Electrophysiological effects of capsaicin on spontaneous activity of rabbit atrioventricular node cells

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Abstract: To study the electrophysiological effects of capsaicin on spontaneous activity of rabbit atrioventricular (AV) node cells, parameters of action potential in AV node were recorded using intracellular microelectrode technique. Capsaicin (1~30 µmol/L) not only decreased the amplitude of action potential, maximal rate of depolarization \( V'_{\text{max}} \), velocity of diastolic (phase 4) depolarization, and rate of pacemaker firing, but also prolonged the duration of 90% repolarization of action potential (APD \(_{90}\)) in a concentration-dependent manner. Both application of L-type Ca\(^{2+}\) channel agonist Bay K8644 (0.5 µmol/L) and elevation of calcium concentration (5 mmol/L) in superfusate antagonized the effects of capsaicin on pacemaker cells. Pretreatment with ruthenium red (10 µmol/L), a capsaicin receptor blocker, did not affect the effects of capsaicin on AV node cells. Capsaicin exerted an inhibitory action on spontaneous activity of AV node cells in rabbits. These effects were likely due to reduction in calcium influx, but were not mediated by VR1.

Key words: capsaicin; atrioventricular node; calcium currents; electrophysiology
phase 0 depolarization in partially depolarized papillary muscles, which was likely due to a decrease of calcium inflow. However, little is known about the effects of capsaicin on atrioventricular (AV) node. In the present study, we observed the electrophysiological effects of capsaicin on AV nodes of rabbits and investigated the mechanism(s) involved.

1 MATERIALS AND METHODS

1.1 AV node preparation. Male rabbit (n=30, weighing 2.5±0.2 kg, Grade II, Certificate No.04037, provided by Experimental Animal Center of Hebei Province) was killed with a single blow on the head and the heart was removed and placed in cold (0~4ºC) oxygenated (95% O₂ and 5% CO₂) Krebs-Henseleit (K-H) solution. The right ventricle was slit open near the apex, and the incision was carefully extended toward the tricuspid valve following the chordae tendineae of the papillary muscles. The tricuspid valve was cut through and the right atrium slit open on its lateral wall to expose the right side of the interatrial septum. The area of the AV node was identified using anatomical landmarks. The final preparation contained right atrial tissues consisting of the interatrial septum, the septal leaflets of the tricuspid valve, and the small portion of the central fibrous body. The preparation was pinned down on a thin silicon disc with endocardial surface oriented upwards and kept in a thermostat-controlled superfusion system.

1.2 Superfusing solution. The K-H solution was prepared with deionized, distilled water and had the following composition (in mmol/L): NaCl 118.0, NaHCO₃ 25.0, KCl 4.7, MgSO₄ 1.6, CaCl₂ 2.5, KH₂PO₄ 1.2, and glucose 11.1. It was oxygenated with 95% O₂ and 5% CO₂ and maintained at 36.0±0.5ºC with pH of 7.32±0.03.

1.3 Electrical recording. The transmembrane potentials were recorded by means of 3 mol/L KCl-filled micropipettes (a tip resistance of 10~20 MΩ.), coupled to a high input impedance amplifier (MEZ 8201, Nihon Kohden). The amplified signals were fed to the A/D converter and processed by a microcomputer. Maximal diastolic potential (MDP), amplitude of action potential (APA), maximal rate of depolarization (V′ max), velocity of diastolic (phase 4) depolarization (VDD), rate of spontaneous firing (RSF) and 90% of duration of action potential (APD₉₀) were analyzed with a program of sampling and processing cardiac transmembrane potential designed by our department.

1.4 Experimental protocols. The experiment started after the preparations were equilibrated for 60 min in the K-H solution at a perfusion rate of 4 ml/min. The experiments consisted of 4 groups: (1) The effects of capsaicin on spontaneous activity of AV node cells. After recording 3 control action potentials (APs), capsaicin (1, 10, 30 µmol/L) was applied, respectively. APs were then recorded at 5, 10, 15, 20 and 25 min after capsaicin administration; (2) The effects of Bay K8644 (0.5 µmol/L) on the response of AV node cells to capsaicin (10 µmol/L). The effects of capsaicin (10 µmol/L) alone were observed. Then after superfusion of Bay K8644 (0.5 µmol/L) for 10 min, capsaicin (10 µmol/L) was added to the superfusate containing Bay K8644 and the APs were recorded; (3) The effects of high Ca²⁺ (5 mmol/L) on the actions of capsaicin (10 µmol/L). The effects of capsaicin (10 µmol/L) alone were observed. Then, normal K-H solution was replaced by high Ca²⁺ (5 mmol/L) K-H solution for 10 min. Afterwards, capsaicin (10 µmol/L) alone were administered and APs were recorded; (4) The effects of ruthenium red (RR, 10 µmol/L) on the response of AV node cells to capsaicin (10 µmol/L). The effects of capsaicin (10 µmol/L) alone were observed. Then after superfusion of RR (10 µmol/L) for 15 min, capsaicin (10 µmol/L) was added to the superfusate containing RR and APs were recorded. In each experiment, the preparation was washed with K-H solution after application of drugs to observe the recovery of AP.

1.5 Drugs. Drugs used in this study included capsaicin, ruthenium red and Bay K8644 (Sigma Chemical Co, USA). Capsaicin was dissolved in distilled water containing 10% ethanol and 1% Tween-80 and then diluted to final concentration with saline. Bay K8644 was prepared as stock solution in alcohol, which in its final concentration did not exceed 0.1%. Ruthenium red was dissolved in distilled water.

1.6 Statistical analysis. All data were presented as mean±SE. Statistical differences were evaluated by paired Student’s t test. Differences between groups were assessed using unpaired t test. Statistical significance was set at P<0.05.

2 RESULTS

2.1 Action potential configurations of AV node cells

Using microelectrode, action potentials from spontaneous active node cells were recorded. AV node cells had a mean rate of spontaneous activity of (84±12) beat/min. The mean MDP was (~62±1) mV, and most cells exhibited relatively slow action potential upstroke [V′ max = (13±1)V/s]. The mean APA was (92±6) mV, and APD₉₀ was (112±15) ms (Fig. 1, Table 1).
2.2 Effects of capsaicin on spontaneous activity of AV node cells

Compared with the control group, capsaicin (1–30 µmol/L) decreased VDD, RPF and $V_{\text{max}}$ in a concentration-dependent manner. Capsaicin (30 µmol/L) not only prolonged APD$_{90}$ but also induced a significant reduction in APA (Table 1, Fig. 1). The changes in RPF induced by capsaicin paralleled to those of VDD. The above-mentioned effects occurred after 5 min of superfusion of capsaicin and reached the peak within 20–25 min. The vehicle had no effect on the parameters of AP of AV node cells.

2.3 Effects of Bay K8644 on capsaicin-induced changes in AP

L-type calcium channel agonist Bay K8644 (0.5 µmol/L) significant increased VDD, RPF and $V_{\text{max}}$. Upon the application of Bay K8644, the effects of capsaicin (10 µmol/L) were significantly reduced (Table 2). The vehicle had no effect on the parameters of APs of AV node cells.

2.4 Effects of high Ca$^{2+}$ concentration on the response of AV node cells to capsaicin

Elevation of Ca$^{2+}$ concentration (5 mmol/L) in superfusate increased VDD, RPF, $V_{\text{max}}$ and reversed the inhibitory action of capsaicin (10 µmol/L) (Table 2).

2.5 Effects of ruthenium red on the response of AV node cells to capsaicin

VR1 blocker RR (10 µmol/L) itself had no effect on AP. Pretreatment with RR (10 µmol/L) failed to affect the above-mentioned effects induced by capsaicin (10 µmol/L) (Table 2).

3 DISCUSSION

The atrioventricular (AV) node is the primary conduction pathway between the atria and ventricles and subserves an important filtering function by virtue of its rate-dependent properties. AV node cells have been subdivided into three regions called AN (atrio-nodal), N (nodal) and NH (atrio-His) cells based upon activation time and transmembrane electrical characteristics.[12,14] The electrophysiologic features of N and NH cells are of low resting potential and low maximum upstroke velocity.[14] Action potential con-
Table 2. Effects of Bay K8644 (Bay, 0.5 μmol/L), high Ca\(^{2+}\) (5 mmol/L) and ruthenium red (RR, 10 μmol/L) on capsaicin (10 μmol/L)-induced effects on spontaneous activity of rabbit atrioventricular node cells (n=6)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDP (mV)</th>
<th>APA (mV)</th>
<th>(V_{\text{max}}) (V/s)</th>
<th>VDD (mV/s)</th>
<th>RPF (beat/min)</th>
<th>APD(_{90}) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-60.6±1.7</td>
<td>84.8±3.5</td>
<td>10.6±1.0</td>
<td>18.6±3.0</td>
<td>82.0±12.6</td>
<td>121.5±3.5</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>-60.6±1.7</td>
<td>84.1±3.4</td>
<td>9.2±1.0(^*)</td>
<td>13.5±2.4(^*)</td>
<td>70.6±12.6(^*)</td>
<td>129.3±4.6(^*)</td>
</tr>
<tr>
<td>Bay</td>
<td>-60.9±2.3</td>
<td>87.0±3.0</td>
<td>12.5±1.1(^*)</td>
<td>21.9±4.0(^*)</td>
<td>97.7±15.2(^*)</td>
<td>120.3±4.2</td>
</tr>
<tr>
<td>Bay+capsaicin</td>
<td>-62.6±2.3</td>
<td>83.9±3.3</td>
<td>10.9±1.2</td>
<td>16.0±3.4(^*)</td>
<td>81.3±14.0(^*)</td>
<td>128.5±5.0(^*)</td>
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<tr>
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<td>85.0±2.2</td>
<td>11.5±1.3</td>
<td>20.1±1.4</td>
<td>91.3±5.6</td>
<td>106.3±4.0</td>
</tr>
<tr>
<td>Capsaicin</td>
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<td>82.3±3.0</td>
<td>9.7±1.5(^*)</td>
<td>17.1±1.6(^*)</td>
<td>82.0±5.5(^*)</td>
<td>114.5±5.5</td>
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<tr>
<td>High Ca(^{2+})</td>
<td>-60.6±3.2</td>
<td>87.5±3.1</td>
<td>15.1±1.7(^*)</td>
<td>25.5±1.9(^*)</td>
<td>119.3±7.9(^*)</td>
<td>99.8±3.5</td>
</tr>
<tr>
<td>High Ca(^{2+})+capsaicin</td>
<td>-60.7±2.7</td>
<td>82.7±3.0</td>
<td>9.9±1.5(^*)</td>
<td>16.4±1.5(^*)</td>
<td>87.1±6.0(^*)</td>
<td>111.6±3.9</td>
</tr>
<tr>
<td>Control</td>
<td>-61.1±2.3</td>
<td>88.0±3.7</td>
<td>12.9±1.5</td>
<td>18.9±1.7</td>
<td>84.6±13.6</td>
<td>118.0±7.0</td>
</tr>
<tr>
<td>Capsaicin</td>
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<td>86.5±4.1</td>
<td>10.2±1.1(^*)</td>
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<td>74.1±15.2(^*)</td>
<td>129.3±11.5</td>
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<td>14.2±2.0</td>
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<td>81.8±13.8</td>
<td>119.5±5.7</td>
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<tr>
<td>RR+capsaicin</td>
<td>-62.4±2.3</td>
<td>86.2±3.1</td>
<td>9.8±1.2(^+)</td>
<td>14.0±1.6(^+)</td>
<td>70.1±14.2(^+)</td>
<td>133.5±13.4</td>
</tr>
</tbody>
</table>

\(*P<0.05, **P<0.01, compared with control group. \(\dagger\)P<0.05, compared with Bay group. \(\ddagger\)P<0.05, compared with high Ca\(^{2+}\) group. \(^*\)P<0.05, compared with RR group.

...tion of neuropeptide from the vanilloid-sensitive nerves\(^{24,25}\). On the other hand, some capsaicin-induced effects did not follow the typical features of vanilloid receptors\(^{22}\). The inorganic dye ruthenium red (RR), a vanilloid receptor blocker, was able to block the capsaicin-induced responses\(^{22}\). But in our experiments, RR failed to abolish the electrophysiological effects of capsaicin on spontaneous activity of AV node cells, suggesting that VR1 may not mediate the inhibitory effects of capsaicin.

In this study, action potential duration of AV node cells was prolonged as the concentration of capsaicin was increased. This effect may be related to a reduction in potassium currents. Castle reported that capsaicin inhibits three distinct K\(^+\) currents (\(I_{\text{to}}, I_{K}\) and \(I_{Kt}\)) on cardiac cells\(^{6}\). The mechanism merits further investigation.

In summary, capsaicin exhibited an inhibitory effect on the spontaneous activity of AV node cells in rabbits. These effects are likely due to the reduction in calcium influx, but were not mediated by VR1.

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