Original Article

Physical and Pharmacological Regulation of Atrial Natriuretic Peptide Release from the Isolated, Blood-Perfused Right Atrium of the Dog

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Abstract: Physical and pharmacological interventions in atrial natriuretic peptide (ANP) release were investigated using the canine isolated, blood-perfused right atrium preparation. Plasma ANP concentration was measured by radioimmunoassay. ANP secretion rate was calculated from plasma ANP concentration in venous blood leaving the right atrium preparation and blood flow through the right coronary artery. When perfusion pressure was increased stepwise from 50 to 200 mmHg, ANP secretion rate was increased. ANP secretion rate was correlated to perfusion pressure and blood flow. ANP secretion rate was increased by atrial muscle distension by a 15-g weight loading and also by atrial tachycardia paced at a rate of 200 beats/min. Acetylcholine (5 μg) injected into the right coronary artery increased ANP secretion rate, which was blocked by 10 μg of atropine. Increased ANP secretion rate by 3 μg of phenylephrine was blocked by prazosin, but unaffected by atenolol. Isoprenaline (0.03 μg) increased the ANP secretion rate, which was blocked by atenolol, but not by prazosin. Increases in sinoatrial rate by noradrenaline and isoprenaline were abolished by atenolol, but not by prazosin. Phenylephrine did not increase the sinoatrial rate. These results suggest that 1) the perfusion pressure of the right atrium is closely related to ANP secretion as well as atrial muscle distension and 2) ANP secretion is stimulated via alpha- and beta-adrenoceptors as well as muscarinic receptors.

Key words: Atrial natriuretic peptide, Perfusion pressure, Blood flow, Muscarinic receptor, Alpha-adrenoceptor, Beta-adrenoceptor

INTRODUCTION

Atrial natriuretic peptide (ANP) is a circulating hormone, regulating body fluid volume and blood pressure1-4. Plasma ANP concentration changes in various heart diseases including congestive heart failure5-7 and paroxysmal atrial tachyarrhythmias8,9. However, the exact mechanism involved in the regulation of ANP secretion from the atria is still unclear. One of the most convincing mechanisms for ANP secretion is atrial distension10-13. Atrial muscle stretch14,15 is caused by pressure loading11,12 or volume overload1,10. Elevated intraatrial pressure must be a cause of an increase in plasma ANP concentration in patients with congestive heart failure5,6,10. Frequency of atrial contraction, i.e., atrial tachycardia is also known to increase plasma ANP levels17-19. In addition to these physical factors, vasoactive substances, such as acetylcholine via muscarinic receptors, noradrenaline and phenylephrine via alpha-1 adrenoceptors, and vasopressin, have been reported to increase ANP secretion from cultured rat atrial myocyte20,21 and from isolated rat atria22. In vivo heart, however, influences of hemodynamic changes, such as increases in blood pressure, heart rate and
intraatrial pressure, induced by these vasoactive substances on ANP release could not be excluded\textsuperscript{23-25}. Furthermore, even in isolated rat atria conflicting results of the effects of muscarinic cholinergic stimulation and of beta-adrenoceptor stimulation were reported by Schiebinger et al.\textsuperscript{26}.

Recently, we prepared an isolated, blood-perfused right atrium preparation of the dog which permit to measure ANP concentration of venous blood immediately leaving the atrium\textsuperscript{19}. This venous blood is not mixed with any systemic venous blood and contains approximately 15 times higher plasma ANP concentration compared with those of venous blood leaving the ventricular tissue and of arterial blood of the donor dog perfusing the isolated heart preparation; 1680 pg/ml in venous blood from the right atrium versus 111 pg/ml in venous blood from the papillary muscle and 106 pg/ml in arterial blood\textsuperscript{19}. In the present experiments by using this isolated, blood-perfused right atrium preparation, we examined the influences of physical factors such as perfusion pressure, blood flow, atrial rate and resting tension on ANP release from the right atrium preparation, and then pharmacologically analyzed the effects of humoral factors such as acetylcholine, noradrenaline, phenylephrine and isoprenaline.

**Methods**

Experiments were carried out on the canine isolated, blood-perfused right atrium preparation. Details of the methods have been described previously\textsuperscript{19}.

A mongrel dog of either sex, weighing approximately 10 kg, was 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 solution kept at about 4°C. The right atrium preparation consists of the entire right atrial free wall, and the right coronary artery was cannulated. The right atrium preparation was placed on a stainless steel mesh in a double-wall glass jacket maintained at 38°C by circulating warm water. The right atrium was not loaded or distended by any weight during the experiments, except the experiment of tension loading. For distension of the atrial muscle, the edge of the right atrium free wall contacted with the right ventricle was fixed to the support bar, the appendage of the right auricle was connected to a force displacement transducer (Dia Medical, DRM-T20) with a silk thread, and then loaded by a 15-g weight. The sinoatrial rate (SAR) of the right atrium preparation was measured with a cardiotachograph (San-ei Instruments, 1321) triggered by the atrial electrogram obtained from bipolar electrodes attached to the right atrium close to the sinoatrial node. The SAR was 97±5 beats/min at control (n=6). For electrical driving of the right auricle at a constant rate of 100 or 200 beats/min by a stimulator (Dia Medical, DHM-226-3), the sinus node artery was ligated at the portion as close as possible to the sinoatrial node area and the SAR decreased to below 50 beats/min.

For comparison, the papillary muscle preparation, which consists of the anterior papillary muscle of the right ventricle attached to the interventricular septum, was prepared and the anterior septal artery was directly cannulated. The papillary muscle preparation was placed in another double-wall glass jacket. The papillary muscle preparation was electrically driven at a fixed rate of 2 Hz (120 beats/min) through bipolar stimulating electrodes placed at the base of the papillary muscle. Developed tension (DT) under a 2-g weight loading was monitored by a force displacement transducer (Dia Medical, DRM-T200 and DRM-T20).

Both right atrium and papillary muscle preparations were simultaneously cross-circulated through each cannulated artery with 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. 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). To maintain the appropriate perfusion pressure, the pneumatic resistance was changed, resulting in a change of blood flow. The higher the perfusion pressure, the greater the blood flow. Since an increase in perfusion pressure caused a decrease in spontaneous sinoatrial rate (SAR) and vice versa\(^2\), the right atrium was electrically paced at 100 beats/min during experiments of changing perfusion pressure, as mentioned above.

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.

Adult mongrel dogs of either sex, weighing 14–23 kg, were used as donor dogs, which were anesthetized initially with pentobarbital sodium, 30 mg/kg 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-eki Instruments, 361–6).

Plasma ANP concentration was measured by radioimmunoassay. The venous blood sample leaving the right atrium preparation, which consists of the blood passed through only the right atrial muscle and is not mixed with any venous blood returned from the great veins, was collected. The ANP concentrations of the venous blood leaving the right atrium and, for comparison, those of the venous blood leaving the papillary muscle preparation and of arterial blood leaving the pneumatic resistance were measured, respectively. The ANP concentration of the arterial blood indicates the circulating plasma ANP concentration. All of the blood conducted to the reservoir. 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 0.05M sodium phosphate buffer (pH 7.4) containing 0.1% BSA, 0.15M NaCl, 0.1% Triton X-100, 0.01% EDTA and 0.1% sodium azide (RIA buffer). Two hundred \(\mu l\) of the diluted plasma sample was mixed with 100 \(\mu l\) of antiserum (total volume: 300 \(\mu l\)) and incubated for 24 hours. Then, after addition of 100 \(\mu l\) of iodinated tracer, it was mixed and incubated for 24 hours. The entire procedure was done at 4°C in polystyrene tubes. Free and bound fractions were separated by 500 \(\mu l\) of 25% polyethylene glycol in the presence of 1% \(r\)-globulin (100 \(\mu l\)). 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. H. Matsuo (Department of Biochemistry, Miyazaki Medical College, Miyazaki, Japan).

Drugs used were acetylcholine chloride (Daichi, Tokyo, Japan), atenolol (Sigma, St. Louis, USA), atropine sulfate (Nakarai, Tokyo, Japan), \(l\)-isoprenaline hydrochloride (Nikken-Kagaku, Tokyo, Japan), \(l\)-noradrenaline bitartrate (Wako, Osaka, Japan), phenylephrine hydrochloride (Wako, Osaka, Japan) and prazosin hydrochloride (Taitoh-Pfizer, Tokyo, Japan). The drugs were directly injected i.a. into the right coronary artery of the right atrium preparation. From plasma ANP concentration, blood flow measured directly by collecting blood in a plastic tube for one minute, and hematocrit (Ht), ANP secretion rate was calculated by the following equation.

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\text{ANP secretion rate (pg/min) = } \left[\text{ANP concentration of the venous blood from the right atrium} - \text{ANP concentration of the arterial blood of the donor dog}\right] \times \frac{\text{Blood flow (ml/min)}}{[1-\text{Ht}(%)/100]}
\]
ANP secretion rate was used as an indicator modified by physical and pharmacological factors.

Statistical analysis was done by a paired t-test to determine differences in plasma ANP concentrations of blood samples and in ANP secretion rates. P<0.05 was considered statistically significant.

RESULTS

ANP concentration of venous blood leaving the right atrium

After one hour equilibration, the right atrium preparation showed spontaneous sinoatrial rates of 97±5 beats/min, blood flow of 3.2±0.5 ml/min and an averaged weight of 11±2 g (n=6), while the papillary muscle preparation electrically driven at 2 Hz (120 beats/min) and loaded by a 2-g weight showed developed tension of 3.4±0.4 g, blood flow of 6.5±0.8 ml/min and an average weight of 32±3 g (n=6). Plasma ANP concentration of venous blood leaving the right atrium preparation was 1650±190 pg/ml (n=6). On the other hand, plasma ANP concentration of venous blood through the papillary muscle preparation of the right ventricle was 103±17 pg/ml (n=6). Moreover, plasma ANP concentration of arterial blood of the donor dog perfusing simultaneously the right atrium and papillary muscle preparations was 105±15 pg/ml (n=6).

Correlation between perfusion pressure and ANP release

Since the change in perfusion pressure induced the change in sinoatrial rate (SAR), the right atrium was electrically paced at a fixed rate of 100 beats/min. ANP secretion rate was 1800±100 pg/min (n=3, Fig. 1, lower panel) at 120 mmHg of perfusion pressure, at which blood flow through the right atrium preparation was 1.5 ml/min (Fig. 1, upper panel). When the perfusion pressure was decreased to 50 mmHg, the blood flow was decreased to 0.85 ml/min and ANP secretion rate was 1530±190 pg/min during the first 5 min after changing perfusion pressure. When the perfusion pressure was stepwise increased up to 200 mmHg, the ANP secretion rates were increased in response to increases in

![Fig. 1. Effects of change in perfusion pressure on ANP secretion rate. Blood flow (upper panel) was simultaneously changed when perfusion pressure was changed. ANP secretion rate was measured at control and during the first 5 min after the change in the perfusion pressure.](image-url)
perfusion pressure. Finally, when the perfusion pressure was decreased to 120 mmHg as in the control, ANP secretion rate was simultaneously decreased to a value similar to the control value. The blood flow simultaneously increased with the increase in the perfusion pressure (Fig. 1, upper panel). Thus, as shown in Figure 2 A, the ANP secretion rate well correlated to the blood flow similar to the perfusion pressure. In these experiments, plasma ANP concentration was decreased in response to the increase in perfusion pressure, which reflected the increased blood flow (Fig. 2 B).

**Influence of atrial tachycardia on ANP secretion rate**

When the right atrium was electrically driven at a rate of 100 beats/min, almost the same rate as spontaneous sinoatrial rates (98±3 beats/min, n=3), after ceasing the sinoatrial node activity by selective ligation of the sinus node artery, ANP secretion rate was not significantly different from that at spontaneous sinoatrial rates (2250±150 vs 2310±90 pg/min, Fig. 3, left two columns). When the driving rate was increased to 200 beats/min, the ANP secretion rate was increased during the first 10 min and the following 10 min (Fig. 3, center two columns). The blood flow was not changed (3.7 vs 3.5 and 3.5 ml/min), reflecting the increase in plasma ANP concentration as reported previously. The increased ANP secretion rate returned to the basal value after stopping the high rate stimulation (Fig. 3, the right column).

**Influence of atrial muscle distension on ANP release**

When the right atrium preparation was distended by a 15-g weight loading of the resting tension, ANP secretion rate was significantly increased during the first 10 min from 2550±120 to 3210±210 pg/min (n=3, Fig. 4). The blood flow (2.2 vs 2.1 ml/min) and the SAR (98 vs 100 beats/min) were not changed by this atrial muscle distension. Increased ANP secretion rate returned to the basal value after ceasing the loading (Fig. 4, left column, 2320±180 pg/min, n=3).

**Effects of acetylcholine on ANP release**

When acetylcholine (ACh), 3 µg, was injected i.a. into the right coronary artery, ANP secretion rate significantly increased by approximately 20% during the first 5 min, as shown in Figure 5. The blood flow was increased twice. When atropine, 10 µg, was i.a. injected into the right coronary artery 5 min before the following injection of ACh, atropine itself did not affect the ANP secretion rate during the 5 min, but markedly suppressed ACh-induced increase in ANP secretion rate (Fig. 5).
Fig. 3. Effects of atrial rate on ANP secretion rate. The right atrium was spontaneously beating at 98±3 beats/min (open column) and then the preparation was electrically driven at a constant rate of 100 beats/min (shadowed column). Then, the atrial rate was abruptly increased up to 200 beats/min (hatched columns). ANP secretion rates were measured during the first 10 min after changing driving rate. Data were obtained from 3 experiments. *P<0.05, **P<0.01 vs control.

Fig. 4. Effects of resting tension on ANP secretion rate. The right atrium was loaded by a 15-g weight. ANP secretion rate was measured during the first 10 min after changing resting tension. Data were obtained from 3 experiments. **P<0.01 vs control.
Effects of adrenergic agents on ANP release

In the control, noradrenaline (NA), 0.3 µg, phenylephrine (PE), 3 µg, and isoprenaline (IPN), 0.05 µg, injected i.a. into the right coronary artery, increased the ANP secretion rate by approximately 18, 14 and 24%, respectively, as shown in Figure 6A. Although the blood flow was not significantly changed, sinoatrial rate was significantly increased by NA and IPN up to 160 beats/min, but not by PE. In the presence of 10 µg of prazosin, an alpha-1 adrenoceptor blocking drug (Fig. 6B), although prazosin itself unaffected ANP secretion rate, PE could not increase the ANP secretion rate, but NA and IPN increased ANP secretion rate. NA and IPN increased the sinoatrial rate in the presence of prazosin as in the control. In the presence of 100 µg of atenolol, a beta-1 adrenoceptor blocking drug (Fig. 6C), despite the fact that atenolol itself did not affect the ANP secretion rate, IPN did not increase the ANP secretion rate, but NA and PE still increased as in the control. At that time, the increase in sinoatrial rate by NA and IPN was completely abolished.

Discussion

ANP was exclusively secreted from the atrium, but not from the ventricle. As discussed in the previous study\textsuperscript{19}, the past \textit{in vitro}\textsuperscript{1,10,13,18} and \textit{in vivo}\textsuperscript{6,11,12} experiments could suggest that ANP was released from the heart into the circulation, but could not identify whether circulating ANP originates from the atrium. In our experiments, however, the plasma ANP concentration of venous blood from the atrium was about 15 times higher than the circulating plasma ANP concentration, while plasma ANP concentration of venous blood from the ventricle was identical to the circulating plasma ANP concentration.
Quite recently, the ventricle was reported to be an important source of ANP in congestive heart failure\(^{28}\), but our present results suggested that at least normal or unfailing ventricular muscles did not secrete any ANP.

The ANP secretion rate from the right atrium is closely related to the perfusion pressure (Fig. 1). Since an increase in perfusion pressure caused a increase in blood flow, ANP secretion rate was rather closely related to the blood flow (Fig. 2A). The relationship between perfusion pressure and blood flow was linear up to 150 mmHg, but at 200 mmHg of perfusion pressure the blood flow abruptly increased (Fig. 1, upper panel). Nonetheless, ANP concentration of the venous blood was reciprocally and linearly decreased in relation to increase in blood flow, i.e., perfusion pressure. In any case, the perfusion pressure of the atrium may be one of the most important mechanisms of ANP release. Perfusion pressure means blood pressure (mean arterial pressure) \textit{in vivo}. Thus, the present study may simply suggest that blood pressure must be closely related to ANP secretion. Plasma ANP concentration is higher in patients with essential hypertension\(^{29,30}\).

Atrial muscle distension is the most convincing physiological mechanism of ANP secretion\(^{10-15}\). The present experiment clearly confirmed that atrial muscle distension increased ANP secretion from the right atrium (Fig. 4). Plasma ANP concentration of the venous blood from the right atrium was increased, but the blood flow was unaffected by the atrial muscle distension. Thus, the increase in ANP secretion by atrial muscle distension did not result from the increase in coronary blood flow. The increase in coronary perfusion pressure, i.e., distension of \textit{arterial} wall of the sinus node artery, a branch of the right coronary artery perfusing the sinoatrial node area, reciprocally decreased sinoatrial rate \textit{via} baroreceptors of the sinus node artery\(^{27}\). Although the change in the sinoatrial rate is opposite direction for increasing ANP secretion\(^{18,19}\), the right atrium preparation was electrically paced at a fixed rate of 100

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**Fig. 6.** Effects of noradrenaline (NA), phenylephrine (PE) and isoprenaline (IPN) injected i. a. into the right coronary artery on ANP secretion rate at control and in presence of prazosin or atenolol. **P<0.01 vs at control.**
beats/min during experiments changing the perfusion pressure. Therefore, influences of atrial rate on ANP secretion was not involved in the results from changes in perfusion pressure. These results may suggest that the baroreceptors of the sinus node artery may be responsible for the ANP secretion from the right atrium.

ANP secretion rate from the atrium was increased by acetylcholine injected i.a. into the right coronary artery, which was blocked by atropine, suggesting the stimulation of muscarinic receptors. In cultured atrial muscle cells and in isolated perfused rat hearts, acetylcholine increased ANP release, which was abolished by atropine. Recently, however, Schiebinger et al. reported in isolated rat atria that methacholine, a muscarinic cholinergic agonist, itself failed to increase ANP secretion, but suppressed the norepinephrine-induced increase in ANP secretion. In the present experiment, although ANP secretion rate was increased, the plasma ANP concentration was decreased after i.a. injection of acetylcholine. Since the blood flow through the right atrium was concomitantly and transiently increased by acetylcholine, the increase in blood flow might increase the ANP secretion rate. Atropine given i.a. into the right coronary artery suppressed the acetylcholine-induced coronary vasodilator effect as well as ANP secretion rate, both of which were mediated via muscarinic receptor stimulation. In the preliminary study, however, we observed that acetylcholine increased the ANP release from the right atrium cross-circulated at a constant flow rate of 3 ml/min of the right coronary artery. Thus, our findings are in agreement with those obtained by Raskoaho et al., but not with those of Schiebinger et al., despite that in both experiments isolated rat atria were superfused at constant flow.

Phenylephrine increased the ANP secretion rate (Fig. 6). Phenylephrine increased the plasma ANP concentration of the venous blood from the right atrium, while it only slightly decreased blood flow and little affected sinoatrial rate. Phenylephrine-induced increase in ANP secretion was abolished by prazosin, an alpha-1 adrenoceptor blocking agent which itself did not affect ANP secretion, but was unaffected by atenolol, a beta-1 adrenoceptor blocking agent. These findings are in agreement with previous in vitro and in vivo studies suggesting alpha-1 adrenoceptor stimulation of ANP release. Although in in vivo experiments the hemodynamic changes by phenylephrine, such as mean arterial pressure, left ventricular end-diastolic pressure and right atrial pressure, could not be excluded, the increase in ANP secretion in response to the direct alpha-1 adrenoceptor stimulation on the atrial cells secreting ANP should be due to activation of the polyphosphoinositol system.

Noradrenaline also increased ANP secretion rate, but it was not affected by either prazosin or atenolol (Fig. 6). Plasma ANP concentration of the venous blood from the right atrium was increased by noradrenaline, but blood flow was little affected. Noradrenaline-induced increase in sinoatrial rate was abolished by atenolol, but not by prazosin. Noradrenaline slightly decreased blood flow in the presence of atenolol, while it slightly increased in the presence of prazosin. These results indicate that noradrenaline increased ANP secretion via the stimulations of both alpha-1 and beta-1 adrenoceptors. Thus, noradrenaline could increase the ANP secretion rate by the beta-1 adrenoceptor stimulation directly and/or through the increase in sinoatrial rate even after blockade of alpha-1 adrenoceptors by prazosin, and vice versa. Alpha-1 adrenoceptor mediated increase in ANP secretion by noradrenaline has been reported in either in vitro or in vivo experiments, as similar to phenylephrine.

On the effects of beta-adrenoceptor stimulation on ANP secretion, some of them are in conflict. In contrast to previous in vitro studies, Schiebinger et al. demonstrated...
trated that the beta-adrenoceptor stimulation by isoprenaline and noradrenaline increase the ANP secretion from the isolated rat atria. Since atrial rate was fixed at 200 beats/min by electrical pacing in their experiments, isoprenaline- or noradrenaline-induced increases in ANP secretion may be due to direct beta-adrenoceptor stimulation of the atrial granule cells, but not due to secondary increased atrial rate. In the present experiments, although isoproterenol increased ANP secretion, which was suppressed by atenolol but not by prazosin, the effects of the increased sinoatrial rate by beta-1 adrenoceptor stimulation could not separate from the mechanisms of isoprenaline- and noradrenaline-induced increase in ANP secretion. In the preliminary experiment, when the right atrium was electrically stimulated at a rate of 150 beats/min, which alone increased ANP secretion rate significantly by approximately 30%, isoprenaline increased ANP secretion rate by about 20% without any change in the atrial rate. These findings suggest that beta-adrenoceptor stimulation increases ANP secretion directly through the stimulation of beta-adrenoceptor on the ANP secreting cells in the atrium and via an increase in atrial rate by beta-adrenoceptor stimulation of the sinoatrial pacemaker cells.

In conclusion, the present results suggest that 1) the perfusion pressure of the right atrium is closely related to ANP secretion as well as atrial muscle distension and 2) ANP secretion is stimulated via alpha- and beta-adrenoceptors as well as via muscarinic receptors.

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Regulation of ANP Release


