Selective Attenuation of Isoproterenol-Stimulated Arrhythmic Activity by a Partial Agonist of Adenosine A₁ Receptor

Yejia Song, MD; Lin Wu, MD; John C. Shryock, PhD; Luiz Belardinelli, MD

Background—The goal of this study was to examine the hypothesis that a partial agonist of the adenosine A₁ receptor (A₁/AdoR) may cause a greater attenuation of catecholamine-induced ventricular arrhythmic activity than of contractility. Methods and Results—The effects of CVT-2759 and adenosine, a partial and a full agonist of the A₁/AdoR, on isoproterenol-stimulated arrhythmic activity and contractility of guinea pig isolated ventricular myocytes were determined. CVT-2759 (10 μmol/L) and adenosine (10 μmol/L) significantly inhibited isoproterenol-induced arrhythmic activity (aftercontraction and transient inward current) but did not reduce the amplitudes of twitch shortening and L-type Ca²⁺ current. Increasing the concentration of the full agonist adenosine from 10 to 100 μmol/L, however, caused significant attenuation of twitch shortening as well as aftercontractions, whereas increasing the concentration of the partial agonist CVT-2759 from 10 to 100 μmol/L did not. CVT-2759 also significantly inhibited isoproterenol-induced spontaneous ventricular beats in isolated hearts. In contrast to adenosine, CVT-2759 neither activated adenosine-sensitive K⁺ current nor shortened the duration of the atrial APD. Conclusions—The present results support the hypothesis and suggest a potential role for a partial agonist of the A₁/AdoR in the treatment of cardiac arrhythmias. (Circulation. 2002;105:118-123.)

Key Words: adenosine □ arrhythmia □ contractility □ myocytes

A denosine modulates the functions of cardiac myocytes by stimulating adenosine A₁ receptors (A₁/AdoRs). Adenosine activates an adenosine-sensitive K⁺ current [Iₖ(Ado), also known as Iₖ(ACCh)] and thereby shortens the atrial action potential duration (APD). Adenosine has little or no direct effect on ventricular myocardiun of most mammalian species, including the guinea pig. However, adenosine antagonizes β-adrenergic stimulation of L-type Ca²⁺ current [Iₖ(Ca,L)], transient inward current (Iₛ), delayed afterdepolarizations (DADs), cell twitch shortening, and aftercontractions of ventricular myocytes.

Adenosine is considered a safe antiarrhythmic drug, because its action is brief. Because adenosine acts on all 4 subtypes of adenosine receptors and is a full agonist, however, side effects of adenosine are common. Therefore, use of adenosine is limited to a hospital setting. Considering the high efficacy and the frequency of side effects of adenosine, a selective and partial agonist of the A₁/AdoR should provide advantages relative to adenosine in the treatment of cardiac arrhythmias.

A partial agonist is a low-efficacy ligand that elicits a submaximal response (compared with a full agonist) when bound to receptors at maximal occupancy. In tissues with low amplification in the signal transduction path from receptor activation to functional response, a partial agonist is ineffective in causing a response. Therefore, a partial agonist causes fewer responses in the intact organism than a full agonist and is potentially a more selective drug. The N⁶ heterocyclic 5'-modified adenosine derivative [5-{6-[(3R)oxol-an-3-yl]amino}purin-9-y][3S,2R,4R,5R]-3,4-dihydroxyoxolan-2-yl)methoxy]-N-methylcarboxamide (CVT-2759) is a newly synthesized partial agonist of the A₁/AdoR. We hypothesized that CVT-2759 may selectively attenuate the proarrhythmic effect of a β-adrenoceptor agonist without significantly affecting either the contractility of ventricular myocytes or the basal electrical activity of atrial myocytes. This hypothesis is based on observations that adenosine decreases catecholamine-induced arrhythmic activity more than contractility and that its potency to inhibit isoproterenol-stimulated Iₖ(Ca,L) is 10-fold greater than its potency to activate Iₖ(Ado). The hypothesis was further examined in this study. The effects of CVT-2759 on (1) isoproterenol-stimulated arrhythmic activity (DADs, Iₛ, and aftercontractions) and contractility [assessed by measuring twitch shortening and Iₖ(Ca,L)] of ventricular myocytes and (2) the action potentials and Iₖ(Ado) of atrial myocytes were determined and compared with those of adenosine. The antiarrhythmic effect of CVT-2759 was also examined in isolated, perfused hearts.
Methods

Chemicals
CVT-2759 was a gift from CV Therapeutics. A stock solution of CVT-2759 (100 mmol/L) was made by dissolving the compound in DMSO. The stock solution was diluted in Tyrode’s solution for use in experiments. The final content of DMSO in Tyrode’s solution during experiments was no more than 0.1%. All other chemicals were purchased from Sigma.

Isolation of Hearts and Myocytes
Use of animals was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 86-23, 1985) and was approved by the Institutional Animal Care and Use Committee of the University of Florida. Hearts of adult Harlan guinea pigs of either sex were isolated and perfused via the aorta. For isolation of single myocytes, hearts were perfused with warm (35°C) and oxygenated solutions as follows: (1) Tyrode’s solution containing (in mmol/L) 140 NaCl, 4.6 KCl, 1.8 CaCl2, 1.1 MgSO4, 10 glucose, and 5 HEPES, pH 7.4, for 5 minutes; (2) Ca2+-free solution containing (in mmol/L) 100 NaCl, 50 KCl, 2 MgSO4, 10 glucose, 5 HEPES, 20 tauroine, and 5 pyruvate, for 5 minutes; (3) Ca2+-free solution, containing collagenase (120 U/mL) and albumin (2 mg/mL) for 20 minutes. At the end of the perfusion, the atria and ventricles were separated, minced, and gently shaken for 10 minutes in solution 3 to free single cells for study.

Measurements of Transmembrane Potential and Current
Myocytes were placed into a recording chamber and superfused with Tyrode’s solution at 35°C. Drugs were applied via the superfusate. Transmembrane voltages and currents were measured with glass microelectrodes filled with solution containing (in mmol/L) 120 potassium aspartate, 20 KCl, 1 MgCl2, 4 NaATP, 0.1 NaGTP, 10 glucose, and 10 HEPES (pH 7.2). Microelectrode resistance was 1 to 3 MΩ. An Axopatch-200 amplifier, a DigiData-1200A interface, and pCLAMP6 software (Axon Instruments) were used to perform electrophysiological measurements. The electrode capacitance, whole-cell capacitance, and series resistance were maximally compensated. The liquid junction potential was corrected. Measurements were made when the response to a drug had reached a stable maximum.

For recording Ical and Ii, myocytes were voltage-clamped at a holding potential of ~40 mV to inactivate the fast Na+ channels. A 500-ms depolarizing pulse to 0 or +20 mV was applied at a frequency of 0.5 Hz. The amplitude of Ical was measured from the zero current to the maximal inward current during the depolarizing pulse, and the amplitude of Ii was measured from the holding current to the peak inward deflection after return to the holding potential. Values of the amplitude of Ical and Ii were normalized by the whole-cell capacitance (30 to 40 pF, read from the capacitance meter of the amplifier) and expressed as pA/pF. For measurement of IhKAdo, a 4-second ramp pulse from −120 to +20 mV was applied at a frequency of 0.1 Hz. The increment of the current elicited by this voltage protocol in the presence of adenosine or CVT-2759 was determined as the amplitude of IhKAdo. To induce ventricular depolarizations, a 5-ms depolarizing pulse was applied at a frequency of 0.5 to 1 Hz. The APD was measured at 50% (APD50) and 90% (APD90) repolarization.

Measurement of Cell Contraction
Cell twitch shortening was elicited by the same procedure as used to induce action potentials. The amplitude of twitch shortening was determined by a video motion detector (Crescent Electronics) and was used as an index of cell contractility.11 Action potentials and amplitude of cell twitch shortening were recorded simultaneously on a chart recorder (Gould 2200B). In this study, a twitch shortening denotes a normal systolic contraction, whereas an aftercontraction denotes a contraction that occurs during diastole and is triggered by events following the preceding normal contraction. The amplitude of twitch shortening and aftercontraction was measured from maximal cell relaxation to peak contraction and was calculated as an average of 10 consecutive events.

Ventricular Pacing and Measurement of Electrogram
Isolated hearts were perfused with warm (36±0.5°C) modified Krebs-Henseleit solution at a rate of 10 mL/min. The Krebs-Henseleit solution contained (in mmol/L) 117.9 NaCl, 2.5 CaCl2, 4.8 KCl, 1.28 MgSO4, 1.2 KH2PO4, 0.5 Na2-EDTA, 0.14 ascorbic acid, 5.5 glucose, 2 pyruvate, and 25 NaHCO3. pH 7.4, gassed with 95% O2 and 5% CO2. Drugs were delivered via the perfusion line. Parts of the atrial tissues, including the region of the sinoatrial node, were removed. A pacing electrode was initially placed in the atrial septum. Pacing stimuli were provided by a stimulator (Grass) as 3-ms pulses at a frequency of 3 Hz. Electrograms were recorded on a chart recorder (Gould RS3400). To facilitate ventricular pacing, a complete block of atrioventricular conduction was necessary. This was achieved by injecting a small amount (20 μL) of 70% ethanol into the region of the atrioventricular node.12 Attainment of third-degree atrioventricular block was indicated by a complete dissociation of atrial and ventricular depolarizations in the electrogram.13 After complete atrioventricular block was produced, the pacing electrode was moved to the ventricular septum. Normal and spontaneous ventricular beats were identified as pacing-induced and non–pacing-induced ventricular depolarizations, respectively.

Statistical Analysis
Data are expressed as mean±SEM. Values of n indicate the number of cells or hearts studied. Percentage inhibition by CVT-2759 or adenosine of the effects of isoproterenol was calculated with the formula [(isoproterenol−CVT-2759 or adenosine/(isoproterenol−control))/100, where isoproterenol, CVT-2759 or adenosine, and control indicate measurements obtained in the presence of isoproterenol alone, isoproterenol plus CVT-2759 or adenosine, and in the absence of drugs, respectively. The paired Student’s t test was used for statistical analysis of paired data, and the 1-way repeated-measures ANOVA followed by Student-Newman-Keuls test was applied for multiple comparisons. A value of P<0.05 was considered statistically significant.

Results

Differential Attenuation of Twitch Shortening and Aftercontraction
Under present experimental conditions, a low concentration (15 mmol/L) of isoproterenol stimulated cell twitch shortening without inducing arrhythmic activity, whereas a high concentration (30 mmol/L) of isoproterenol induced DADs and aftercontractions as well as increasing the amplitude of twitch shortening. Therefore, by use of 15 and 30 mmol/L isoproterenol, we were able to examine the effects of CVT-2759 on twitch shortening of ventricular myocytes in the absence and presence of arrhythmic activity, respectively. Figure 1 shows the results obtained in the absence of arrhythmic activity. The amplitude of twitch shortening was increased by isoproterenol (15 mmol/L) from 2.6±0.3 to 5.8±0.7 μm (n=9, P<0.05). Neither DADs nor aftercontractions were observed. CVT-2759 (10 μmol/L) attenuated isoproterenol-stimulated twitch shortenings by 27±2%, decreasing the amplitude of twitch shortening to 4.9±0.5 μm (P<0.05). This effect of CVT-2759 (and all other effects of CVT-2759 observed in the present study) was reversed on washout of drug. CVT-2759 (10 μmol/L) alone had no effect on the resting membrane potential (−84±2 versus −84±1 mV, n=7).

Figure 2 shows data obtained in the presence of arrhythmic activity. In these experiments, isoproterenol (30 mmol/L) not
The actions of CVT-2759 on isoproterenol-stimulated twitch shortening were compared with those of the full agonist adenosine in the absence and presence of aftercontractions. In a group of 4 myocytes, isoproterenol (15 nmol/L) increased the amplitude of twitch shortening from 1.3 ± 0.2 to 5.4 ± 0.5 μm (P < 0.05) but did not induce aftercontractions. Adenosine at 10 μmol/L caused a 48 ± 4% attenuation of isoproterenol-stimulated twitch shortening, from 5.4 ± 0.5 to 3.5 ± 0.5 μm (P < 0.05). The amplitude of twitch shortening was further decreased to 2.1 ± 0.4 μm when the concentration of adenosine was increased to 100 μmol/L (P < 0.05).

In another group of myocytes (n = 8), isoproterenol (30 nmol/L) increased the amplitude of twitch shortening from 2.1 ± 0.4 to 5.0 ± 0.5 μm (P < 0.05) and induced aftercontractions. Adenosine at 10 μmol/L decreased the amplitude of aftercontractions from 0.68 ± 0.20 to 0.02 ± 0.01 μm (P < 0.05) but had no effect on the amplitude of twitch shortening (4.9 ± 0.5 versus 5.0 ± 0.5 μm) (Figure 2, A and C). However, 100 μmol/L adenosine reduced the amplitude of twitch shortening significantly, from 5.0 ± 0.5 to 3.7 ± 0.3 μm (P < 0.05) (Figure 3, A and C).

**Differential Attenuation of I_{Ca(L)} and I_{si}**

β-Adrenergic stimulation of I_{Ca(L)} increases Ca²⁺ influx into myocytes and thereby enhances cell contractility. Conversely, Ca²⁺ overloading of myocytes may cause Ca²⁺ release from the sarcoplasmic reticulum (SR) during diastole, which leads to induction of arrhythmic activity, such as aftercontractions and I_{si}. Although an A₁AdoR agonist is expected to attenuate isoproterenol-stimulated I_{Ca(L)}, the results shown in Figures 1 to 3 indicate that the effect of CVT-2759 on I_{Ca(L)} may be greater in the absence than in the presence of arrhythmic activity. CVT-2759 (10 μmol/L) attenuated isoproterenol-stimulated I_{Ca(L)} by 25 ± 3% when arrhythmic activity was absent (Figure 4). When the depolarizing pulses were applied to a higher potential (+20 mV) to facilitate the induction of I_{si}, isoproterenol (30 nmol/L) induced I_{si} as well as causing the expected increase of I_{Ca(L)}. The amplitude of I_{Ca(L)} became smaller when I_{si} appeared (not shown). CVT-2759 (10 μmol/L) suppressed I_{si} but not I_{Ca(L)}. In fact, I_{Ca(L)} was slightly increased after inhibition by CVT-2759 of I_{si} in some cells (Figure 5A). The effects of CVT-2759 were antagonized by the A₁AdoR antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX, 100 nmol/L, Figure 5A). In summary, isoproterenol increased the amplitude of I_{Ca(L)} from 29 ± 3 to 65 ± 14 pA/pF (n = 5, P < 0.05) and induced I_{si} with an amplitude of 14 ± 4 pA/pF. CVT-2759 decreased the amplitude of I_{si} to 3 ± 1 pA/pF (P < 0.05) but did not significantly reduce the amplitude of I_{Ca(L)} (64 ± 10 pA/pF) (Figure 5B).

**Lack of Effect on Atrial Myocytes**

CVT-2759 (10 μmol/L) did not significantly shorten the APD of atrial myocytes (Figure 6, A and B). The APD_{50} and APD_{90} were 96 ± 16 and 145 ± 21 ms in the absence and 91 ± 16 and 142 ± 21 ms in the presence of CVT-2759 (n = 6, P > 0.05). In contrast, in the same cells, adenosine (10 μmol/L) markedly shortened the APD_{50} and APD_{90} to 28 ± 4 and 43 ± 4 ms, respectively (P < 0.05).
Application of a ramp voltage-clamp pulse from −120 to +20 mV to atrial myocytes in the absence of drug elicited a background inward-rectifying K⁺ current (I_{K1}). Because the current-voltage relationships for I_{K(Ado)} and I_{K1} are similar, activation of I_{K(Ado)} is determined as an increase of the current in the presence of CVT-2759 or adenosine. The currents recorded in the absence of drug and in the presence of CVT-2759 (10⁻⁶ mol/L) were not significantly different (Figure 6C). The amplitude of the current in the presence of adenosine (10⁻⁶ mol/L), however, was significantly greater than the amplitude of the background current (Figure 6C), indicating an activation of I_{K(Ado)}. When measured at 0 mV, the amplitudes (n=4) of the background current and the currents in the presence of CVT-2759 and adenosine were 183±75, 200±70 (P<0.05 versus control), and 603±75 (P<0.05 versus control) pA, respectively (Figure 5D).

Inhibition of Spontaneous Ventricular Beats

The antiarrhythmic effect of CVT-2759 was further tested in isolated hearts by determining the effect of CVT-2759 on
iso-isoproterenol-induced spontaneous ventricular beats (Figure 6). Spontaneous ventricular beats were observed only in the presence of isoproterenol (30 nmol/L). CVT-2759 (10 μmol/L) alone had no effect on paced beats (not shown) but significantly reduced isoproterenol-induced spontaneous beats from 118±12 to 64±16 bpm (n=6, P<0.05).

Discussion

The results of this study confirm the hypothesis that the partial A_1 AdoR agonist CVT-2759 can selectively attenuate the proarrhythmic effect of isoproterenol to induce aftercontractions, DADs, and I_Ca,L without significantly reducing the contractility of ventricular myocytes and without affecting the electrical activity of atrial myocytes.

Potential Mechanism for the Differential Anti—β-Adrenergic Actions

The differential anti-β-adrenergic actions of CVT-2759 may involve the regulation of intracellular Ca^{2+} release. Release of Ca^{2+} from the SR during diastole is thought to be a common mechanistic step in the induction of aftercontractions and I_Ca,L.14 Because CVT-2759 inhibits both aftercontractions and I_Ca,L, it is likely that CVT-2759 attenuates isoproterenol-stimulated diastolic Ca^{2+} release. Consistent with this assumption, ryanodine (100 nmol/L), an inhibitor of SR Ca^{2+} release,15 caused an inhibitory effect similar to that of CVT-2759 on isoproterenol-stimulated DADs and aftercontractions.9 Diastolic Ca^{2+} release from the SR has been shown to cause a reduction of cell twitch shortening.17,18 We found that the amplitude of twitch shortening following an aftercontraction was smaller than that without a preceding aftercontraction (Figure 3, A and B). There is evidence that spontaneous Ca^{2+} release may cause a refractory period in Ca^{2+} release from the SR.19 and Ca^{2+} released from the SR may inactivate L-type Ca^{2+} channels.20 These observations suggest that diastolic Ca^{2+} release may reduce both intracellular Ca^{2+} release and extracellular Ca^{2+} entry during systole and thereby decrease the amplitude of cell twitch shortening. Thus, reduction by ryanodine of the diastolic Ca^{2+} release resulted in an increase of the twitch shortening.17,18 The underlying mechanism by which CVT-2759 attenuated isoproterenol-induced diastolic Ca^{2+} release is most likely inhibition of isoproterenol-stimulated cAMP formation and protein phosphorylation.5,13 Although the mechanisms of the actions of CVT-2759 and ryanodine are not the same, inhibition by CVT-2759 of diastolic Ca^{2+} release could also be expected to facilitate twitch shortening and I_Ca,L. Thus, the moderate, direct inhibitory effect of CVT-2759 on isoproterenol-stimulated twitch shortening (Figure 1) and I_Ca,L (Figure 4) may be well compensated by the facilitatory effect of a reduction of diastolic Ca^{2+} release on twitch shortening and I_Ca,L.

Although an inhibition of diastolic Ca^{2+} release from the SR alone can explain the selective anti-β-adrenergic actions of CVT-2759, a direct inhibition of the sodium-calcium exchange current cannot be ruled out as a potential mechanism to explain the attenuation by CVT-2759 of isoproterenol-induced I_Ca,L.21

Comparison of Actions of CVT-2759 and Adenosine

The actions of CVT-2759 were similar to those of adenosine at a low concentration. The difference was that when their concentrations were increased from 10 to 100 μmol/L, adenosine further attenuated the twitch shortening, whereas CVT-2759 did not (Figure 3). In other words, at a high concentration, the selectivity of action of adenosine decreases, whereas the selectivity of action of CVT-2759 remains. This is because the partial agonist CVT-2759 causes only a submaximal response7,8 compared with the full agonist adenosine. Although it is a low-efficacy agonist, however, CVT-2759 significantly attenuated isoproterenol-stimulated ventricular arrhythmic activity in intact hearts (Figure 7) as well as in isolated cells. These results suggest that CVT-2759 can be an effective antiarrhythmic drug.

Lack of Effect on Atrial Myocytes

Another major difference between the actions of CVT-2759 and adenosine was that adenosine activated I_K(Ado) and shortened the atrial APD, whereas CVT-2759 had little effect on atrial myocytes (Figure 6). Thus, CVT-2759 is a more selective antiarrhythmic drug than adenosine, not only because it causes less inhibition of twitch shortening of ventricular myocytes but also because it does not affect the action potentials of atrial myocytes. The lack of effect of CVT-2759 on atrial action potentials is probably due to a lack of effect of the drug on I_K(Ado). The differential effects of CVT-2759 on I_K(Ado) and on isoproterenol-stimulated I_Ca,L and I_h were expected and can be explained by the receptor reserve theory. That is, it has been shown that a higher occupancy of A_1 AdoRs is required for activation of I_K(Ado) than for antagonism of β-adrenergic stimulation.10 Thus, the full agonist...


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