Human Atrial Natriuretic Peptide Suppresses Torsades de Pointes in Rabbits

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Background  The increase in inward current, primarily L-type Ca2+ current, facilitates torsades de pointes (TdP). Because human atrial natriuretic peptide (ANP) moderates the L-type Ca2+ current, in our study it was hypothesized that ANP counteracts TdP.

Methods and Results  We tested the effect of ANP, guanosine 3’,5’-cyclic monophosphate analogue (8-bromo cGMP) and hydralazine on the occurrence of TdP in a rabbit model. In control rabbits, administration of methoxamine and nifekalant almost invariably caused TdP (14/15). In contrast, ANP (100μg·kg⁻¹·min⁻¹) markedly abolished TdP (2/15), whereas hydralazine failed to show a comparable anti-arrhythmic action (10/15). TdP occurred only in 1 of 15 rabbits treated with 8-bromo cGMP. Presence of early afterdepolarization-like hump in the ventricular monophasic action potential was associated with the occurrence of TdP.

Conclusion  Results suggest that ANP affects TdP in the rabbit model, and that this anti-arrhythmic effect of ANP is not necessarily shared by other vasodilating agents. (*Circ J 2008; 72: 820–824)

Key Words:  Aferdopolarization; Human atrial natriuretic peptide; Repolarization; Torsades de pointes

The decrease in outward current and the increase in inward current facilitate the genesis of afterdepolarization related with torsades de pointes (TdP). Some pharmacological interventions, such as a calmodulin antagonist, protein kinase A inhibitor, or potassium channel opener, have been shown to decrease afterdepolarizations. However, clinical application of these agents requires further assessment of their biological safety at the therapeutic dose. Synthetic human atrial natriuretic peptide (ANP), however, is currently available for the treatment of various forms of cardiac failure. Because ANP modifies the L-type Ca²⁺ channel through the enhanced intracellular production of guanosine 3’,5’-cyclic monophosphate (cGMP), it is conceivable that ANP has an inhibitory effect on TdP. In an attempt to search for a therapeutic means against TdP, we tested the effect of ANP on the occurrence of TdP in a rabbit model.

Methods  

Animal Preparation  Eighty-five male Japanese white rabbits (2.5 to 3.5 kg) were anesthetized with intravenous ketamine (35 mg/kg) and xylazine (5 mg/kg). Additional doses (ketamine 15 mg/kg, xylazine 1 mg/kg) were given if necessary to maintain appropriate anesthesia. Anesthetic and surgical procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 85-23, revised 1996). Rabbits were ventilated with room air through an artificial respirator via a tracheal cannula. Arterial blood pressure was monitored using a left femoral artery cannula attached to a Statham pressure transducer (Amplifier AP621G, Nihon Kohden, Tokyo, Japan). Body temperature was maintained at about 37°C with electrical blanket. Arterial blood gases and electrolytes were periodically determined, and pH within physiological range. Two surface electrocardiograms (ECG) were continuously monitored; they were stored in a personal computer together with arterial blood gases and pH for subsequent analysis (PowerLab 8-channel System, Tokyo, Japan).

According to an in vivo animal model of TdP established by Carlson et al, we administered nifekalant chloride concurrently with methoxamine, an alpha-stimulant. Alpha adrenoreceptor stimulation is known to increase L-type Ca²⁺ current and decrease transient outward current. Arrhythmogenicity of the present animal model might be partly attributable to these modifications of ionic channels by methoxamine. Nifekalant has been reported to block the rapid component of delayed rectifier ( IKr ), the inward rectifier ( IK1 ), and the transient outward ( Ito ) K⁺ channels. We used nifekalant because this is the only class III agent readily obtainable for intravenous administration in Japan. The current study was carried out in 3 stages.

Experimental Protocol  

Study 1. Treatment With ANP, Hydralazine or Saline (n=45)  The protocol of this part is schematically shown...
in Fig 1. After a 10-min period of stabilization, methoxamine was intravenously administered at a rate of 70 nmol·kg⁻¹·min⁻¹. Ten min infusion of methoxamine was followed by the administration of nifekalant chloride (0.2 mg·kg⁻¹·min⁻¹). Then, both agents were continuously given until TdP induction or for 20 min. Fifteen rabbits were treated with a synthetic human ANP (Carpertide; Sunthry, Gunma, Japan) dissolved in saline solution (10 µg·kg⁻¹·min⁻¹, ANP group). To investigate whether any ANP-related changes in the incidence of TdP were due to its unique effect on ventricular electrophysiologic properties or were caused simply by vasorelaxation, 15 rabbits were treated with hydralazine (2 mg·kg⁻¹·min⁻¹ dissolved in 0.9% saline; Sigma, St Louis, MO, USA: hydralazine group). The remaining 15 rabbits were given saline solution at a rate of 0.5 ml/min (control group). Treatment by ANP, hydralazine, or saline started 5 min later than that of methoxamine, ie, 5 min before the administration of nifekalant.

**Study 2. Treatment With Analogue of Cyclic GMP, 8-Bromo cGMP (n=15)** To know whether the effect of ANP on TdP was mediated by cGMP, the effect of a membrane-permeable analog of cGMP (8-bromo cGMP) on the occurrence of TdP was investigated. Most of the procedures were the same as in Study 1; however, instead of ANP or hydralazine, 8-bromo cGMP was intravenously given at a rate of 70 ng·kg⁻¹·min⁻¹.

**Study 3. Recording of Monophonic Action Potential (n=25)** This part was designed to confirm the association of TdP and early afterdepolarization (EAD) in the present animal model. The heart was exposed by a midsternal incision, and was suspended in a pericardial cradle. The monophasic action potential (MAP) of the left ventricular epicardial surface was recorded using a contact electrode with an interelectrode distance of 3 mm. MAP signals were amplified with frequency range of 0.1 to 10 kHz (AB-601G; Nihon Kohden, Tokyo, Japan). MAPs were accepted when the amplitude was stable and over 10 mV. The duration of MAP at 90% repolarization (MAP90) was measured. In 25 open-chest rabbits, MAP recordings were performed in the baseline state and during the treatment with saline (n=12), ANP (n=9), or 8-bromo cGMP (n=4) at the same dose as in Study 1.

**Definitions and Measurements**
Tdp was defined as the episode of 6 or more consecutive ventricular beats with polymorphic QRS configuration. We included episodes of polymorphous ventricular tachycardias lacking complete twisting QRS morphology into Tdp because of the limited number of ECG leads. Heart rates and QT intervals were obtained from the measurements of 3 consecutive RR intervals or those of 3 beats in either lead with more prominent T waves, respectively. The QT interval was corrected according to the Bazett formula. The measurements of variables in the baseline state were made before the administration of methoxamine. Measurements during the treatment with each agent were performed after 5 min from the onset of administration of nifekalant. After this period, frequent premature ventricular contractions or Tdp interfered with the measurements of electrocardiographic variables in control rabbits.

**Statistics**
Results were expressed as mean±SD. Significance of temporal changes in the parameters was tested by paired t-test. Comparison of relative changes in the variables from the baseline state among the 4 groups in Study 1 and Study 2 was made by 1-way analysis of variance. In the presence of significant F values, further comparison between pairs of groups was done by the Bonferroni method. The incidence of Tdp during the treatment was compared with that of the saline group using Fisher’s exact test. Probability values less than 0.05 were considered to indicate significance.

**Results**

**Study 1**

Incidences of Tdp in Study 1 and Study 2 are collective-
ly shown in Fig 2. In control rabbits, concomitant administration of methoxamine and nifekalant almost invariably caused TdP (14/15). The time to TdP from the onset of the administration of nifekalant ranged from 80 s to 770 s (589±214 s). Electrophysiologic variables in Study 1 and Study 2 are listed in Table 1. Prolongation of the QT interval and bradycardia preceded the appearance of TdP.

TdP occurred only in 2 of 15 rabbits in the presence of ANP (p<0.01 vs control), whereas hydralazine did not markedly suppress TdP (incidence of TdP: 10/15). There were significant inter-group differences in the changes in heart rate, systolic blood pressure, or QT interval. In control rabbits, treatment with methoxamine and nifekalant reduced the heart rate from 207±37 /min to 99±16 /min (p<0.01). Both ANP and hydralazine attenuated the reduction of the heart rate. Increase in systolic blood pressure in these groups was smaller than that in control rabbits. Prolongation of QT interval was minimized either by ANP or hydralazine.

Study 2
The effect of 8-bromo cGMP on TdP and QT interval were similar to those of ANP (Fig 2). TdP occurred only in 1 of 15 rabbits (p<0.01 vs control in Study 1). Prolongation of QT interval was relatively small (from 296±37 ms to 417±62 ms, p<0.01).

Study 3
Demonstrable recordings of ECG and MAP in rabbits from the saline group and ANP group are shown in Fig 3.

![Fig 3. Demonstrable electrocardiograms (ECG) and monophasic action potential (MAP) in Study 3. Panel A indicates baseline state. Early afterdepolarization-like hump, as indicated by arrows, was observed during administration of methoxamine and nifekalant in control rabbits (Panel B).](image)

![Fig 4. Electrocardiograms (ECG) and monophasic action potential (MAP) during the treatment with atrial natriuretic peptide (ANP). When ANP was given, prolongation of MAP90 was attenuated. In some rabbits, torsades de pointes (TdP) did not appear despite the presence of early afterdepolarization (EAD)-like hump (Panel A). Neither TdP nor EAD-like-hump was seen in other rabbits (Panel B).](image)

### Table 2 QTc, MAP90, and the Incidence of EAD-Like Hump and TdP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>QTc, ms</th>
<th>MAP90, ms</th>
<th>Hump</th>
<th>TdP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n=12)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>324±45</td>
<td>259±47 (216±41 beats/min)</td>
<td>100% (12/12)</td>
<td>92% (11/12)</td>
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<tr>
<td>Treatment</td>
<td>496±88</td>
<td>444±88 (102±21 beats/min)</td>
<td>90% (11/12)</td>
<td>84% (10/12)</td>
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<td>ANP (n=9)</td>
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</tr>
<tr>
<td>Baseline</td>
<td>301±44</td>
<td>255±40 (211±34 beats/min)</td>
<td>44% (4/9)</td>
<td>22% (2/9)</td>
</tr>
<tr>
<td>Treatment</td>
<td>412±70**</td>
<td>380±71 (115±38 beats/min)</td>
<td>40% (4/10)</td>
<td>17% (2/12)</td>
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<tr>
<td>8-Br cGMP (n=4)</td>
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</tr>
<tr>
<td>Baseline</td>
<td>322±91</td>
<td>271±30 (192±39 beats/min)</td>
<td>0% (0/4)</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>387±30*</td>
<td>353±41 (118±31 beats/min)</td>
<td>0% (0/4)</td>
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</table>

*Abbreviations see in Table 1.
*p<0.05 vs saline, **p<0.01 vs saline.
and Fig.4. Electrophysiologic variables and the incidence of EAD-like hump or TdP are shown in Table 2. EAD-like hump was found in all 12 rabbits treated with saline, and TdP occurred in all of them. When ANP or 8-bromo cGMP was added to nifekalant and methoxamine, EAD-like hump during phase 3 depolarization was detected only in 4 of 9 rabbits with ANP and in 2 of 4 rabbits with 8-bromo cGMP. In accordance with the scarcity of EAD-like hump, TdP appeared in 15% of rabbits during the treatment with ANP or 8-bromo cGMP (2/9 and 0/4).

Discussion

Observations of the Present Study

Major findings of the present study were that: (1) ANP eliminated TdP, whereas hydralazine failed to show a significant effect; (2) 8-bromo cGMP prevented TdP to the comparable degree to that of ANP; and (3) appearance of TdP was closely associated with the advent of possible EAD during the phase 3 repolarization.

Studies of the ionic mechanisms of EADs have shown that either L-type Ca2+ current, persisting Na+ current, or Na+/Ca2+ exchange current is responsible for Ca2+ loading. Diverse ionic mechanisms, such as decreased K+ currents or lingering non-inactivating Ca2+ or Na+ currents, might cause EAD. Also, some studies using selective inhibitors have appreciated the molecular background for EAD, such as involvement of calmodulin and calmodulin kinase. In the present study, we found that ANP inhibits TdP in an established rabbit model.

ANP is a potent intrinsic vasodilator, diuretic, and natriuretic. ANP also has multimodal effects on cardiac electrophysiology, including potentiation of the acetylcholine-stimulated K+ current and attenuation of the catecholamine-induced L-type Ca2+ current. Some investigations indicated that inhibition of the L-type Ca2+ current is via a cGMP-dependent mechanism in isolated rabbit ventricular cells. In human atrial cells, inhibition of the calcium-independent transient outward K+ current and delayed rectifier K+ current have been shown. The effect of ANP or cGMP on arrhythmias is explained by various aspects such as modification of above-mentioned ionic channels and Na+/Ca2+ exchanger. Furthermore, some recent experimental studies have indicated the favorable effect of natriuretic peptide on the regulation of cardiac ryanodine receptor and sarcoplasmic reticulum Ca2+ ATPase expression. Accordingly, it is conceivable that ANP and cGMP attenuate the arrhythmogenicity in failing heart characterized by the impairment of the function of sarcoplasmic reticulum.

The effect of ANP on arrhythmia was conflicting depending on the model. In a study using canine ischemic hearts, ANP decreased the incidence of ventricular extrasystoles and ventricular fibrillation. However, 2 other reports failed to detect any favorable effect on ventricular arrhythmias in either during coronary occlusion or during reperfusion. In a study by Osie et al, ANP suppressed cesium (non-specific potassium channel blocker) induced ventricular tachyarrhythmias in rabbits. They have suggested that antiarrhythmic action is attributable to the reduction of pressure overload by ANP. The degrees of hemodynamic alterations caused by ANP or hydralazine were substantially the same in our study. However, in our previous study ANP showed an appreciable influence on the canine ventricular electrophysiologic properties, whereas hydralazine did not, despite the similar hemodynamic changes. Based on the observations in the present study, together with the earlier findings, we consider that electrophysiologic effect of ANP is mediated by cGMP-mediated pathway but is not explained solely by its vasodilating effect.

Implications and Limitations

In patients treated with ANP, many factors such as modifications of ionic currents might affect electrophysiologic property of myocardium and its response to ANP. Thus, extrapolation of the results obtained in intact animal hearts to diseased human hearts is limited. Nevertheless, because ANP is an intrinsic humoral agent without pronounced adverse actions, ANP might be considered as a possible choice for the treatment of clinical TdP.

An earlier study has reported that ANP can prevent the shortening of the effective refractory period and MAP duration in the rapid atrial stimulation animal model. Thus, atrial arrhythmia as well as EAD-related ventricular tachyarrrhythmia might be treated by ANP. Finally, because of the inter-species discrepancies of experimental observations and the scarcity of data for the whole heart clinical usefulness of ANP on TdP requires further confirmation.

Conclusion

The results suggested that ANP has an inhibitory effect on TdP, and that this anti-arrhythmic effect of ANP is not necessarily shared by other vasodilating agents. Anti-arrhythmic action of ANP may be, if not solely, via inhibition of the cGMP-mediated pathway.

References


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