ELECTROPHYSIOLOGICAL EFFECTS OF NICARDIPINE HYDROCHLORIDE ON THE ISOLATED SINOATRIAL AND ATRIOVENTRICULAR NODES OF THE RABBIT

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The electrophysiological effects of nicardipine-HCl, a new calcium antagonist and potent vasodilator, were studied in the isolated sinoatrial (SA) and atrioventricular (AV) nodes of the rabbit in an oxygenated Tyrode solution at 35°C using an intracellular microelectrode technique. Nicardipine-HCl decreased the spontaneous rate of the SA node and prolonged sinus recovery time, dose-dependently. The effective refractory period (ERP) and functional refractory period (FRP) of the AV node and AV conduction time (A-H interval) were also prolonged by nicardipine-HCl in a dose-dependent manner. In conclusion, nicardipine-HCl has electrophysiological effects on the SA and the AV node similar to those of other calcium antagonists in the excised and superfused rabbit heart.

NICARDIPINE hydrochloride (YC-93), a new derivative of 1, 4-dihydropyridine, 2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylic acid 3-[2-(N-benzyl-N-methylamino)]-ethyl ester 5-methyl ester hydrochloride, is a calcium antagonist and a potent vasodilator.3–6

The electrophysiological effects of 3 other calcium antagonist, diltiazem, verapamil and nifedipine, on the isolated sinoatrial (SA) and atrioventricular (AV) nodes of the rabbit have been reported previously.7–12 However, few studies have described the electrophysiological effects of nicardipine-HCl on isolated SA and AV nodes.13

This paper describes the effects of nicardipine-HCl on the isolated SA and AV nodes of the rabbit.

METHODS

Under pentobarbital anesthesia (30 mg/kg, i.v.) and artificial respiration, the rabbit atrium was excised and cut into 2 portions: preparation I, the sinoatrial node with the crista terminalis; preparation II, the right atrium, the AV node and the His bundle. The tissues were superfused in a Tyrode solution equilibrated with 95% O₂ and 5% CO₂. The temperature was maintained at 35.0 ± 0.5°C.

Using the conventional microelectrode technique, spontaneous cycle length (SCL) and sinus recovery time (SRT) were measured in isolated SA nodal preparation I and effective and functional refractory periods (ERP and FRP) and atrioventricular conduction time (A-H interval) in isolated AV nodal preparation II.

Key Words:
Nicardipine
SA node automaticity
ERP of AV node
Calcium antagonist

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### Table I Effects of Nicardipine-HCl in Different Concentrations on the Sinoatrial Node in the Rabbit

<table>
<thead>
<tr>
<th>Concentration (g/ml)</th>
<th>Spontaneous cycle length</th>
<th>Overdrive stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average sinus slowing (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>1 x 10^{-7}</td>
<td>6.4 ± 3.9 **</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.001 ns</td>
<td></td>
</tr>
<tr>
<td>5 x 10^{-7}</td>
<td>20.4 ± 10.1 **</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.001 **</td>
<td></td>
</tr>
<tr>
<td>1 x 10^{-6}</td>
<td>78.5 ± 36.8 §§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.05 §§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td></td>
<td>Control (mean ± SD)</td>
<td>After (mean ± SD)</td>
</tr>
<tr>
<td>120.0 ± 10.0</td>
<td>129.5 ± 9.5 **</td>
<td></td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>119.6 ± 5.5</td>
<td>248.4 ± 95.2 *</td>
<td></td>
</tr>
<tr>
<td>p &lt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant level in analysis of variance: *p < 0.05, **p < 0.01, ns = not significant. Complete arrest in: ¶ = 5 experiments, § = 2 experiments.

Abbreviations: %SRT = sinus node recovery time expressed as percentage of spontaneous cycle length, n = number of experiments

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**Figure 1.** Effects of nicardipine-HCl at 10^{-7}, 5 x 10^{-7} and 10^{-6} g/ml on the spontaneous rate of sinoatrial (SA) node in the rabbit (expressed as percent of spontaneous rate before nicardipine-HCl). Fifteen minutes after the addition of nicardipine-HCl, the frequency of spontaneous firing decreased in a dose-dependent manner. The thick lines denote mean values. At concentration of 10^{-6} of nicardipine-HCl, the dotted line denotes mean values obtained from all cases including those which lost their automaticity and of which the spontaneous rate is assumed to be zero% of the control values.

Transmembrane resting and action potentials were recorded with glass microelectrodes with resistances of 10–20 MΩ from the cells of the SA node in preparation I and from those of the His bundle in preparation II. The electrode was connected to a high input impedance preamplifier (WPI model 750) by a silver-silver chloride wire.

For the surface recordings from the crista terminalis in preparation I and the right atrium in preparation II, a close bipolar surface lead constructed with a pair of silver wire electrodes was used with a 1-mm interelectrode distance which was connected to input of a differential preamplifier (WPI DAM-6A).

The recording equipment consisted of preamplifiers, a monitoring cathode-ray oscilloscope (Nihonkoden VC-7) and a minograph 800 (Siemens Elemen). The recordings were taken at a paper speed of 100 mm/sec for SCL and SRT, 250 mm/sec for refractory periods and 1000 mm/sec for A-H interval.

The preparations were driven with rectangular pulses of twice-diastolic-threshold voltage and 2-msec duration, generated by an electronic stimulator (Nihonkoden MSE-40) and applied with an isolation transformer and platinum wire electrodes.

The modes of stimulation were: 30-sec overdrive from the crista terminalis at a rate of 180–300 beats/min for determining SRT, and one premature stimulus (S2) with varied coupling intervals at every 8th basic stimulus (S1) from the right atrium to determine refractory periods of the AV node.

SRT was the time interval between upstrokes of the last stimulated and the first recovering
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Fig. 2. Effects of $10^{-6}$ g/ml of nicardipine-HCl on the spontaneous rate (expressed as percent of spontaneous rate before nicardipine-HCl) of the isolated rabbit sinoatrial node.

Fig. 3. Effects of nicardipine-HCl $10^{-7}$ and $5 \times 10^{-7}$ g/ml on the prolongation of sinus recovery time in excised rabbit sinoatrial (SA) nodal preparations. In the latter, 5 experiments are excluded because they lost automaticity after overdrive. Open circles denote mean values.

spontaneous action potentials. Max SRT was determined as the longest SRT among 3 trials of overdrive at rates of 180, 240 and 300 beats/min, and was expressed as a percentage of spontaneous cycle length (%SRT).

In the measurements of ERP, FRP and A-H interval, the basic cycle length ($S_1-S_2$) was set as 500 msec, short enough to dominate the automaticity of the AV node preparation. The coupling interval ($S_1-S_2$) was decreased in 10 msec steps from the basic cycle length until AV conduction block occurred. Then, the ERP was sought from the last conducted $S_1-S_2$, decreasing the coupling interval by 1 msec.

Nicardipine-HCl was added into the Tyrode tissue bath in a final concentration of $10^{-7}$ to $10^{-6}$ g/ml.

All measurements were repeated 15 min after the addition of nicardipine-HCl.

The t-test was used to assess the statistical significance in these experimental studies. The statistical significance of changes was tested by analysis of variance according to Newman-Keuls' multiple comparison.

RESULTS

Effects on the SA Nodes

The number of experiments, average sinus rate slowing and the rate of %SRT prolongation before and after nicardipine-HCl in different concentrations are shown in Table I.

After 15 min the solutions of $10^{-7}$, $5 \times 10^{-7}$ and $10^{-6}$ g/ml of nicardipine-HCl suppressed the frequency of the spontaneous firing. The spontaneous rate of the SA node after nicardipine-HCl (expressed as a percentage of the spontaneous rate before nicardipine-HCl) in different concentrations are plotted in Fig. 1. On the average, the rate was decreased by 6.4% from the control values at a concentration of $10^{-7}$ g/ml, by 20.4% at $5 \times 10^{-7}$ g/ml and by 24.7% at $10^{-6}$ g/ml. At the last concentration, the slowing is more marked (−78.5%) if the rates of the arrested 5 preparations are assumed to be −100%. The effect of this agent is dose-dependent.
TABLE II  EFFECTS OF NICARDIPE-HCl ON EFFECTIVE AND FUNCTIONAL REFRACTORY PERIODS AND A-H INTERVAL OF THE ATRIOVENTRICULAR NODE IN THE RABBIT

<table>
<thead>
<tr>
<th>Concentration (g/ml)</th>
<th>ERP (msec)</th>
<th>FRP (msec)</th>
<th>A-H Interval (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C N Δ</td>
<td>C N Δ</td>
<td>C N Δ</td>
</tr>
<tr>
<td>1 × 10^{-7}</td>
<td>6 125 146 +21</td>
<td>190 203 +13</td>
<td>53 55 +2</td>
</tr>
<tr>
<td>±SD</td>
<td>±12 ±17 ±14</td>
<td>±13 ±10 ±10</td>
<td>±3 ±2 ±1</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.025</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>3 × 10^{-7}</td>
<td>6 142 176 +34</td>
<td>196 226 +30</td>
<td>54 59 +5</td>
</tr>
<tr>
<td>±SD</td>
<td>±39 ±30 ±26</td>
<td>±22 ±26 ±22</td>
<td>±3 ±3 ±2</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.025</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>5 × 10^{-7}</td>
<td>5 140 272 +32</td>
<td>204 301 +97</td>
<td>53 65 +12</td>
</tr>
<tr>
<td>±SD</td>
<td>±36 ±53 ±47</td>
<td>±35 ±47 ±32</td>
<td>±8 ±10 ±4</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.005</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1 × 10^{-6}</td>
<td>9 140 396 256</td>
<td>187 354 167</td>
<td>53 70 17</td>
</tr>
<tr>
<td>±SD</td>
<td>±47 ±117 ±95</td>
<td>±16 ±79 ±81</td>
<td>±3 ±9 ±7</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>&lt; 0.005</td>
</tr>
</tbody>
</table>

Significant level in analysis of variance: *p < 0.05, **p < 0.01, ns = not significant.

Four experiments each in § and ¶ were excluded because they showed Wenckebach phenomenon after nicardipine-HCl.

Abbreviations: ERP = effective refractory period; FRP = functional refractory period; C = control; N = after nicardipine-HCl; Δ = amounts of changes; n = number of experiments

Fig.4. Effects of nicardipine-HCl on sinus recovery time (SRT) in an excised rabbit sinoatrial (SA) nodal preparation. Overdrive stimulation at a rate of 240 beats/min was given to the crista terminals (CT) for 30 sec. In the control (upper panel), the (%) SRT was 119%. Fifteen minutes after the addition of nicardipine-HCl in a concentration of 5 × 10^{-7} g/ml (lower panel), the SA node lost its automaticity with overdrive stimulation. The SA node responded only to electrical stimulation (lower right). The voltage calibration on the right end in each panel is 50 mV for the SA nodal action potential.

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![Diagram showing atrial and His bundle action potentials with S1-S2 intervals and His bundle potentials labeled H1 and H2.](image)

Figure 5. Effects of nicardipine-HCl on atrioventricular nodal (AVN) refractory periods in an excised rabbit AVN preparation driven electrically at a basic cycle length (BCL) of 500 msec. The upper tracings in both panels show the extracellular action potentials of the right atrium, and the lower tracings show the intracellular action potentials of the His bundle. The upper panel shows a control record and the lower panel the same record after exposure to $5 \times 10^{-7}$ g/ml of nicardipine-HCl. Figures denote time in milliseconds.

- His = His bundle action potential
- $S_1$ = stimulus artifact
- $S_2$ = extrastimulus artifact
- $H_1$ = His bundle electrogram
- $H_2$ = His bundle electrogram in response to extrastimulus $S_2$

The time course of changes in the spontaneous rate of the SA node at a concentration of $10^{-6}$ g/ml of nicardipine-HCl is shown in Fig. 2. The spontaneous rate of the SA node was suppressed and the automaticity was lost in 5 of 7 preparations within 15 min, and in all within 20 min.

Changes of %SRT at concentrations of $10^{-7}$ and $5 \times 10^{-7}$ g/ml of nicardipine-HCl are shown in Fig. 3. The SRT was prolonged slightly ($120.7 \pm 10.0$ to $129.2 \pm 9.5\%$) at $10^{-7}$ g/ml, and markedly ($119.6 \pm 5.5$ to $248.4 \pm 95.2\%$) at $5 \times 10^{-7}$ g/ml. At the latter concentration, SA nodes lost their automaticity after overdrive in 5 of 10 preparations. A representative example of prolongation of the SRT (postoverdrive arrest) after nicardipine-HCl is shown in Fig. 4. Before nicardipine-HCl, %SRT was 119% (normal). At $5 \times 10^{-7}$ g/ml of nicardipine-HCl, no recovery of SA node automaticity was seen after 30 sec of overdrive with 240 beats/min stimulation.

**Effects on the AV Node**

Nicardipine-HCl prolonged the refractory period of the isolated AV node (Table II).

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Figure 5 shows a representative case. In this preparation, an extra stimulus to the atrium with a coupling interval of 144 msec ($S_1-S_2$: 144 msec) was propagated to the His bundle through the AV node, while an extra stimulus with a coupling interval of 143 msec ($S_1-S_2$: 143 msec) was not conducted. After the injection of nicardipine-HCl at a concentration of $5 \times 10^{-7}$, an extra stimulus with a coupling interval of 200 msec ($S_1-S_2$: 200 msec) was propagated to the His bundle, and that of 198 msec ($S_1-S_2$: 198 msec) was already blocked. That is, the ERP was prolonged from 143 to 198 msec.

Figure 6 shows the AV conduction curve of another representative case. It is apparent that nicardipine-HCl prolonged the ERP and FRP at a concentration of $5 \times 10^{-7}$ g/ml. The differences in amounts of prolongation in the refractory periods at concentrations of $10^{-7}$, $3 \times 10^{-7}$, $5 \times 10^{-7}$ and $10^{-6}$ g/ml, were statistically significant (Table II). Thus, the effects of this agent on the AV node were dose-dependent.

Prolongation of the A-H interval at $10^{-7}$, $3 \times 10^{-7}$, $5 \times 10^{-7}$ and $10^{-6}$ g/ml of nicardipine-HCl was also statistically significant (Table II and Fig. 5).
Fig. 6. Conduction curve of atrioventricular node (AVN) of a typical experiment before and after addition of $5 \times 10^{-7}$ g/ml nicardipine-HCl (basic cycle length 500 msec). The time interval between responses of the His bundle to basic and extra stimuli ($H_1 - H_2$) is plotted against that of the right atrium ($A_1 - A_2$). The effective refractory period (ERP) and functional refractory period (FRP) of AVN were prolonged from the control value by 108 to 253 msec, and 191 to 314 msec, respectively.

DISCUSSION

Takenaka's hypothesis that the calcium antagonistic activity of nicardipine-HCl on vascular smooth muscle was more potent than that of other calcium antagonists such as nifedipine, verapamil, and diltiazem, and concluded that the calcium antagonistic effect of nicardipine-HCl was more effective on the blood vessels than on the heart.

Diltiazem, verapamil, and nifedipine exert similar suppressive effects on the sinoatrial (SA) nodal function and the atrioventricular (AV) nodal function in the excised rabbit heart.

In our experiments, nicardipine-HCl decreased the spontaneous rate of the SA node and prolonged sinus recovery time (SRT). These effects are similar to those of the other calcium antagonists mentioned above. Comparative effects of $10^{-7}$, $5 \times 10^{-7}$ and $10^{-6}$ g/ml of nicardipine-HCl and of nifedipine on the average sinus slowing (%) are shown in Fig. 7. At a concentration of $10^{-7}$ of nicardipine-HCl and nifedipine the average sinus slowing (%) is 6.4% and 8.0%, respectively. At $5 \times 10^{-7}$ g/ml, it is 20.4% and 25.7%; at $10^{-6}$ g/ml, 78.5% and 55%. It is apparent that nicardipine-HCl has a potent effect on isolated SA nodes similar to that of nifedipine.

Kawai et al. and Taira have reported that diltiazem, nifedipine, verapamil, and nicardipine-HCl caused atrial standstill at high doses. In our experiments, at a concentration of $5 \times 10^{-7}$ g/ml nicardipine-HCl caused complete arrest after overdrive in 5 of 10 preparations, although the sinus cycle length (SCL) was not prolonged (Fig. 4). At $10^{-6}$ g/ml the automaticity of the SA node was lost in all preparations within 20 min. The time course (Fig. 2) shows that the SA nodes lost their automaticity rather suddenly. In patients receiving nicardipine-HCl the mean plasma concentration of this drug was approximately $10^{-7}$ g/ml. On the basis of these reports, it seems that nicardipine-HCl in clinically practical doses does not cause atrial standstill.

Nicardipine-HCl $10^{-7}$ g/ml prolonged the ERP of the AV node less than did the other 3 calcium antagonists. The ERP was lengthened from 125 to 146 msec (116.8% of the control value) with nicardipine-HCl, 106 to 161 msec (151.8%) with diltiazem, 125 to 224 msec

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(179.2%) with verapamil and 99 to 152 msec (153.5%) with nifedipine. Nicardipine-HCl lengthened the ERP and FRP of the AV node and AV conduction time (AH interval) dose-dependently (Table II). After 10⁻⁶ g/ml nicardipine-HCl, 4 of 9 experiments showed Wenckebach phenomenon even with a basic atrial drive of 120 beats/min. Thus, nicardipine-HCl has definite effects on isolated AV nodes in high doses.

Nicardipine-HCl has a similar chemical structure to nifedipine and is claimed to have a more potent vasodilating action than diltiazem, verapamil and nifedipine. As we have reported, nifedipine is the most potent vasodilator among these last 3 calcium antagonists. Its direct suppressive effect on the AV node was overcome probably by reflex sympathetic drive, and the ERP of AV nodes was shortened in the clinical dosing. In this regard, electrophysiologic effects of nicardipine-HCl are nearer to those nifedipine than to those of verapamil or diltiazem. In clinically practical doses, nicardipine-HCl may have no antiarrhythmic properties.

In conclusion, nicardipine-HCl has electrophysiologic effects on isolated SA and AV nodes similar to those of other calcium antagonists. In the clinical setting, however, it can be used as a potent vasodilator with the least untoward effects on SA and AV nodal function.

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