Inhibitory Effects of KW-3902, a Selective Adenosine A1-Receptor Antagonist, on the Adenosine-Induced Shortening of Action Potential Duration in Guinea Pig Atrial Muscles

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ABSTRACT—We investigated the effects of KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine), a newly-synthesized selective adenosine A1-receptor antagonist, on the shortening of action potential duration (APD) in guinea pig atria exposed to adenosine. The APD shortening by adenosine was inhibited by KW-3902 at higher than 10^{-8} M, but not by 10^{-5} M of KF17837, an adenosine A2-receptor antagonist. These results support the notion that the APD shortening by adenosine in atria is mediated via adenosine A1-receptors. The potency of KW-3902 in antagonizing the APD-shortening were similar to those in antagonizing the negative inotropic and chronotropic action of adenosine in the isolated right atria, suggesting that these responses to adenosine are mediated via the receptors of the same type.

Keywords: KW-3902, Adenosine A1-receptor antagonist, Electrophysiology

In atrial preparations, adenosine has been shown to modulate the electrophysiological properties of myocytes. For example, adenosine increases the specific outward potassium current (I_{KADDO}) (1) and decreases the basal and isoproterenol-induced calcium inward current (I_{Ca}) (2–4). It has been assumed that these electrophysiological effects of adenosine are involved in the shortening of the atrial action potential duration (APD) (3, 5) and are executed through the adenosine A1-receptor (6, 7). However, the effect of a specific adenosine A1-receptor antagonist has not been reported prior to the present experiment. KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine) is a newly-synthesized selective adenosine A1-receptor antagonist (8), which exhibits diuretic and renal protective effects (9, 10). The K_i values for adenosine A_1 and A_2-receptors are 1.3±0.12 and 380±30 nM, respectively (8). The purposes of the present study were 1) to reexamine, by using the specific adenosine antagonist, the receptor subtype of adenosine receptors responsible for the APD-shortening and 2) to compare the potency of KW-3902 in antagonizing the APD-shortening with those in antagonizing the negative inotropic and chronotropic action of adenosine, in order to determine if the receptors involved in these responses are of the same type. Moreover, we investigated the effect of glibenclamide, an ATP-sensitive potassium current (I_{K,ATP}) inhibitor, on the APD shortening by adenosine since adenosine is known to increase the I_{K,ATP} in ventricular myocytes (11) and coronary arteries (12).

Male Hartley guinea pigs (Japan Shizuoka Laboratory animal Center, Inc., Hamamatsu), weighing 250–300 g, were used for the present study. Under pentobarbital (50 mg/kg, i.p.) anesthesia, the heart was rapidly excised. For the mechanical study, the spontaneously beating right atrium was isolated. The preparation was mounted in an organ bath filled with Krebs-Ringer solution gassed with 95% O_2 and 5% CO_2, maintained at 36.0–37.0°C. The composition of Krebs-Ringer solution was as follows: 120 mM NaCl, 4.0 mM KCl, 1.3 mM MgSO_4, 1.2 mM NaH_2PO_4, 1.2 mM CaCl_2, 25.2 mM NaHCO_3 and 5.0 mM glucose. After a 2-hr period of equilibrium, 1 μM of dipyridamole (an adenosine uptake inhibitor) and 1 μM of erythro-9-(2-hydroxy-3-nonyl)-adenine hydrochloride (an adenosine deaminase inhibitor) were applied to the Krebs-Ringer solution at 30 min before the experiment, in order to sustain the effect of adenosine. For the mechanical study, the spontaneously beating right atrium was isolated. The preparation was mounted in an organ bath filled with Krebs-Ringer solution gassed with 95% O_2 and 5% CO_2, maintained at 36.0–37.0°C. The composition of Krebs-Ringer solution was as follows: 120 mM NaCl, 4.0 mM KCl, 1.3 mM MgSO_4, 1.2 mM NaH_2PO_4, 1.2 mM CaCl_2, 25.2 mM NaHCO_3 and 5.0 mM glucose. After a 2-hr period of equilibrium, 1 μM of dipyridamole (an adenosine uptake inhibitor) and 1 μM of erythro-9-(2-hydroxy-3-nonyl)-adenine hydrochloride (an adenosine deaminase inhibitor) were applied to the Krebs-Ringer solution at 30 min before the experiment, in order to sustain the effect of adenosine. Rate and tension were monitored with a force-displacement transducer (model TB-611T; Nihon Kohden, Tokyo) and recorded on a pen recorder (type 3066; Yokogawa Hokushin Electric, Tokyo). For the electrophysiological study, a small
piece of free wall (3 x 3 mm) was cut from the right atrial muscle near the sino-atrial node. The free wall preparation was pinned to the bottom of an organ bath and superfused continuously at a constant rate of 5 ml min⁻¹ with Krebs-Ringer solution gassed with 95% O₂ and 5% CO₂, maintained at 36.0–37.0°C. The preparation was driven at 1 Hz by rectangular pulses, 1 msec in duration and 1.5 times threshold voltage (model SEZ-3301, Nihon Kohden), delivered through bipolar platinum electrodes. Conventional microelectrode penetration was made into the endocardial surface using a glass microelectrode filled with 3 M KCl. The microelectrodes were selected so that the tip resistance was 10 to 30 MΩ. The output of a microelectrode preamplifier with high input impedance and capacity neutralization (model MEZ-7101, Nihon Kohden) was fed into a storage oscilloscope (model 5113; Tektronix, Beaverton, OR, USA) for display of action potential. The variables measured were as follows: resting membrane potential (RP); action potential amplitude (APA); maximum upstroke velocity of the action potential (Vmax), which was determined by an electronic differentiating circuit that was linear up to 1,000 V/s; and action potential duration at 50% or 90% repolarization (APD50 or APD90). All the experimental results were obtained from a single continuous impalement. The action potentials were recorded before, 5–15 min after cumulative addition of adenosine (10⁻⁷–10⁻⁴ M) and after removal of adenosine. In another series of experiments, to determine the effects of KW-3902 and glibenclamide per se, 10⁻⁸ M, 10⁻⁷ M and 10⁻⁶ M of KW-3902 or 10⁻⁵ M of glibenclamide were simultaneously applied with the adenosine uptake inhibitor and the adenosine deaminase inhibitor.

In the mechanical study in isolated right atria, the basal values of developed tension and atrial rate were 75 ± 18 mg and 147 ± 5 beats/min (n = 5), respectively. Adenosine concentration-dependently decreased the developed tension, with an IC50 (50% inhibitory concentration) of 411 ± 78 nM, and attenuated the atrial rate, with an IC25 (25% inhibitory concentration) of 419 ± 12 nM. KW-3902 at 10⁻⁸–10⁻⁷ M shifted to the right the concentration-response curves for both the developed tension and the atrial rate. The pA2 values for KW-3902 in the developed tension and the atrial rate were 8.93 and 8.89, respectively. In the electrophysiological study, adenosine, in a range of concentrations between 10⁻⁷ and 10⁻⁴ M, caused no statistically significant changes in RP, APA and Vmax.

Concentration-dependent shortenings of APD50 and APD90 were observed following exposure to adenosine. During rinsing, the APD was restored. These changes in parameters are shown in Table 1. The basal variables of action potential, such as RP, APA, Vmax and especially APD, were not changed by an adenosine uptake inhibitor plus an adenosine deaminase inhibitor, KW3902 or glibenclamide. The change of APD90 values were as follows: 97.5 ± 4.44 to 93.6 ± 5.39 msec (an adenosine uptake inhibitor and an adenosine deaminase inhibitor), 94.1 ± 3.66 to 88.5 ± 3.81 msec (10⁻⁸ M KW-3902), 84.9 ± 4.42 to 84.1 ± 5.27 msec (10⁻⁷ M KW-3902), 95.6 ± 4.83 to 98.9 ± 3.58 msec (10⁻⁶ M KW-3902) and 93.3 ± 4.14 to 87.1 ± 3.49 msec (10⁻⁵ M glibenclamide).

Table 1. Electrophysiological effects of adenosine on the variables of action potential in guinea pig atrial muscles

<table>
<thead>
<tr>
<th>Condition</th>
<th>RP (mV)</th>
<th>APA (mV)</th>
<th>Vmax (V/s)</th>
<th>APD50 (msec)</th>
<th>APD90 (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-87.5 ± 0.9</td>
<td>110.0 ± 1.3</td>
<td>161.1 ± 9.6</td>
<td>48.7 ± 6.0</td>
<td>93.6 ± 5.4</td>
</tr>
<tr>
<td>10⁻⁷ M</td>
<td>-87.3 ± 0.9</td>
<td>110.2 ± 1.5</td>
<td>163.5 ± 8.4</td>
<td>48.9 ± 6.3</td>
<td>92.1 ± 5.7</td>
</tr>
<tr>
<td>3 x 10⁻⁷ M</td>
<td>-87.9 ± 1.1</td>
<td>111.1 ± 1.4</td>
<td>162.3 ± 8.3</td>
<td>48.9 ± 6.5</td>
<td>92.5 ± 5.7</td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>-86.9 ± 0.8</td>
<td>110.1 ± 1.0</td>
<td>160.5 ± 8.8</td>
<td>45.3 ± 6.7</td>
<td>84.7 ± 7.6</td>
</tr>
<tr>
<td>3 x 10⁻⁶ M</td>
<td>-87.2 ± 1.0</td>
<td>110.1 ± 1.5</td>
<td>161.1 ± 9.1</td>
<td>40.5 ± 7.2</td>
<td>77.4 ± 8.7</td>
</tr>
<tr>
<td>10⁻⁵ M</td>
<td>-86.7 ± 1.1</td>
<td>107.7 ± 1.7</td>
<td>159.0 ± 9.0</td>
<td>30.8 ± 6.3</td>
<td>60.7 ± 8.6**</td>
</tr>
<tr>
<td>3 x 10⁻⁵ M</td>
<td>-86.1 ± 0.6</td>
<td>105.7 ± 1.9</td>
<td>165.0 ± 10.7</td>
<td>22.1 ± 4.5*</td>
<td>48.3 ± 6.2**</td>
</tr>
<tr>
<td>10⁻⁴ M</td>
<td>-85.3 ± 0.5</td>
<td>104.0 ± 1.9</td>
<td>165.0 ± 11.1</td>
<td>15.7 ± 2.5**</td>
<td>36.8 ± 3.7**</td>
</tr>
<tr>
<td>Wash out</td>
<td>-86.8 ± 0.6</td>
<td>112.0 ± 1.0</td>
<td>166.9 ± 10.3</td>
<td>49.2 ± 5.1</td>
<td>93.9 ± 4.74</td>
</tr>
</tbody>
</table>

Values are presented as means ± S.E. from 6 preparations. RP: resting membrane potential, APA: action potential amplitude, Vmax: maximum upstroke velocity of the action potential, APD50, APD90: action potential duration at 50% or 90% repolarization. *P < 0.05, **P < 0.01, when compared with the pre-values by the ANOVA followed by the Dunnett’s test.
value for KW-3902 was estimated to be 9.06. Thus, the participation of adenosine A1-receptors in the APD shortening by adenosine was confirmed in the atrial muscles by using KW-3902, a specific adenosine A1-receptor antagonist. Moreover, the present results demonstrate that the pA2 values for KW-3902 are similar to each other in the experiments examining the negative inotropic action (pA2 = 8.93), the negative chronotropic action (pA2 = 8.89) and the APD-shortening action (pA2 = 9.06, APD90) induced by adenosine. It is, therefore, suggested that the adenosine A1-receptors executing the above three responses are receptors of the same type.

KF17837, an adenosine A2-receptor antagonist (13) (pA2 value obtained from rat aorta is 5.93; T. Mori et al., unpublished observation), at up to 10^-5 M did not influence the APD shortening by adenosine. The final values of APD90 attained by 10^-7 M, 3 x 10^-7 M, 10^-6 M, 3 x 10^-6 M, 10^-5 M, 3 x 10^-5 M and 10^-4 M of adenosine were 98.3±1.06%, 98.7±1.31%, 89.8±4.09%, 81.6±5.66%, 63.7±6.63%, 50.9±4.57% and 39.0±2.23% in the absence of KF17837 and 98.9±0.82%, 96.2±1.43%, 89.9±1.67%, 78.0±3.23%, 61.2±4.88%, 45.88±5.95% and 35.8±4.86% in the presence of KF17837, respectively. These results further support the notion that the APD shortening by adenosine is mediated via A1-receptors.

The concentration-dependent shortening by adenosine of APD in atrial muscles has been reported to be due to the increase of the I_{KADO} (1, 6) and decrease of basal I_{Ca} (2, 3). On the other hand, it is reported that the effect of adenosine on APD shortening in ventricular myocytes and on the K^+ inward current in coronary arteries are predominantly due to activation of I_{KATP} (11, 12). The present results indicate that adenosine has little or no interaction with I_{KATP} in the atrial muscle, because 10^-5 M glibenclamide did not affect the APD shortening by adenosine (Fig. 1). Thus, our present results suggest that the APD shortening in atria is mediated by I_{KADO}, and possibly by I_{Ca}, but not by I_{KATP}.

In conclusion, the present study, performed with the selective adenosine receptor antagonist confirmed that the negative inotropic and chronotropic action and the APD shortening by adenosine in atria are mediated via adenosine A1-receptors, which presumably couple to adenosine activated K channels. Moreover, the present results suggest that the adenosine receptors responsible for the APD-shortening are of the same type as those for the negative inotropic and chronotropic action.

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REFERENCES


