rectum, while 50 μl was injected intravenously into intact rats to determine the effect of the fractions on fractional sodium excretion. The natriuretic response was measured in conscious, fasted rats equilibrated at least 6 hours before the injections. Fractional sodium excretion is expressed as the percent change (mean ± standard error) from 20-minute urine samples collected before and after injection of the test substance. The glomerular filtration rate and blood pressure did not change and the PBS vehicle was inactive. Values in parentheses give the number of observations. (B) Dose-response curve of active Sephadex G-75 fractions as smooth muscle relaxants. Peak 1 (60 ml) and peak 2 (80 ml) from (A) were tested on rabbit aorta and chick rectum. Glyceryl trinitrate (10 ng) caused a 25-mm relaxation of the rabbit aorta strips. This experiment was repeated three times, each with similar results.

chromatography. Further purification is required to determine whether the biological effects of atrial extract are elicited by a single substance. The isolated smooth muscle preparations used in this study provide a rapid and simple in vitro bioassay for the quantitative detection of bioactive substances derived from the atria during such purification efforts. Such a volume-regulating system in the atria could explain the decrease in vascular resistance and the natriuresis and diuresis caused by distention of the atria (*3*, *6*).

The relation of the bioactive atrial substance to other reported natriuretic factors derived from plasma and urine extracts is uncertain. The "natriuretic hormone" described by de Wardener (*7*) has a molecular weight of less than 500, is not sensitive to proteolytic digestion, and lacks amino acids. An apparently different natriuretic factor, purified from the plasma of salt-loaded dogs, is a heat-stable, acidic peptide of low molecular weight that appears to be derived from a larger precursor (*8*). This natriuretic factor, however, has been shown to potentiate vasoconstriction by norepinephrine, whereas our rat atrial extract actually reversed norepinephrine-induced contraction of the isolated blood vessel segment (Fig. 1).

We propose that mammalian atria have the potential to regulate fluid volume. The atria are in an ideal location to detect volume changes and appear to be the source of substances that influence renal function and vascular tone (Fig. 3). We envision that the atria detect an increase in the extracellular fluid volume and respond by releasing bioactive substances into the bloodstream that stimulate a rapid loss of fluid through the urine and peripheral vasodilation.

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Dicarboxylic Aciduria: Deficient [1-^{14}C]Octanoate Oxidation and Medium-Chain Acyl-CoA Dehydrogenase in Fibroblasts

Abstract. *Dicarboxylic aciduria, an inborn error of metabolism in man, is thought to be caused by defective β-oxidation of six-carbon to ten-carbon fatty acids. Oxidation of [1-^{14}C]octanoate was impaired in intact fibroblasts from three unrelated patients with dicarboxylic aciduria (19 percent of control), as was the activity of medium-chain (octanoyl-)acyl-CoA dehydrogenase in the supernatants of sonicated fibroblast mitochondria (5 percent of control). These data confirm that dicarboxylic aciduria is caused by an enzyme defect in the β-oxidation cycle.*

Dicarboxylic aciduria (DCA) is a metabolic disorder of man characterized by episodic hypoglycemia without accompanying ketosis and the excretion of elevated amounts of C_6–C_{10} ω-dicarboxylic acids in the urine (*1–3*). These dicarboxylic acids, primarily adipic (hexanedioic), suberic (octanedioic), and sebacic (decanedioic), are formed by microsomal ω-oxidation of fatty acids of either medium- or long-chain length which are then shortened to medium-chain dicarboxylic acids by mitochondrial β-oxidation (*4*). Although excretion of these dicarboxylic acids has been found in patients with other metabolic derangements (*5*, *6*), nine to ten patients with DCA have been identified in whom other entities can be excluded on clinical and biochemical grounds (*1–3*). The presence of C_6–C_{10} DCA without associated ketosis has suggested that mitochondrial β-oxidation of fatty acids is impaired in these patients, since this catabolic pathway is responsible for the stepwise degradation of C_4–C_{18} fatty acids to acetyl coenzyme A (acetyl-CoA) in mammals, as well as the production of acetoacetate and β-hydroxybutyrate. Increased fatty acid ω-oxidation and the resultant formation of dicarboxylic acids has been observed both in vivo and in vitro when the catabolism of fatty acids is impaired either by toxins or by inherited enzymatic deficiencies (*6*). The most important enzyme of β-oxidation with activity toward fatty acids of medium-chain length is the mitochondrial acyl-CoA dehydrogenase (E.C. 1.3.99.3) that demonstrates specificity for C_4–C_{14} acyl-CoA's with maximal activity toward C_6–C_{10} acyl-CoA's, with separate enzymes showing maximal activities toward the analogous short-chain (E.C. 1.3.99.2) and long-chain (E.C. 1.3.99.4) substrates (*7*). This enzyme, termed Y_1-fatty acyl-CoA, general acyl-CoA, or medium-chain acyl-CoA dehydrogenase (MCADH) is probably essential for normal functioning of the β-oxidation pathway (*7*); deficient activity

Table 1. The oxidation of ^{14}C-labeled substrates by human diploid skin fibroblasts. Culture of normal diploid skin fibroblasts and ^{14}C-substrate oxidation studies were performed as described (*15*), with the cells (passages 7 to 12) being harvested 8 days after subculture (1:4). All assays were done in triplicate and all values are expressed as means ± standard error of the mean. The final concentration and specific activity of each substrate were as follows: [1-^{14}C]butyrate, 1 m*M*, 10 mCi/mmole; [1-^{14}C]octanoate, 1 m*M*, 4.7 mCi/mmole; [1-^{14}C]palmitate, 0.2 m*M*, 5.0 mCi/mmole; [1,4-^{14}C]succinate, 2.0 m*M*, 1.0 mCi/mmole; [2-^{14}C]leucine, 1.5 m*M*, 0.5 mCi/mmole. The results obtained with five normal cell lines were compared to those from the three DCA cell lines.

Cell line	$^{14}CO_2$ production by intact fibroblasts per hour (pmole/mg protein)				
	[1-^{14}C]-butyrate	[1-^{14}C]-octanoate	[1-^{14}C]-palmitate	[2-^{14}C]-leucine	[1,4-^{14}C]-succinate
Normal control	2450 ± 143	2070 ± 204	102 ± 15	779 ± 67	945 ± 71
DCA	1760 ± 150	386 ± 69	59 ± 12	736 ± 83	979 ± 128
Percent of control	72	19	58	94	116