Release of Atrial Natriuretic Peptide from the Isolated, Blood-Perfused Right Atrium of the Dog

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Abstract—Release of atrial natriuretic peptide (ANP) was investigated using the isolated right atrium (RA) cross-circulated with heparinized arterial blood of the donor dog. ANP concentration of the blood was measured by radioimmunoassay. The plasma ANP concentration of the arterial blood of the donor dog (ANP-D) which perfused the RA preparation was 111 ± 18 pg/ml ($n=4$), while the ANP concentration of venous blood leaving the RA preparation (ANP-A) was 1680 ± 220 pg/ml ($n=4$). In comparison, the plasma ANP concentration of venous blood leaving the isolated papillary muscle of the right ventricle (ANP-V) was 106 ± 19 pg/ml ($n=4$), which was not significantly different from the ANP-D concentration. When the right atrium was electrically driven at a rate of 100 beats/min, almost the same rate as the spontaneous sinoatrial rate of 98 ± 6 beats/min ($n=4$), the ANP-A concentration was not changed (1630 ± 160 pg/ml, $n=4$). When the driving rate was increased to 200 beats/min, the ANP-A concentration was significantly increased to 2250 ± 130 pg/ml ($n=4$). These results suggest that ANP is exclusively secreted from the atrium, but not from the ventricle, and that release of ANP is directly related to atrial rate.

Atrial natriuretic peptide (ANP) is synthesized, stored in, and released from cardiac atria; and it plays a role in the body fluid volume and blood pressure homeostasis as a circulating hormone (1–5). One of the most well-known convincing mechanisms for the ANP release is atrial distension (5–10). Elevated intraatrial pressure must be a cause of the increase in plasma ANP levels in animals with experimental heart failure (11, 12) and in patients with congestive heart failure (13–15). It is also known that plasma ANP levels are elevated during atrial tachycardia and cause polyuria (16, 17). The mechanism whereby ANP release is enhanced during atrial tachycardia is still unknown. Elevated atrial pressure during atrial tachycardia (5, 18) and/or atrial frequency *per se* (19) have been proposed to be responsible for the increase in ANP release. In any case, there has been little direct and quantitative data for the ANP released from the atria into

circulation, even though a number of reports on circulating plasma ANP concentration have been presented (5–18).

In the present experiments, we attempted to measure the ANP concentration of venous blood leaving the atrial preparation, not mixed with systemic venous blood. For this purpose, the right atrium of the dog was isolated and perfused through the right coronary artery with heparinized arterial blood of the donor dog (20). The venous blood samples from the right atrial preparation consisted of blood that passed and leaked only through the right atrial muscle. Thus, it is not mixed with any venous blood returned from the great veins. For comparison, the ANP concentration of blood leaving the ventricular tissue, i.e., the papillary muscle preparation (21) and that of the arterial blood of the donor dog perfusing both preparations, were measured. We also examined direct effects of atrial tachycardia electrically induced on ANP release from the

right atrium.

Materials and Methods

Experiments were carried out using the right atrium (RA) (20) and papillary muscle (PM) (21) preparations perfused with heparinized arterial blood of a donor dog. A schematic circulation diagram is shown in Fig. 1.

The blood-perfused RA and PM preparations: The RA and PM preparations were obtained from a mongrel dog of either sex, weighing approximately 10 kg, anesthetized with sodium pentobarbital (30 mg/kg, i.v.), given sodium heparin (500 U/kg, i.v.) and exsanguinated. The heart was excised and plunged into cold Tyrode's solution kept at about 4°C; then both preparations were made. The RA preparation consisted of the entire right atrial free wall, and the right coronary artery was cannulated (Fig. 1A). The PM preparation consisted of the anterior papillary muscle of the right ventricle attached to the interventricular septum; and the anterior septal artery, the nutrient artery to the papillary muscle, was directly cannulated (Fig. 1B). The RA preparation was placed on stainless

steel mesh in a double-wall glass jacket, not loaded nor distended by any weight, while the PM preparation, placed in another jacket, was under a 2-g weight load. Both jackets were maintained at 38°C by circulating warm water. Both the RA and PM preparations were simultaneously cross-circulated through each cannulated artery with the heparinized arterial blood of a donor dog at a constant perfusion pressure of 120 mmHg with a Cole-Parmer Masterflex peristaltic pump and a Starling pneumatic resistance placed parallel to the perfusion system (Fig. 1C). Venous blood from the preparations and excess arterial blood passing through the pneumatic resistance was once collected in a blood reservoir and returned to the donor dog through the jugular vein. The rate of blood flow (BF) through the anterior septal or right coronary arteries was measured using an electromagnetic flowmeter attached to each perfusion circuit (Nihon Kohden, MVF-1100). The sinoatrial rate (SAR) of the RA preparation was measured with a cardi tachograph (San-ei Instruments, 1321) triggered by the atrial electrogram obtained from bipolar electrodes attached to the right atrium close to the sinoatrial node.

Fig. 1. The isolated, blood-perfused right atrium (A) and papillary muscle (B) preparations of the dog and a schematic circulation diagram (C).

Electrical driving of the right auricle was done using a stimulator (Dia Medical, DHM-226-3) at rates of 100 or 200 beats/min after the sinus node artery was ligated at a portion as close as possible to the sinoatrial node area, and the SAR decreased to below 50 beats/min. The PM preparation was electrically driven at a fixed rate of 2 Hz (120 beats/min) through bipolar stimulating electrodes placed at the base of the PM. Developed tension (DT) of the PM preparation under a 2-g weight loading was monitored by a force displacement transducer (Dia Medical, DRM-T200 and DRM-T20).

Adult mongrel dogs of either sex, weighing 14–23 kg, were used as donor dogs, which were anesthetized initially with 30 mg/kg of pentobarbital sodium, i.v., and given an additional 4–5 mg/kg every hour. The animals received an initial dose of 500 U/kg of heparin sodium, followed by 200 U/kg every hour. Respiration was controlled using an animal respirator (Harvard Apparatus, model 607). Systemic blood pressure and heart rate of the donor dog were monitored continuously with a polygraph (San-ei Instruments, 361-6).

Radioimmunoassay of ANP: Blood samples for measurements of plasma concentration of ANP were obtained at each stopcock on venous lines leaving the RA preparation (Stopcock 1 for ANP-A) and the PM preparation (Stopcock 2 for ANP-V), and on an arterial line leaving the pneumatic resistance (Stopcock 3 for ANP-D), all of which conducted blood to the reservoir (Fig. 1C). Blood samples were collected into plastic syringes and transferred to chilled polystyrene tubes containing EDTA and trasylol. Plasma was immediately separated by centrifugation (3000 rpm for 10 min at 4°C). Plasma samples were diluted 6 times with 0.05 M sodium phosphate buffer (pH 7.4) containing 0.1% BSA, 0.15 M NaCl, 0.1% Triton X-100, 0.01% EDTA and 0.1% sodium azide (RIA buffer). Two hundred μ l of the diluted plasma samples were mixed with 100 μ l of antiserum (total volume: 300 μ l) and incubated for 24 hr. Then, 100 μ l of iodinated tracer was added, mixed and incubated for 24 hr. The entire procedure was done at 4°C in polystyrene tubes. Free and bound fractions were sepa-

rated by 500 μ l of 25% polyethyleneglycol in the presence of 1% γ -globulin (100 μ l). After centrifugation at 3000 rpm for 20 min, the supernatant was aspirated in vacuum and the radioactivity in precipitates was measured. The synthetic hANP, iodinated hANP and antiserum were kindly supplied by Dr. Hisayuki Matsuo, (Department of Biochemistry, Miyazaki Medical College, Miyazaki, Japan).

Statistical analysis was done by the paired *t*-test to determine differences between ANP-A and ANP-D, between ANP-A and ANP-V, between ANP-D and ANP-V, and between ANP-A under spontaneous SAR and atrial pacing at 100 or 200 beats/min. $P < 0.05$ was considered statistically significant.

Results

One hour after equilibration, the right atrium (RA) preparation showed spontaneous sinoatrial rates of 98 ± 6 beats/min, blood flow of 3.3 ± 0.7 ml/min and an average weight of 12 ± 2 g ($n=4$), while the papillary muscle (PM) preparation electrically driven at 2 Hz (120 beats/min) and loaded by 2-g weight showed developed tension of 3.5 ± 0.5 g, blood flow of 6.5 ± 0.8 ml/min and an average weight of 33 ± 2 g ($n=4$). At that time, plasma ANP concentration of arterial blood of the donor dog (ANP-D) perfusing simultaneously the RA and PM preparations was 111 ± 18 pg/ml ($n=4$). On the other hand, plasma ANP concentration of venous blood leaving the RA preparation (ANP-A) was 1680 ± 220 pg/ml ($n=4$), while plasma ANP concentration of venous blood through the PM preparation of the right ventricle (ANP-V) was 106 ± 19 pg/ml ($n=4$). The former was approximately 15 times higher than the ANP-D ($P < 0.01$), but the latter was not significantly different from the ANP-D (Table 1).

The sinus node artery, a main branch of the right coronary artery, was ligated at a portion as close as possible to the sinoatrial node area. Thereby, spontaneous sinoatrial node activity was suppressed to the SAR below 50 beats/min, while the blood flow of the RA preparation was only slightly decreased to 3.1 ± 0.8 ml/min ($n=4$). When the right atrium was electrically driven at a rate of 100 beats/min,

Table 1. Plasma ANP concentrations of blood samples

Blood samples	Plasma ANP concentration (pg/ml, n=4)
Arterial blood of the donor dog (ANP-D)	111± 18
Venous blood leaving the right atrium beating spontaneously (ANP-A)	1680±220 ^a
Venous blood leaving the papillary muscle electrically driven at 2 Hz (ANP-V)	106± 19 ^b
Venous blood leaving the right atrium electrically driven at 100 beats/min	1630±160 ^c
Venous blood leaving the right atrium electrically driven at 200 beats/min	2250±150 ^d

^asignificantly different from ANP-D ($P<0.01$). ^bsignificantly different from ANP-A ($P<0.01$) but not from ANP-D. ^cnot significantly different from ANP-A. ^dsignificantly different from ANP-A of the RA preparation electrically driven at 100 beats/min ($P<0.01$).

Fig. 2. Time course of effect of atrial rate on release of ANP.

almost the same rate as spontaneous sinoatrial rates, the ANP-A concentration was 1630 ± 160 pg/ml ($n=4$). This was not significantly different from the ANP-A concentration at spontaneous sinoatrial rates. When the driving rate was increased to 200 beats/min, the

ANP-A was increased to 2250 ± 150 pg/ml ($n=4$) (Table 1). In Fig. 2 is shown a typical experimentally determined time course of ANP secretion following changing atrial rates, in which an increased ANP concentration during higher atrial rates of 200 beats/min gradually

returned to the basal value after stopping the high rate stimulation.

Discussion

In the present experiments, it was directly and quantitatively demonstrated that ANP was released from the right atrium into irrigating blood, but little from the ventricle. It has been reported that the rat atrium superfused *in vitro* (19, 22) and the rat heart-lung preparation perfused through coronary arteries (6) secreted ANP into perfusates and that circulating plasma ANP concentration was increased by atrial distension, i.e., an increase in atrial pressure (5–10), and by atrial tachycardia (16, 17), both of which resulted from congestive heart failure (11–15). However, these results could not identify whether circulating ANP was directly released from the atrium. Currie et al. (4) reported that extracts of rabbit atria contained both high and low molecular weight bioactive atrial peptides, but the coronary venous effluent contained only the low molecular weight peptide in the isolated perfused rabbit heart. Ledsome et al. (7, 8) demonstrated that plasma ANP was higher in the coronary sinus blood than in the femoral artery. These results strongly suggested that ANP was released from the heart into the circulation, but it is still uncertain whether circulating ANP originates from the atrium, since the coronary sinus blood is mainly of the venous blood from the ventricles. Raine et al. (15) reported that a step-up in plasma ANP concentration was seen between the systemic venous blood and the blood in the right atrium and between the pulmonary and systemic arterial blood, directly suggesting the release of ANP from the atria. However, Akabane et al. (10) failed to show any difference in plasma ANP concentrations between blood from the pulmonary artery and that from the aorta. Since the blood in the atrial cavity or pulmonary artery comes not only from the coronary sinus blood but also from the systemic circulation via both vena cavae and the venous blood from the atrial muscle enters directly into atrial cavities as thebesian flows, it is very difficult or inappropriate to use plasma ANP concentrations in the atrium for comparing the amounts of ANP released from the atrium into the circulation in

response to various stimulations.

In the present experiments, nutrient blood passing through the right atrium was collected immediately after leaving the RA preparation without any dilution with systemic blood, and thereby the amounts of ANP released from the right atrium into the perfused blood could be determined. The plasma ANP concentration of venous blood leaving the atrium (ANP-A) was approximately 1600 pg/ml. For comparison, the plasma ANP concentration of venous blood from the ventricle (ANP-V) was about 100 pg/ml. On the other hand, plasma ANP concentration of the arterial blood of the donor dog (ANP-D), which perfused simultaneously both the right atrium (RA) and papillary muscle (PM) preparations, was about 100 pg/ml. The ANP-D, the ANP concentration of the circulating blood of our donor dogs, was slightly higher than, but within the range of, circulating plasma ANP concentrations previously reported in dogs (7–10, 12). According to Ledsome et al. (7, 8), the plasma concentration of ANP in coronary sinus blood was only about 1.5 to 2 times higher than in arterial blood. In our present experiments, plasma ANP concentration of venous blood from the atrium (ANP-A) was about 15 times higher than in arterial blood (ANP-D), suggesting that the ANP was directly released from the right atrium. Furthermore, the ANP-V was identical to the ANP-D, suggesting that the ventricular muscle does not secrete ANP. In addition, ANP concentration in the circulating blood (ANP-D) might be derived from the atrium (ANP-A). In our cross-circulation system, there are three atria: the isolated right atrium preparation and the right and left atrium of the donor dog. If we suppose that the left atrium releases equal amounts of ANP to that from the right atrium, though it has been anatomically shown that there are less secretory granules in the left atrium; the blood flow through each atrium is 3–4 ml/min, as obtained in the present experiments; and the circulating blood volume (cardiac output) is 700–1000 ml (ml/min) in a donor dog weighing about 15 kg, the calculated circulating plasma ANP concentration might be 30–50 pg/ml, and this value is a little less than the ANP-D. These results strongly indicate

that the atria is a source of ANP as a circulating hormone, at least under normal conditions.

Atrial tachycardia enhanced release of ANP from the right atrium when the atrial rate was electrically increased from 100 to 200 beats/min. Since the ANP-A concentration was identical between the right atria beating spontaneously at 98 ± 6 beats/min ($n=4$) and that driven electrically at 100 beats/min, electrical stimulation itself must not have affected the ANP release. Paroxysmal supraventricular tachycardia, including atrial fibrillation and flutter, have been shown to increase circulating plasma ANP concentration (16, 17), although Akabane et al. (10) failed to show a positive correlation between heart rate and plasma ANP levels. The mechanism whereby ANP release is increased by atrial tachycardia is still a matter of discussion (5, 18, 19). ANP release may be enhanced by an elevation of right atrial pressure during atrial tachycardia (5, 18). Schiebinger and Linden (19, 22) suggested that the frequency of atrial contraction directly influenced the rate of ANP release from rat atria, although the influence of resting tension could not be completely excluded (19). On the other hand, changes in resting tension affected ANP release in either non-paced or electrically paced left atria, indicating that beating is not necessary for stretch-induced ANP release (22). From our present experiment, where the right atrium was not loaded by any weight and was not distended, we conclude that atrial tachycardia directly increased ANP release from the right atrium.

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