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SODIUM ION STIMULATES THE RELEASE OF ATRIAL NATRIURETIC POLYPEPTIDES (ANP) FROM RAT ATRIA

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SUMMARY. The release of atrial natriuretic polypeptides from spontaneously beating isolated rat atria was found to be sensitive to the increase in the concentration of sodium ion. The osmotic pressure, when produced by pharmacologically inactive choline chloride, also increased the release of ANP but substantially less than the sodium ion. Sodium ion and osmotic pressure stimulated the release of ANP in the hyperosmotic but not in the hypo-osmotic range. Neither stretch nor several neurotransmitters tested had any effects on the rate of ANP secretion.

Mammalian cardiac atria contain polypeptides with powerful natriuretic, diuretic, and smooth muscle relaxing activity (1). The peptides are called either atrial natriuretic polypeptides (ANP) or atrial natriuretic factor (ANF). All these polypeptides are derived from a common precursor, called pronatriodilatin (2), atriopeptigen (3), or gamma-ANP (4), which is located in specific atrial granules (5). A large number of homologous ANP have been isolated from rat and human atria (4,6-12). It has been shown that the atrial granules of various animal species contain large amounts of ANP-like immunoreactivity (13). ANP have also been found in the brain area controlling vasomotor responses (14-16). Previously, increased atrial distension has been suggested to be the stimulus for the release of ANP from the atria (17-19). With a sensitive and specific radioimmunoassay for ANP (20), we found

ABBREVIATIONS: ANP: atrial natriuretic polypeptides, RIA: radioimmunoassay.

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that increasing the concentration of sodium ion to hyperosmotic direction significantly increased the release of ANP from rat atria in vitro. This is the first direct positive stimulus reported for ANP release.

MATERIALS AND METHODS

Incubation of isolated rat atria

Male rats (290-390 g) of Sprague-Dawley strain were used. After decapitation of the animals, the hearts were excised and transferred into warm (30 °C) physiological buffer solution (154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 5.0 HEPES, 0.12 mM $MgCl_2$, 2.5 mM glucose, pH 7.4). The atrial block (both the atria and the auricles) was excised as an entity from the ventricular tissue under a microscope and placed in a tissue bath (30 ml, the buffer solution being that mentioned above with the exception that it contained only 115 mM NaCl, pH 7.4, at 33 °C). The temperature of the bath was kept constant by means of a water jacket. In the first set of experiments, a calculated amount of NaCl in 1 ml of buffer solution was cumulatively added to the bath every 15 min. In the second set of experiments, a calculated amount of choline chloride (195 mM ChCl, 115 mM NaCl, 5.6 KCl, 2.2 $CaCl_2$, 5 mM HEPES, 0.12 $MgCl_2$, 2.5 mM glucose) was cumulatively added to bath every 15 min causing an increase in the osmotic pressure. The osmotic pressures were measured by an osmometer (Knauer Halbmikro Osmometer). The atrial block was beating spontaneously during the experiments; the frequency varied from 60/min to 300/min.

Radioimmunoassay procedure.

The radioimmunoassay utilized a specific antiserum raised in rabbits against carbodiimide conjugate of synthetic rat pronatriodilatin fragment 101-126 and bovine thyroglobulin. Details of the radioimmunoassay procedure have been described previously (20).The intra- and interassay coefficients of variation were <10% and <15%, respectively. 250 ul samples from the incubation media (12.5-50 µl equivalents) were drawn at 5 min intervals and they were subjected directly to radioimmunoassay. Serial dilutions of the incubation media produced parallel displacement curves. The secreted immunoreactivity has been shown to correspond to 25-35 amino acid peptides (20).

Statistical analysis of results

One way analysis of variance continued by a modified Student's t-test (Bonferroni) was used.

RESULTS

We studied the release of ANP from rat atria using spontaneously beating atria in a tissue bath. Fig. 1 clearly shows that when the increasing osmotic pressure is brought about by sodium chloride, the ANP releasing system reacts sensitively to a small

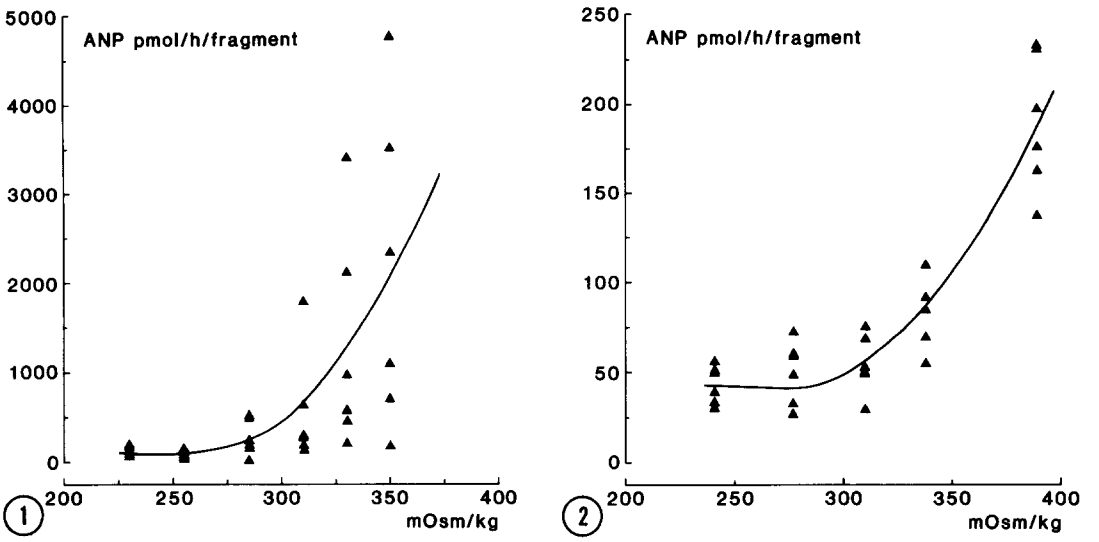

Fig. 1. The amount of ANP (Atrial Natriuretic Polypeptides) secreted from rat atria in vitro after increasing the osmolality with NaCl, see Methods. A polynome of second order was fitted to the data (R=0.9332).

Fig. 2. The amount of ANP (Atrial Natriuretic Polypeptides) secreted from rat atria in vitro after increasing the osmolality with choline chloride (NaCl 115 mM), see Methods. A polynome of second order was fitted to the data (R=0.9320).

increase in osmotic pressure in the hyperosmotic direction. Fig. 2 indicates that increasing the osmotic pressure from the iso-osmotic state increases the release of ANP from atria even when the concentration of sodium chloride is low (115 mM). Although the increase is much stronger in the case of NaCl than in the case of ChCl, it appears that the ANP releasing system is also sensitive to changes in osmotic pressure. The different secretion rates at comparable osmotic pressures in both groups of experiments give evidence for the premise that the secretion of ANP is not stimulated by the chloride ion. In the hypo-osmotic range, the secretion rates do not differ from those at the iso-osmotic states in respective groups.

Table 1 shows the results of statistical analyses in the experiments made with sodium chloride and Table 2 the results in the experiments in which the osmotic pressure was raised by choline chloride.

Table 1. The statistical analyses of the observations shown in Fig. 1. One way analysis of variance continued by a modified Student's t-test (Bonferroni)

Osmotic pressure		Secretion (pmol/h/fragment)			
Group	(mOsm/kg)	n	mean	SD	SEM
1.	230	6	110	46	19
2.	255	6	85	51	21
3.	285	6	255	197	80
4.	310	6	548	639	261
5.	330	6	1290	1241	507
6.	350	6	2108	1780	727

Groups	t modif.	p	Bonf.
1. and 6.	3.732	0.0039	**
2. and 5.	2.251	0.0481	*
2. and 6.	3.778	0.0036	**
3. and 6.	3.461	0.0061	**
4. and 6.	2.914	0.0155	*

(*, $p<0.05$; **, $p<0.01$)

Controls. The secretion rate of ANP was under 100 pmol/h/fragment in the experiments made in normal buffer solution (NaCl 154 mM) (n=3) during the same period of time as the experiments described in Fig. 1 were made.

Table 2. The statistical analyses of the observations shown in Fig. 2. One way analysis of variance continued by a modified Student's t-test (Bonferroni)

Osmotic pressure		Secretion (pmol/h/fragment)			
Group	mOsm/kg	n	mean	SD	SEM
1.	241	6	43	11	4
2.	277	6	50	17	7
3.	310	6	54	16	7
4.	338	6	83	18	7
5.	389	6	190	38	16

Groups	t modif.	p	Bonf.
1. and 4.	3.093	0.0114	*
1. and 5.	11.367	0.0000	***
2. and 4.	2.545	0.0291	*
2. and 5.	10.818	0.0000	***
3. and 4.	2.237	0.0493	*
3. and 5.	10.510	0.0000	***
4. and 5.	8.274	0.0000	***

(*, $p<0.05$: ***, $p<0.001$)

DISCUSSION

The present results show that the sodium ion and the osmotic pressure are direct stimuli for the release of ANP from rat atria. In actual conditions, sodium ion and osmotic pressure are closely related because both plasma and interstitial fluid contain mainly sodium and chloride ions. Before these findings, we studied the effects of stretch and several neurotransmitters and hormones on the release of ANP from rat atria. The experimental protocols were designed on the basis of reported results (17,18,21). However, we were unable to find any significant changes in the rate of secreted ANP after having added 30 μM doses of adrenaline, noradrenaline, acetylcholine, 5-HT, dopamine, histamine, or vasopressin to the bath. Neither static nor dynamic stretch, with or without noradrenaline, applied to atria gave any positive results.

Large amounts of ANP have been found in all atrial compartments (13). In normal physiological conditions, the venous pressure is low and stable in both atria, particularly in the left atrium (22). If the stimulus for the ANP release was atrial stretch, the pressures should vary within a wider range in order that the releasing system would be functional. Therefore, it is more reasonable that the ANP releasing system is sensitive to changes in concentration of sodium ion and to osmotic pressure, changes in whole circulation, rather than to atrial stretch. Also the reported responses of ANP secretion to atrial stretch have been minute (18) in relation to the amount of ANP stored in the heart. Stretch, a previously suggested physiological stimulus for the release of ANP, may in fact mechanically mimick the effects of hyperosmotic pressure on atrial cells. As the sodium ion and the osmotic pressure are stimuli for the ANP release the role of ANP would be to regulate long-term changes in the cardiovascular

system. If atrial stretch would effect on the release of ANP through visceral nerves it would mean a fast reacting mechanism to any change blood pressure, caused by e.g. a change in position. The functional role of ANP-containing neurons in the brain (14-16) is at present unknown.

ANP may change our concept of cardiovascular homeostasis in the near future and may give a new tool to manipulate the blood pressure under pathophysiological conditions.

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