Atrial Pacing Stimulates Secretion of Atrial Natriuretic Polypeptide Without Elevation of Atrial Pressure in Awake Dogs With Experimental Complete Atrioventricular Block

Kazunobu Nishimura, Toshihiko Ban, Yoshihiko Saito, Kazuwa Nakao, and Hiroo Imura

To clarify whether or not tachycardia stimulates the secretion of atrial natriuretic polypeptide (ANP) without elevation of atrial pressure, we examined the effects of atrial pacing on ANP secretion in awake dogs with normal sinus rhythm and with complete atrioventricular block (CAVB), which was produced surgically by heat cauterization of His' bundle. In four dogs with normal sinus rhythm, atrial pacing increased the atrial rate from 146±20 to 260±10 beats/min, with marked elevation of right atrial pressure (from 0.2±0.1 to 3.9±0.8 mm Hg) and left atrial pressure (from 0.2±0.1 to 8.6±2.8 mm Hg). Along with the hemodynamic changes, the ANP level in plasma obtained from the coronary sinus was increased from 405±99 to 849±199 pg/ml (p<0.01). In five dogs with CAVB, the ANP level was also significantly increased from 730±82 to 1,137±35 pg/ml (p<0.01) by rapid atrial pacing (from 164±20 to 317±30 beats/min) in spite of the lack of any appreciable changes in either left or right atrial pressure. Furthermore, in the CAVB group, while the ventricular rate was increased by ventricular pacing from 52±5 to 146±16 beats/min, atrial pacing was performed simultaneously without atrioventricular (A-V) sequential form. Even under this condition, the ANP level was increased from 480±172 to 626±223 pg/ml (p<0.05) without any substantial changes in atrial pressure. Such effects of rapid atrial pacing on ANP secretion were suppressed after infusion of autonomic blocking agents. These results demonstrate that atrial pacing per se stimulates ANP secretion from the heart without elevation of atrial pressure and indicate that this change is mediated by the autonomic nervous system. (Circulation Research 1990;66:115-122)

Accumulating evidence indicates that atrial natriuretic polypeptide (ANP) with high and low molecular weights is produced in the atrium and that α-ANP with 28 amino acids is secreted through the coronary sinus from the heart and circulates in the body as a hormone. Intravenous administration of α-ANP causes rapid diuresis, natriuresis, and vasodilation. These results indicate that the heart is an endocrine organ involved in regulation of body fluid as well as a pumping organ. The plasma level of ANP is elevated during episodes of paroxysmal supraventricular tachycardia or atrial fibrillation, which are often associated with polyuria. In addition, several investigators have reported that atrial pacing increases the plasma ANP concentration in humans and experimental animals. The tachycardia-induced ANP secretion has been generally explained by an elevation of atrial pressure during tachycardia. On the other hand, it is widely accepted that an increased frequency of depolarization stimulates the hormone secretion in various hormone-producing cells. These findings raise the possibility that an increase in heart rate is one stimulus for ANP secretion. However, since atrial pacing is usually accompanied by an elevation of atrial pressure due to mistiming of atrial contraction, it is difficult to examine the effects of atrial pacing on ANP secretion without alteration of atrial pressure in animals with a normal sinus rhythm. In the present study, we experimentally prepared dogs with complete atrioventricular block (CAVB) in which atrial pressure was constant during atrial pacing and examined the effects of atrial pacing on ANP secretion by use of this model.
rate that normal ventricular contraction could follow. In five of the dogs in the CAVB group, atrial pacing was performed at twice the resting atrial rate. Furthermore, after the dogs in the CAVB group underwent ventricular pacing at the same rate as the atrial rate for 60 minutes, atrial pacing was performed simultaneously without an atroventricular (A-V) sequential form (atrial plus ventricular pacing). All atrial pacings were sustained for 30 minutes and were followed by a recovery period of 30 minutes.

In the other three dogs with CAVB, the influence of autonomic blocking agents on tachycardia-induced ANP secretion was examined. The ventricular rate was maintained by ventricular pacing at the fixed rate throughout the experiment so that a reduction in the ventricular rate caused by injection of adrenergic antagonists would not affect the hemodynamics. Atrial pacing was first performed for 20 minutes in a manner similar to the protocol of atrial plus ventricular pacing and was followed by a recovery period of 30 minutes. Then a bolus injection of 0.5 mg/kg propranolol (Sumitomo Pharmacy, Osaka, Japan), 1.0 mg/kg phenolamine (Japan CIBA-GEIGY, Takarazuka, Japan), and 0.1 mg/kg atropine sulfate (Tanabe Pharmacy, Osaka, Japan) was given, followed by drip infusion of the same three blocking agents (10 μg/kg/min propranolol, 20 μg/kg/min phenolamine, and 2 μg/kg/min atropine sulfate) until the end of the protocol. After drip infusion had been maintained for 15 minutes, atrial pacing was repeated in the same manner.

**Blood Sampling**

Blood samples for measurement of the plasma concentration of ANP were obtained from the coronary sinus at two points before pacing and every 10 minutes during the pacing and the recovery period. They were also obtained from the aorta just before pacing, 20 minutes after initiation of pacing, and 20 minutes after the end of pacing. Each 1 ml of blood withdrawn from the coronary sinus and the aorta was replaced by infusion of an equal volume of physiological saline. The blood samples were transferred to chilled disposable tubes containing aprtenin (1,000 kallikrein-inactivator units/ml) and EDTA (1 mg/ml) and immediately centrifuged at 4°C. Aliquots of plasma were stored at −20°C until the assay.

**Radioimmunoassay**

The plasma ANP concentration was measured by radioimmunoassay as previously described. This radioimmunoassay recognizes a carboxy-terminal fragment of ANP, α-ANP (17–28); the minimal detectable quantity of ANP is 1 pg/tube. The 50% binding intercept of the standard curve was 20 pg/tube. Intra-assay and interassay variances were 7.0% and 12.3%, respectively.

**Data Analysis**

Data obtained from the pacing and recovery periods were compared with the mean values of two prepacing
TABLE 1. Changes in Atrial Natriuretic Polypeptide Level at Coronary Sinus and in Hemodynamics During Atrial Pacing

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Preparing</th>
<th>Pacing</th>
<th>Postpacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
<td>405±99</td>
<td>849±199*</td>
<td>476±258</td>
</tr>
<tr>
<td>LAP (mm Hg)</td>
<td>0.2±0.1</td>
<td>8.6±2.8*</td>
<td>1.1±0.4</td>
</tr>
<tr>
<td>RAP (mm Hg)</td>
<td>0.2±0.1</td>
<td>3.9±0.8*</td>
<td>0.3±0.5</td>
</tr>
<tr>
<td>AR (beats/min)</td>
<td>146±20</td>
<td>260±10*</td>
<td>144±9</td>
</tr>
<tr>
<td>VR (beats/min)</td>
<td>146±20</td>
<td>260±10*</td>
<td>144±9</td>
</tr>
<tr>
<td>CAVB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
<td>730±82†</td>
<td>1,137±35*</td>
<td>750±63</td>
</tr>
<tr>
<td>LAP (mm Hg)</td>
<td>8.4±2.2‡</td>
<td>8.0±2.8</td>
<td>8.6±2.7</td>
</tr>
<tr>
<td>RAP (mm Hg)</td>
<td>3.4±1.2‡</td>
<td>3.6±1.3</td>
<td>3.4±1.5</td>
</tr>
<tr>
<td>AR (beats/min)</td>
<td>164±20</td>
<td>317±30*</td>
<td>172±25</td>
</tr>
<tr>
<td>VR (beats/min)</td>
<td>52±5</td>
<td>50±5</td>
<td>51±5</td>
</tr>
<tr>
<td>CAVB and ventricular pacing</td>
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<td></td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
<td>480±172</td>
<td>626±223‡</td>
<td>445±171</td>
</tr>
<tr>
<td>LAP (mm Hg)</td>
<td>7.3±2.9</td>
<td>7.3±2.7</td>
<td>7.1±3.2</td>
</tr>
<tr>
<td>RAP (mm Hg)</td>
<td>1.4±0.4</td>
<td>1.7±0.3</td>
<td>1.6±0.5</td>
</tr>
<tr>
<td>AR (beats/min)</td>
<td>147±14</td>
<td>295±32*</td>
<td>147±14</td>
</tr>
<tr>
<td>VR (beats/min)</td>
<td>146±16</td>
<td>146±16</td>
<td>146±16</td>
</tr>
</tbody>
</table>

Values for pacing and postpacing are at 10 minutes after beginning of pacing and 30 minutes after end of pacing, respectively. Values are mean±SEM.

ANP, atrial natriuretic polypeptide; LAP, left atrial pressure; RAP, right atrial pressure; AR, atrial rate; VR, ventricular rate; CAVB, complete atrioventricular block.

†p<0.01 compared with prepaing values.
‡p<0.05, ‡p<0.01 compared with control group.
§p<0.05 compared with prepaing values.

Results

Effects of Atrial Pacing in Control Group

The changes in the plasma ANP concentration and in hemodynamics are summarized in Table 1 and Figure 2. The atrial rate, which was equal to ventricular rate, was increased by atrial pacing from 146±20 beats/min at control resting level to 260±10 beats/min during pacing. Left atrial pressure and right atrial pressure were significantly elevated (from 0.2±0.1 to 8.6±2.8 mm Hg, p<0.01, and from 0.2±0.1 to 3.9±0.8 mm Hg, p<0.01, respectively) with a trivial fall in systolic arterial pressure. Along with the hemodynamic changes, the ANP level in the coronary sinus was significantly increased from 405±99 to 849±199 pg/ml (p<0.01) at 10 minutes after the start of pacing, maintained a high level during the pacing, and gradually returned to the control level during the recovery period. The ANP level in the aorta was also increased significantly from 143±35 to 290±59 pg/ml (p<0.05).

Effects of Atrial Pacing in CAVB Group

In the CAVB group, the atrium and ventricle contracted independently before the atrial pacing, as clearly indicated in Figure 3. P waves following the spikes during the pacing manifested atrial depolarization. Figure 3 also shows that arterial pressure and left atrial pressure were maintained constant throughout the experiment. The mean left and right atrial pressures were 8.4±2.2 and 3.4±1.2 mm Hg, respectively, higher than those in the control group (p<0.01), and the basal plasma ANP level was also significantly higher (p<0.05) (Table 1).

Figure 4 shows the time course of the plasma ANP concentration and hemodynamics during atrial pacing in the CAVB group. The atrial rate was increased about twofold, from 164±20 to 317±30 beats/min, while ventricular rate was unchanged with the mean rate of 52±5 beats/min throughout the experiment. Even under this condition, the ANP level in the coronary sinus was significantly increased from 730±82 to 1,137±35 pg/ml (p<0.01) at 10 minutes after the onset of pacing and maintained a high level during the pacing. The ANP level in the aorta tended to increase (208±54 to 294±73 pg/ml), but this increase was not statistically significant.

Effects of Atrial Plus Ventricular Pacing in CAVB Group

The ventricular pacing was performed for 60 minutes at the rate of 146±16 beats/min, and then the atrium was also paced independently at about double this rate (from 147±14 to 295±32 beats/min). Hemodynamic data of this protocol were essentially the same as those for atrial pacing alone, except that the basal atrial pressure was slightly lower. Under this condition the
ANP level in the coronary sinus was increased slightly but significantly from 480±172 to 626±223 pg/ml (p<0.05) at 10 minutes after the start of atrial pacing (Figure 5). The peak level of ANP concentration during atrial plus ventricular pacing was lower compared with the protocol of atrial pacing alone (626±223 pg/ml vs. 1,137±35 pg/ml, p<0.05).

Influence of Autonomic Blocking Agents

Figure 6 illustrates the effects of autonomic blocking agents on ANP secretion during rapid atrial pacing. Without autonomic blockades, the plasma ANP level was increased during rapid atrial pacing.
The present study demonstrates that atrial pacing per se stimulates ANP secretion without an elevation of atrial pressure in dogs with CAVB. Recently Schiebinger and Linden reported that an increase in the frequency of pacing from 2 to 4 Hz resulted in about a 46% rise in the ANP level over baseline in atrial strips from rats. Moreover, we have reported that the concentration of ANP significantly increased approximately one and a half times during atrial pacing with the mean rate of 130 beats/min without elevation of atrial pressure in certain patients who underwent heart surgery and had normal cardiac functions. These results are consistent with the present study showing that an increased frequency of heart rate itself can stimulate ANP secretion.

As several investigators have already reported, the plasma ANP level increased during atrial pacing in association with a significant elevation of atrial pressure. Our results from the protocol in dogs with normal sinus rhythm were fundamentally the same as those previously reported. In this condition, however, the ANP secretion induced by atrial pacing is not ascribed to the direct effect of an increase in heart rate or the increased frequency of depolarization, because rapid atrial pacing usually causes an elevation of atrial pressure that is one of the major factors for ANP secretion. The hemodynamic response to atrial pacing in normal hearts has already been examined by several groups. As heart rate increases, the A-wave (reflecting the phase of atrial contraction) is fused with the preceding V wave (reflecting the phase of late ventricular systole), such as that seen in paroxysmal supraventricular tachycardia. Therefore, atrial pressure is elevated during rapid atrial pacing in hearts with a normal sinus rhythm, because the atrial contraction occurs at the ventricular systolic phase when the A-V valve is closed. In contrast, in the CAVB heart, the frequency of A-wave increases during atrial pacing while that of V wave is constant, and A-waves and V waves arise independently. Therefore, atrial pressure is not elevated during rapid atrial pacing, because fusion of both waves hardly occurs. The basal atrial pressure in the CAVB group, however, is set to a higher level compared with the control group due to noncooperation between atrial and ventricular contraction. Goetz and Bond observed similar hemodynamic changes when they evaluated the role of atrial and sinoaortic receptors in dogs with CAVB.

In the present study, in dogs with CAVB the ventricular rate was about 50 beats/min and was much slower than the normal ventricular rate. To further prove that atrial pacing stimulates ANP secretion under the physiological range of ventricular rate without alteration of atrial pressure, we performed atrial pacing without an A-V sequential form after the ventricle was paced at the same rate as resting atrial rate. The slight drop in atrial pressure was shown as a result of ventricular pacing, because ventricular end-diastolic volume might have been reduced by an increase in heart rate in the CAVB heart with low rate. Even under this condition, the plasma ANP level increased without elevation of atrial pressure; however, the peak level of ANP concentration in dogs with CAVB during atrial plus ventricular pacing was lower than that during atrial pacing alone. This observation may be explained by the lower atrial pressure before addition of atrial

**Discussion**

The present study demonstrates that atrial pacing per se stimulates ANP secretion without an elevation of atrial pressure in dogs with CAVB. Recently Schiebinger and Linden reported that an increase in the frequency of pacing from 2 to 4 Hz resulted in about a 46% rise in the ANP level over baseline in atrial strips from rats. Moreover, we have reported that the concentration of ANP significantly increased approximately one and a half times during atrial pacing with the mean rate of 130 beats/min without elevation of atrial pressure in certain patients who underwent heart surgery and had normal cardiac functions. These results are consistent with the present study showing that an increased frequency of heart rate itself can stimulate ANP secretion.

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**Figure 5.** Time course of atrial natriuretic polypeptide (ANP) level and hemodynamics during atrial plus ventricular pacing without atrioventricular (A-V) sequential form in dogs with complete A-V block. Values are mean±SEM. CS, coronary sinus; A, aorta; AR, atrial rate; VR, ventricular rate; SAP, systolic aortic pressure; LAP, left atrial pressure; RAP, right atrial pressure. **p<0.05, **p<0.01 compared with prepacing values.**
pacing. Thus, the effect of atrial pacing on ANP secretion may depend on the level of atrial pressure.

The mechanism or mediating system of frequency-induced changes in ANP secretion is not yet known. A rise in output of the autonomic nerve as a result of increased pacing frequency might increase ANP release. We examined the effects of autonomic blocking agents on ANP release during rapid atrial pacing in this study. A bolus and drip infusion of a combination of α-adrenergic, β-adrenergic, and muscarinic antagonists prevented the increase in ANP secretion by rate-induced changes in our models, in which atrial rate was independent of atrial pressure, although we did not determine which antagonist contributed to the response. However, adrenergic antagonists are nearly always accompanied by changes of wall tension, which highly influence ANP secretion, even in our models. Therefore, it cannot be denied that the wall tension declined after infusion of autonomic blockades, so that ANP secretion was modified by lowered atrial pressure. Up to this time, there has been a controversy as to whether the autonomic nervous system is associated with ANP release for increasing pacing frequency, either in vivo or in vitro. Rankin et al.\textsuperscript{18} reported that the release of ANP with tachycardia by atrial pacing was not influenced by administration of various neurotransmitter blockers (β-adrenergic, muscarinic, or ganglionic) in anesthetized rabbits. Further, Schiebinger and Linden\textsuperscript{28} reported that α-adrenergic, β-adrenergic, and muscarinic antagonists did not block the rise in ANP secretion resulting from an increased pacing frequency in experiments of superfused rat atrial tissues. On the other hand, Schiebinger et al.\textsuperscript{27} also reported that activation of the sympathetic nervous system might enhance ANP secretion in isolated rat atria. This report is compatible with our observation in respect to the potential contribution of the autonomic nervous system to the mechanism of ANP release. Thus, it is possible that the endogenous neurotransmitters of the autonomic nervous system may influence ANP secretion for pacing-induced stimulation on the release mechanism in vivo. In addition, an increase in the frequency of the membrane depolarization of atrial cardiocytes may stimulate ANP secretion in itself. Recently we reported that Bay K 8644, a voltage-sensitive calcium channel agonist, stimulates ANP secretion from the isolated perfused rat heart.\textsuperscript{38} Since it is an acceptable theory that voltage-sensitive calcium channel agonists evoke hormone secretion in various hormone-producing cells,\textsuperscript{20–22} tachycardia-induced ANP secretion may also be explained, at least partly, by calcium mobilization via the voltage-dependent calcium channel. The mechanism of tachycardia-induced ANP secretion must await further examination.

In conclusion, the present study, in which atrial pressure is constant during rapid atrial pacing in dogs
with CAVB, demonstrates that tachycardia induced by atrial pacing per se stimulates ANP secretion from the heart and indicates that this response is mediated by the autonomic nervous system.

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**KEY WORDS** • atrial natriuretic polypeptide • complete atrioventricular block • tachycardia • atrial pacing
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