Cibenzoline Attenuates Upregulation of Kv1.5 Channel Gene Expression by Experimental Paroxysmal Atrial Fibrillation

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SUMMARY

Antiarrhythmic drugs exert their effects by inhibiting the ion channels of cardiomyocytes. However, these effects could also modify the ionic environment around them, and thereby affect the expression of ion channels, leading to biochemical enhancement or attenuation of the antiarrhythmic effects. To test this hypothesis, the physiological and biochemical effects of cibenzoline were evaluated in a rapid atrial pacing model in rats. In rats with rapid atrial pacing, pretreatment with cibenzoline significantly inhibited the increases in Kv1.5 mRNA at 2 hours and immunoreactive protein at 4 hours by 35 ± 15% and 30 ± 10%, respectively. These effects were observed only in the rapid atrial pacing group, not in the sham-operated group. With cibenzoline pretreatment, 4-hour rapid atrial pacing resulted in significant prolongation of the atrial refractory period compared to the untreated group even after removal of cibenzoline. In contrast, the sham and rapid atrial pacing model with and without cibenzoline pretreatment showed similar acute physiological responses to cibenzoline.

In conclusion, in addition to the acute physiological effects, pretreatment with cibenzoline exerted pleiotropic effects of inhibition of Kv1.5 channel upregulation by rapid pacing, implying differences in the cibenzoline effects when administered before and after onset of paroxysmal atrial fibrillation. (Int Heart J 2005; 46: 279-288)

Key words: Atrial fibrillation, Cibenzoline, Kv1.5

ATRIAL fibrillation (AF) represents an important medical problem, not only because of increased incidence in the elderly population, but also as a major cause of embolic stroke. AF often begins as a paroxysmal form, gradually increasing in frequency and duration, and in many cases eventually becoming chronic. Numerous antiarrhythmic drugs have been developed and used to treat the arrhythmia, but clinical efficacy is often less than satisfactory. These drugs aim to
prevent and cure AF by directly inhibiting ion channels present in atrial myocardial cells.

Recent interest has focused on the remodeling by the arrhythmia. This refers to the effects of AF itself on modifying the gene expression of myocardial cells. These biochemical changes promote the sustaining of AF and decrease the efficacy of antiarrhythmic drugs, limiting the clinical utility of antiarrhythmic therapy and also providing researchers with a challenge to solve these problems. The time course of these biochemical changes is often more rapid than expected. Actually, we have previously reported that rapid atrial pacing in rats increased Kv1.5 channel gene expression within a few hours, and shortened action potentials on the biochemical basis in the atrial muscle. Taking this into account, we can hypothesize that for a drug to prove effective for treating paroxysmal AF, it should not only directly and physiologically inhibit ion channels, but also exert biochemical effects preventing the early remodeling that occurs in AF. These assumptions would be supported by the recent studies that showed amiodarone exhibits both of these effects.

The physiological effects of antiarrhythmic agents on ion channels in essence leads to changes in the ionic environment inside myocardial cells. These changes in the ionic environment might indirectly modify the ion channel gene expression in myocardial cells. Thus, also for antiarrhythmic drugs other than amiodarone, such biochemical effects, if any, are also likely to enhance or attenuate their antiarrhythmic effects. Cibenzoline, a multichannel blocker, is one of the drugs that have been shown to be highly effective for treatment of paroxysmal AF. In addition to its physiological effects, cibenzoline might also display some biochemical effects leading to the clinical efficacy. To test this hypothesis, we investigated the effects of cibenzoline on the upregulation of Kv1.5 channel gene expression by paroxysmal AF.

**METHODS**

**Preparation of tachycardia models:** Sprague-Dawley rats (12 weeks-old) were used in the present study. Rats were anesthetized using pentobarbital (50 mg/kg i.p.) and ventilated using a volume-cycled respirator. A quadripolar electrode catheter (1.5 Fr) was introduced through the cervical vein for pacing and recording. Surface ECG lead II and atrial electrogram were recorded. Stimulation was performed with 2-ms rectangular pulses using a digital programmable stimulator (SEN 7203, Nihon Kohden, Tokyo) and a constant current source (SS401J, Nihon Kohden). Stimulation frequency was set at 1,000 beats/min. Sham-operated animals underwent an identical procedure without stimulation. Hearts were removed after predetermined durations of stimulation (2 hours for mRNA assay; 4 hours...
for protein assay and electrophysiological studies). In the cibenzoline pretreatment group, the drug (5 mg/kg) was administered intraperitoneally before rapid atrial pacing and then every 1 hour after the onset of pacing.

**RNA preparation and RNase protection assay:** Right and left atrial appendages were excised and quickly frozen in liquid nitrogen. Total RNA was extracted using the acid guanidinium isothiocyanate method. Levels of the voltage-dependent K⁺ channel mRNA were assayed using the RNase protection assay with an RPA III kit (Ambion, Austin, TX, USA). Probes were prepared as described previously. Hybridization was performed using 10 µg RNA, followed by RNase digestion, and the protected RNAs were transferred to a nylon membrane after running on a denaturing gel. Membranes were incubated with anti-digoxigenin antibody conjugated to alkaline-phosphatase, and protected fragments were subsequently detected using a CDP star (Tropix). Chemiluminescent signals were quantified using a lumino-image analyzer (LightCaptureAE-6960, ATTO, Tokyo). Cyclophilin signals were used as internal controls.

**Western blot analysis:** Membrane fractions of atrial myocardium were prepared as described by Barry, *et al.* Membrane proteins (30 µg) were fractionated on SDS-PAGE gels and transferred to PVDF membranes. The membrane was incubated with rabbit polyclonal anti-Kv1.5 antibody (Alamone Laboratories, CA, USA) and then with goat anti-rabbit immunoglobulin G conjugated to alkaline-phosphatase.

**Electrophysiological study:** To examine the functional aspects of modified gene expression, effective refractory periods were determined in isolated perfused hearts. After 4-h rapid pacing, the hearts were rapidly excised and retrogradely perfused using Tyrode’s solution (NaCl, 136.5 mM; KCl, 5.4 mM; HEPES, 5.5 mM; Na₂HPO₄, 0.33 mM; glucose, 5.5 mM; CaCl₂, 1.8 mM; MgCl₂, 0.53 mM; pH 7.4). After recovery for 30 minutes at sinus rhythm, the effective refractory periods were determined by introducing extrastimuli from the cathode on the left atrial appendage at basic cycle lengths of 200 ms with an output of 10 diastolic thresholds. The anode was placed on the aorta. Effective refractory period was defined as the longest coupling interval that did not capture the atrium.

**Statistical analysis:** To examine mRNA and protein levels for the Kv1.5 channel gene, values from sham-operated animals without cibenzoline were arbitrarily set as 1 unit for quantitative comparisons. Mean levels of mRNA and protein and effective refractory periods were compared using an analysis of variance, and multiple comparisons were made using Bonferroni’s modified *t*-test. Values of *P* < 0.05 were considered statistically significant. Data are expressed as the mean ± standard deviation.
RESULTS

Rapid atrial pacing model: The heart rate and right atrial pressure in the sham and rapid atrial pacing groups, with or without administration of cibenzoline, are shown in the Table. In the sham group, cibenzoline exerted no significant effect on the heart rate or right atrial pressure. However, in the rapid atrial pacing group, cibenzoline significantly increased both the heart rate and right atrial pressure.

Effect of cibenzoline on Kv1.5 mRNA: In the rapid atrial pacing model in rats used in the present study, Kv1.5 mRNA increased transiently with a peak after 2 hours of pacing as reported in our previous study. We evaluated whether pretreatment with cibenzoline would have any effects on the increase in Kv1.5 mRNA. In the sham group, cibenzoline had no significant effect on Kv1.5 mRNA. Compared to the sham group, Kv1.5 mRNA increased by a mean of 135 ± 18% after 2 hours of rapid atrial pacing (Figure 1). However, in the group pretreated with cibenzoline, this increase was attenuated to only 88 ± 24% (Figure 1). Cibenzoline thus significantly inhibited the increase in Kv1.5 mRNA induced by rapid atrial pacing by 35 ± 15%.

Table. Ventricular Rate and Right Atrial Pressure in a Rapid Pacing Model

<table>
<thead>
<tr>
<th>Cibenzoline pretreatment</th>
<th>Sham (-)</th>
<th>Rapid pacing (-)</th>
<th>Sham (+)</th>
<th>Rapid pacing (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular rate (beats/min)</td>
<td>380 ± 25</td>
<td>458 ± 52</td>
<td>372 ± 35</td>
<td>562 ± 45*</td>
</tr>
<tr>
<td>Right atrial pressure (mmHg)</td>
<td>3 ± 1</td>
<td>7 ± 1</td>
<td>3 ± 1</td>
<td>9 ± 1*</td>
</tr>
</tbody>
</table>

Values represent mean ± SD (n = 10 in each group).

*P < 0.05 versus cibenzoline (-) with rapid pacing.

Figure 1. Effects of cibenzoline pretreatment on the increase in Kv1.5 mRNA induced by 2-hour rapid atrial pacing. A: A representative RNase protection assay of Kv1.5 mRNA. Rapid atrial pacing dramatically increased Kv1.5 mRNA. The effect was attenuated by cibenzoline administration before and during rapid atrial pacing. B: % increase in Kv1.5 mRNA by rapid atrial pacing with and without cibenzoline pretreatment as compared with sham-operated group without cibenzoline. Bars and lines represent mean and standard deviation of % increase, respectively. The increase was significantly attenuated by cibenzoline (n = 5 in each, P < 0.05).
Effect of cibenzoline on Kv1.5 immunoreactive protein expression: In the rapid atrial pacing model, Kv1.5 protein was detected as a membrane protein with a peak 4 hours after the start of pacing. We then evaluated whether the attenuation of increases in Kv1.5 mRNA by cibenzoline actually exerted any effect on the expression of Kv1.5 immunoreactive protein. In the sham group, pretreatment with cibenzoline had no effect on Kv1.5 protein. However, compared to the sham group, Kv1.5 protein increased by a mean of 57 ± 10% in the rapid atrial pacing group (Figure 2). With cibenzoline pretreatment, this increase was attenuated to only 40 ± 9% (Figure 2). Cibenzoline thus significantly inhibited the pacing-induced upregulation of Kv1.5 protein by 30 ± 10%.

Effect of cibenzoline on atrial electrophysiology: To evaluate the electrophysiological characteristics associated with the transient increase in Kv1.5 protein at 4 hours, we measured the atrial refractory period in isolated perfused hearts (without cibenzoline) to eliminate the influence of various extrinsic factors occurring in vivo. The atrial refractory period was 39 ± 3 ms in the sham group, which was almost identical to that in the sham group with cibenzoline pretreatment. In contrast, it was significantly shorter (30 ± 3 ms) in the rapid atrial pacing group. However, in the rapid atrial pacing group pretreated with cibenzoline, the atrial refractory period was 35 ± 4 ms despite the absence of cibenzoline in the perfusate, significantly longer than in the rapid atrial pacing group without the pretreatment. This finding indicated that cibenzoline biochemically attenuated the shortening of the refractory period induced by rapid atrial pacing (Figure 3A).

![Figure 2](image-url)
When cibenzoline was added to the perfusate to evaluate the acute effects in each model, prolongation of the atrial refractory period was similar between the groups, with no difference in the acute effects of the drug (Figure 3B). In rapidly paced hearts, the atrial refractory periods at cibenzoline perfusate concentrations of 1 \( \mu M \) and 3 \( \mu M \) were 32 \( \pm \) 3 ms and 39 \( \pm \) 3 ms, respectively. With cibenzoline pretreatment, the values were 38 \( \pm \) 4 ms and 42 \( \pm \) 3 ms, respectively, which were also significantly prolonged compared to rapid pacing without cibenzoline pretreatment. Although the conduction times between the left and right atria increased in a concentration-dependent manner, the degree of prolongation was affected neither by rapid atrial pacing nor by the pretreatment of cibenzoline.

**DISCUSSION**

The major finding of the present study was that cibenzoline pre-treatment inhibits the increase in Kv1.5 gene expression on rapid atrial pacing, resulting in the prolongation of the intrinsic atrial refractory period. As the acute effects of cibenzoline on the atrial refractory period remained unaffected by 4-h rapid pacing and cibenzoline pretreatment, the drug thus affected the atrial electrophysiological characteristics via both physiological (direct action) and biochemical mechanisms (pleiotropic action).
Most antiarrhythmic drugs exert their effects by direct actions on ion channels and pumps in myocardial cells with subsequent changes in the electrophysiological characteristics of the myocardium. Many previous studies have thus focused on the binding of antiarrhythmics to ion channels and the mechanisms of their inhibition from physiological perspectives. However, our understanding of “remodeling”, which involves dynamic changes in the number of ion channels and pumps in diseases, now suggests that physiological models alone are insufficient to fully elucidate the mechanisms of the drug effects in the management of arrhythmia. Two problems would exist when trying to clarify the effects of antiarrhythmic drugs from a remodeling perspective: 1) To what extent our knowledge of the effects of antiarrhythmic drugs in normal myocardial cells can be applied to diseased myocardial cells with different numbers of expressed ion channels and 2) How antiarrhythmic drugs themselves affect the process of remodeling. Assuming on this basis, an understanding of the mechanisms of action from both physiological and biochemical perspectives is necessary to interpret the clinical efficacy of antiarrhythmic drugs.

The present study has investigated the effects of cibenzoline from both of these perspectives. Many studies have shown that cibenzoline directly inhibits several ion channels, and thus belongs to Class IA drugs in the Vaughan-Williams classification of antiarrhythmic drugs. Cibenzoline exerts its effects by primarily acting as a slow kinetic drug on Na channels, but it also inhibits L-type Ca channels, delayed rectifier K channels, transient outward currents, and acetylcholine-sensitive K channels. The electrophysiological net effects are decreased maximum upstroke velocity of action potentials and increased action potential duration and refractory period in the atrial muscle. However, the present study is the first to demonstrate that cibenzoline also had effects on biochemical remodeling by rapid pacing. Cibenzoline inhibits upregulation of the Kv1.5 channel induced by rapid atrial pacing, thereby resulting in a biochemical effect on prolongation of the atrial refractory period. The effect should be noted to be an intrinsic prolongation observed even after removal of cibenzoline. In addition to these novel biochemical effects of cibenzoline, the drug showed acute physiological effects of its own on the atrium, which were not altered by rapid pacing and pretreatment of the drug. Because the two biochemical and physiological effects of cibenzoline could be coexistent, beginning treatment with cibenzoline during sinus rhythm rather than after the development of tachycardia may become advantageous in clinical situations.

Recently, a report has appeared on reevaluation of the antiarrhythmic drug effects from this biochemical perspective. Shinagawa, et al first reported on the biochemical effects of amiodarone on the remodeling of atrial myocytes. In their experimental model, atrial pacing was performed for longer than just the few
hours used in our study, and the ion channel studied was the L-type Ca channel. Pretreatment with amiodarone prevented downregulation of the L-type Ca channel induced by rapid atrial pacing. This resulted in amiodarone exerting a greater effect on prolongation of the refractory period than its physiological effects alone. Because the process of remodeling in atrial cells differs depending on the duration of rapid pacing, our study targeted a transient increase in Kv1.5 channels that occurred within a short time. However, the perspective for evaluating the antiarrhythmic effects was the same as their report.5) Our findings also support the pleiotropic effects of antiarrhythmic drugs, although the effects might differ depending on the duration of the tachycardia and the kind of ion channels. At present, however, research in this area has been lacking, except for amiodarone and cibenzoline in our study.

The mechanisms by which cibenzoline inhibits upregulation of Kv1.5 channels are of great interest, but remain unclear. The same is true for the effects of amiodarone on the prevention of remodeling in L-type Ca channels. This is because the mechanisms of remodeling in atrial myocytes are still themselves not well understood. However, the transcriptional control of Kv1.5 channels has been shown to be closely related to intracellular Ca\(^{2+}\) and mediated by CREB phosphorylation.25) The potent inhibition of Na\(^+\) channels by cibenzoline would decrease intracellular Ca\(^{2+}\) through the Na\(^+\)/Ca\(^{2+}\) exchanger. Moreover, cibenzoline also blocks the L-type Ca channel,14-16) further decreasing intracellular Ca\(^{2+}\) levels. These combined effects would act to attenuate increases in intracellular Ca\(^{2+}\) during rapid atrial pacing, possibly leading to the attenuation of Kv1.5 transcription. During normal sinus rhythm, the decrease in the intracellular Ca\(^{2+}\) would not be so remarkable because of the rate-dependent effects of the drug, and therefore could not affect Kv1.5 gene expression. In contrast, it was difficult to attribute the pleiotropic effects of cibenzoline to the indirect hemodynamic effects of cibenzoline because cibenzoline increased the heart rate and right atrial pressure and caused hemodynamic deterioration.

The present study has several limitations. First, the study was performed in rats, and the data may not be directly extrapolated to humans. In particular, the mechanisms of transcriptional regulation of Kv1.5 channels in humans have not yet been elucidated. Second, the present study focused primarily on Kv1.5 channels, which showed the greatest changes in our previous study,4) but remodeling of other channels may also take place. Thus, the effects of cibenzoline on the formation of other ion channels remain unknown. Third, our study data are based on rapid atrial pacing that lasted for only a few hours. Tachycardia for longer periods of time causes loss of upregulation in Kv1.5 channels and more predominant downregulation of L-type Ca\(^{2+}\) channels. In this case, the inhibition of Kv1.5 channel gene expression by cibenzoline may no longer have any effects on the
atrial refractory period. Despite these limitations, however, our study contributes to a better understanding of the effects of cibenzoline in paroxysmal AF. The results suggest that treatment to prevent rather than abolish paroxysmal AF by cibenzoline may enhance the effect of prolonging the atrial refractory period via modification of atrial remodeling processes. However, consideration should also be given to the potential adverse effects on its hemodynamic function.

**Conclusion:** In conclusion, the results of the present in vivo study suggest that the multichannel blocker cibenzoline inhibited the increase in Kv1.5 gene expression induced by rapid atrial pacing in rats, exerting a biochemically-based prolongation of the atrial refractory period. Antiarrhythmic drugs that interfere with the remodeling process itself may have a beneficial effect on the physiological actions of the drugs.

**References**


