Electrophysiological Effects of Carvedilol on Rabbit Heart Pacemaker Cells

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SUMMARY

The electrophysiological effects of carvedilol, a β -blocking agent with vasodilating actions, have been studied on rabbit pacemaker cells using the whole-cell patch clamp technique. Nystatin-perforated patch recordings from the sinoatrial (SA) and atrioventricular (AV) nodes demonstrated that 1-3 μ M of carvedilol caused a decrease in the spontaneous firing frequency, depolarization of the maximal diastolic potential, and prolongation of the action potential duration in both species. Voltage clamp experiments were performed using SA and AV node myocytes to identify and define the carvedilol-induced changes in the Ca^{2+} current, I_{Ca} , delayed rectifier K^{+} current, I_{K} , and hyperpolarization-activated inward current, I_{f} . In the SA node cells, 1 μ M of carvedilol blocked I_{K} , I_{Ca} , and I_{f} by 72%, 47%, and 22%, respectively. In the AV node cells, the corresponding reductions were 64% (I_{K}) and 46% (I_{Ca}), respectively. In both the SA and AV nodes the decrease in I_{K} appeared to be mainly due to the rapidly activating component of the delayed rectifier, I_{Kr} , since the high dose of carvedilol blocked I_{K} in the SA and AV nodes to a submaximal degree. In conclusion, effective doses of carvedilol have classical class III antiarrhythmic actions and a negative chronotropic effect resulting from the inhibition of I_{K} and I_{Ca} . Both actions may be efficacious for treating supraventricular tachyarrhythmias. (Int Heart J 2007; 48: 347-358)

Key words: Sinoatrial (SA) node, Atrioventricular (AV) node, Whole-cell patch clamp, Ca^{2+} current (I_{Ca}), Delayed rectifier K^{+} current (I_{K}), Hyperpolarization-activated inward current (I_{f}), β -blocking agent

CARVEDILOL is a new type of β -adrenoreceptor blocking agent with multiple potencies. In addition to its nonselective β -adrenergic blocking effect, it also has α_1 adrenergic blocking actions,¹⁾ antioxidant properties,^{2,3)} Ca^{2+} blocking effects,⁴⁾ and inhibitory effects on cardiac remodeling and myocyte apoptosis.⁵⁻⁷⁾ Recently, this agent has attracted increasing attention among cardiologists following positive outcomes in various clinical trials. For example, it has been shown that carvedilol produces dose-related improvements in left ventricular function and a

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reduction in mortality in patients with chronic heart failure,⁸⁻¹²⁾ and decreases secondary cardiac events after myocardial infarction.¹³⁾ Furthermore, it has also been suggested that this agent might have antiarrhythmic properties since it was reported to reduce the occurrence of sudden cardiac death in some clinical studies.^{8,10-12)} Indeed, it has been demonstrated that carvedilol suppresses ventricular arrhythmias in patients with hypertension, angina pectoris, and congestive heart failure, and reduces the frequency of lethal ventricular arrhythmias in severe heart failure.^{12,14)} However, it is not clear whether the reduction in these arrhythmic events is due to a direct action of the drug or secondary effects caused by improved cardiac function.

In animal experimental models, carvedilol has been shown to reduce reperfusion arrhythmias and digitalis-induced ventricular arrhythmias.¹⁵⁻¹⁷⁾ Recently, it has been reported that carvedilol can inhibit the rapidly activating component of the delayed rectifier K⁺ current (I_{Kr}) and L-type Ca²⁺ current (I_{Ca}) in rabbit ventricular myocytes.⁴⁾ Furthermore, it has recently been shown that carvedilol inhibits human ether-a-go-go related gene (HERG) potassium channels.¹⁸⁾ Thus, carvedilol has been recognized to have the ability to slow cardiac repolarization and produce a negative chronotropic effect. Consistent with this, a significant reduction in sinus node beating was observed in patients with acute myocardial infarction.¹³⁾ However, the ionic mechanisms underlying the carvedilol-induced action potential prolongation and negative chronotropic actions have not yet been fully elucidated. Since the pacemaker tissue of the sinoatrial (SA) and atrioventricular (AV) nodes is responsible for the pacemaking activity of the heart and is related to some types of arrhythmias such as sinoatrial nodal reentrant tachycardia, AV nodal reentrant tachycardia, and AV reentrant tachycardia using an accessory pathway, it is important to ascertain the effects of carvedilol on the automaticity of the nodal cells. Hence, we investigated the ionic mechanisms underlying the carvedilol-induced action potential prolongation and negative chronotropic actions using rabbit SA and AV node cells.

METHODS

This investigation conformed to the Guidelines for the Care and Use of Laboratory Animals of Asahikawa Medical College.

Isolation of nodal cells: Rabbits weighing 1.5-2.0 kg were anesthetized with sodium pentobarbital (50 mg/kg). The heart was quickly removed and then perfused retrogradely for 5 minutes on a Langendorff apparatus (37°C, 80 cm H₂O) with a Tyrode's solution containing 1.8 mM CaCl₂ to remove the blood, Ca²⁺-free Tyrode for 10 minutes, and then Ca²⁺-free Tyrode containing 0.04 mg/mL of collagenase (Yakult Co. Ltd., Tokyo). After 8 minutes of enzyme treatment, the ana-

tomical SA and AV nodal regions were removed, and the surrounding tissues were dissected away. The final preparations were cut into small pieces and stirred in Ca^{2+} -free HEPES-buffered Tyrode solution containing 1.0 mg/mL of collagenase (Sigma type 1), 0.3 mg/mL of elastase (Boehringer Mannheim, Germany), and 1% bovine serum albumin (BSA, Sigma Co. Ltd. Fraction V) for the SA nodal cells, and 1.0 mg/mL of collagenase and 1% BSA for the AV nodal cells. Every 3 minutes, aliquots of these solutions were aspirated and placed in KB solution. The isolated cells were collected by centrifugation for 5 minutes, and stored in KB solution with 1% BSA at 4°C.

Solutions and drugs: The HEPES-buffered Tyrode solution contained (in mM): NaCl 143, KCl 5.4, MgCl_2 0.5, CaCl_2 1.8, HEPES 5.0, and glucose 5.5 (pH = 7.4 adjusted with NaOH). The KB solution contained (in mM): KOH 70, L-glutamic acid 50, KCl 40, KH_2PO_4 20, MgCl_2 3.0, taurine 20, HEPES 10, EGTA 0.5, and glucose 10 (pH = 7.4 adjusted with KOH). The pipette filling solution used for nystatin-perforated patches contained (in mM): KCl 140, NaCl 6.0, MgCl_2 1.0, and HEPES 5.0, (pH = 7.2 adjusted with KOH). The pipette filling solution used for the conventional ruptured patch method contained (in mM): K^+ -aspartate 95, KCl 25, HEPES 5.0, EGTA 10, K_2ATP 3.0, CaCl_2 1.0, and MgCl_2 1.0 (pH = 7.2 adjusted with KOH).

Carvedilol (Daiichi Pharmaceuticals., Tokyo) was dissolved in DMSO and stored as a 10 mM stock solution.

Electrophysiological recordings: The electrical recordings were made using an Axopatch-1D amplifier (Axon Instruments, Foster City, CA, USA). The action potential measurements of the nodal cells were performed using the nystatin permeabilized patch method. Nystatin dissolved in DMSO (0.1 mg/ μL) was added to the pipette solution, giving a final concentration of 400 $\mu\text{g/mL}$. The pipette resistance, when filled with this solution, was 1.5-3.0 M Ω . A liquid junction potential of approximately 10 mV was corrected for. All recordings were made at 32-36°C. The electrical signals were analyzed using a pCLAMP7 (Axon Instruments). When possible, pooled data were expressed as the mean $\pm$ S.E. Statistical significance was determined by unpaired or paired *t* tests, as appropriate.

RESULTS

Effects of carvedilol on automaticity and spontaneous action potentials: The effects of carvedilol on the automaticity and spontaneous action potentials of the SA and AV nodal cells were studied using the nystatin-permeabilized patch method. Figures 1 and 2 show representative tracings recorded before and during superfusion with 1 μM of carvedilol. Superfusion with 1 μM of carvedilol caused decreases in the spontaneous firing frequency, maximal diastolic potential, and prolonga-

Figure 1. Effect of carvedilol on spontaneous pacing and action potentials in rabbit SA nodal cells. Representative tracing of action potentials from a single SA nodal cell in the presence and absence of 1 μ M of carvedilol.

Figure 2. Effect of carvedilol on spontaneous pacing and action potentials in rabbit AV nodal cells. The spontaneous electrical activity of an AV nodal cell before and after superfusion with 1-3 μ M of carvedilol.

tion of the action potential duration in both the SA and AV nodal cells. An increase in the concentration of carvedilol to 3 μ M exaggerated these changes, finally leading to the cessation of any automatic activity in both the SA and AV

Figure 3. Effects of carvedilol on the L-type Ca^{2+} current (I_{Ca}) and delayed rectifier K^{+} currents, I_{K} , in rabbit SA nodal cells. The upper panel shows representative recordings of membrane currents in the presence and absence of carvedilol (1 μM). The current-voltage relations for the I_{Ca} in the absence and presence of carvedilol are plotted in the lower left panel ($n = 4$). The lower middle and right panels show representative time-dependent outward current and tail currents under control conditions and after the application of carvedilol (1 μM).

nodal cells (data not shown). In 7 such experiments in the AV nodal cells, superfusion with 1 μM of carvedilol decreased the spontaneous firing frequency, maximal diastolic potential, and maximum rate of depolarization from 214.8 ± 16.7 / min to 170.3 ± 16.5 / min ($P < 0.01$), -59.0 ± 3.0 mV to -55.9 ± 3.6 mV ($P < 0.05$), and 4.17 ± 0.63 V/s to 3.54 ± 0.47 V/s ($P < 0.05$), respectively. The action potential duration at 90% increased from 116.9 ± 7.0 ms to 139.8 ± 10.8 ms ($P < 0.05$).

Figure 4. Effects of carvedilol on the L-type Ca^{2+} current (I_{Ca}) and delayed rectifier K^{+} currents, I_{K} , in rabbit AV nodal cells. The upper panel shows representative membrane currents under control conditions (left) and after the application of carvedilol (1 μM). The voltage clamp protocols were the same as those described in the previous figure. The lower panels show plots of the I_{Ca} , time dependent outward current, and initial magnitude of the deactivating tail currents under control conditions and after the application of 1 μM of carvedilol ($n = 4$).

Similar results were obtained in a total of 2 experiments in the SA nodal cells.

Effects of carvedilol on L-type Ca^{2+} current (I_{Ca}) and delayed rectifier K^{+} currents, I_{K} , in rabbit SA and AV nodal cells: The effects of carvedilol on the L-type Ca^{2+} current (I_{Ca}) and delayed rectifier K^{+} currents, I_{K} , in the SA and AV nodes were investigated using a conventional membrane-rupturing method. Figures 3A and

4A show the changes in the membrane currents obtained from a step depolarization from a holding potential of -40 mV to various potential levels before and during the application of 1 μ M of carvedilol in the SA (3A) and AV (4A) nodal cells. Figures 3B and 4B show the current-voltage relationships for the Ca^{2+} current (I_{Ca}), steady-state currents (I_{ss}), and I_{K} tail. The results of these 4 experiments on the SA nodal cells are summarized as follows. During the control, the peak I_{Ca} recorded at -10 mV measured 306 ± 109 pA. The addition of 1 μ M of carvedilol decreased I_{Ca} to 130 ± 78 pA ($P < 0.01$). The I_{ss} and I_{K} tail recorded during repolarization from -40 to 20 mV decreased from 265 ± 25 pA to 147 ± 17 pA and 85 ± 20 pA to 23 ± 5 pA, respectively ($P < 0.01$ and $P < 0.05$). In the AV nodal cells,

Figure 5. Effect of carvedilol on the hyperpolarization-activated inward current (I_f) in rabbit SA nodal cells. Representative recordings of whole cell currents from a rabbit SA nodal cell. The upper panel shows the currents elicited by hyperpolarizing pulses from -50 to -100 mV in 10 mV decrements. The current-voltage relationships for the I_f in the SA node are plotted in the lower panel ($n = 8$).

Figure 6. Selectivity of the ionic channel block by carvedilol in rabbit SA nodal cells. The axis of the abscissa denotes the concentration of carvedilol. The vertical line denotes the percentage of the inhibition of each current in the presence of carvedilol calculated by the following equation: $(1 - (\text{current elicited after the administration of carvedilol}) / (\text{current elicited in the control state})) \times 100 (\%)$.

I_{Ca} recorded at -10 mV, and the I_{SS} and I_K tails recorded during repolarization from -40 to 20 mV decreased from 495 ± 103 pA to 231 ± 115 pA, 193 ± 10 pA to 135 ± 23 pA, and 114 ± 41 pA to 50 ± 28 pA, respectively ($n = 4$; $P < 0.05$, $P < 0.05$ and $P < 0.01$).

Effects of carvedilol on the hyperpolarization-activated inward current (I_f) in rabbit SA nodal cells: Figure 5A shows the effect of carvedilol on the hyperpolarization-activated inward current (I_f) in the step hyperpolarization from a holding potential of -40 mV in the SA nodal cells. Figure 5B shows the current-voltage relationships for I_f during superfusion with $1 \mu\text{M}$ of carvedilol. As shown in Figure 5B, I_f was slightly reduced by carvedilol. When the data in the 8 experiments were averaged, I_f recorded at -100 mV decreased from 321 ± 111 pA to 238 ± 92 pA ($P < 0.05$).

Selectivity of the ionic channel block by carvedilol in the rabbit SA node: The percentage of inhibition caused by carvedilol for each current was plotted as a function of the drug concentrations in Figure 6. I_K , I_{Ca} , and I_f were measured on repolarization to 20 mV, depolarization to -10 mV, and hyperpolarization to -100 mV from a holding potential of -40 mV, respectively. Carvedilol blocked such ionic currents in a dose-dependent manner in the decreasing order of I_K , I_{Ca} , and I_f in the SA nodal cells. The percentage inhibition was 72%, 47%, and 22%, respectively, at $1 \mu\text{M}$ of carvedilol. In the AV nodal cells, the corresponding reductions were 64% (I_K) and 46% (I_{Ca}), respectively (data not shown).

DISCUSSION

In the present study, we demonstrate that carvedilol suppressed automaticity and repolarization in rabbit nodal cells. This agent prolonged the spontaneous cycle length and reduced the maximum rate of depolarization. In addition, it caused a prolongation of the action potential duration, and decreased the maximal diastolic potential and action potential amplitudes. In the voltage clamp experiments, carvedilol was shown to block I_K , I_{Ca} , and I_f , especially I_K . Therefore, this agent was proven to have negative chronotropic effects and class III antiarrhythmic actions.

It was previously reported that carvedilol blocks I_{Kr} and L type Ca currents in rabbit ventricular myocytes.⁴⁾ Furthermore, carvedilol has been shown to inhibit human ether-a-go-go-related gene (HERG) K^+ channels.¹⁸⁾ However, the mechanisms underlying the possible negative chronotropic actions of this agent have not yet been fully elucidated. This is the first study demonstrating that carvedilol possesses direct negative chronotropic effects. Although we did not conduct experiments using blockers of the slowly activating component of the delayed rectifier, I_{Ks} , the majority of the blocked components by carvedilol were considered to be I_{Kr} since it had been reported that the contribution of I_{Ks} to the spontaneous pacemaker rate was small under control conditions in the rabbit SA node, and I_{Kr} which predominates in the rabbit AV node was blocked to a sub-maximal degree by carvedilol.¹⁹⁻²¹⁾ Cheng, *et al* reported that carvedilol exhibited a bell-shaped frequency and dose-response curve of the action potential duration.⁴⁾ In our experiments, carvedilol prolonged the action potential duration in a dose-dependent fashion in the range of 0.1-3 μM , which was considered to be caused by a greater I_K block in comparison to the I_{Ca} block in that dose range.

The effects of adrenergic stimulation of the SA node have been reported to make the slope of the slow diastolic depolarization steeper, increase the maximum rate of depolarization, and shorten the action potential duration. Hence, one of the mechanisms of the antiarrhythmic action of β -adrenergic blocking agents is considered to be to antagonize those effects in the presence of β -adrenergic stimulation. Indeed, it has been shown that atenolol has no effect on the repolarization of the SA node, whereas it has an inhibitory effect on the acceleration of the repolarization phase in the presence of β -receptor stimulation.²²⁾ On the other hand, some β -adrenergic blocking agents have been demonstrated to have a direct membrane action which differs from the membrane stabilizing action of a Na channel block. Namely, it has been demonstrated that pindolol and propranolol cause a prolongation of the action potential duration and negative chronotropic effect in the rabbit SA node, although the concentration used in that study was 5-50 times higher than that in clinical use.²³⁾ The study also demonstrated that both

drugs inhibited the slow inward current and time-dependent outward current, but produced different actions on I_f (propranolol inhibited I_f , but pindolol did not affect it). Furthermore, the α_1 - and β -adrenoreceptor blocking drugs labetalol and medroxalol have been shown to reduce the maximum rate of depolarization and prolong the action potential duration in the rabbit SA node.²⁴⁾ Thus, it has been suggested that some β -adrenoreceptor blocking drugs have a potency equal to class III or class IV antiarrhythmic actions.

In the present study, the effect of carvedilol on the action potential was evident at 0.1 μ M. The therapeutic range of the plasma concentration of carvedilol has been reported to be 0.1-0.6 μ M.^{25,26)} Therefore, carvedilol is considered to have a direct membrane action like that of class III antiarrhythmic drugs, with a negative chronotropic effect. On the other hand, the contribution of the I_f block in the negative chronotropic effect caused by carvedilol may be small based on our experimental results.

It is well-known that nodal tissue is related to some types of reentrant tachyarrhythmias such as AV nodal reentrant tachycardia, AV reentrant tachycardia using an accessory pathway, and sinus nodal reentrant tachycardia. Furthermore, it has recently been suggested that tissue similar to nodal cells is present in the pulmonary veins in experimental animals and in human pulmonary vein preparations in patients with atrial fibrillation.²⁷⁻²⁹⁾ Karle, *et al* reported that the potency of carvedilol for an I_{Kr} block encoded by the HERG might be beneficial for ventricular arrhythmias, thereby resulting in the positive outcomes of various clinical trials in patients with chronic heart failure and postmyocardial infarction.¹⁸⁾ Since supraventricular tachyarrhythmias leading to atrial fibrillation can affect cardiac function, carvedilol's effect on I_K and I_{Ca} observed in our study, at least in part, may be responsible for the good prognosis in various clinical trials. Of course, carvedilol has a number of ancillary activities, including antioxidant, anti-inflammatory, and antiapoptotic properties in addition to the well-known properties,^{2-3,5)} and hence, some unknown antiarrhythmic activities might exist. Further studies will be required to elucidate these points.

In conclusion, effective doses of carvedilol have classical class III antiarrhythmic actions and a negative chronotropic effect resulting from the inhibition of I_K and I_{Ca} in rabbit SA and AV node cells. Both actions may be effective in the treatment of supraventricular tachyarrhythmias in the clinical setting.

Limitations: It has been demonstrated that the electrical characteristics, including the ion channel activity of the SA and AV nodes, are heterogeneous.³⁰⁻³²⁾ In the present study, we attempted to isolate and use spindle and ovoid cells of the SA and AV nodes, respectively. However, some rod cells, which are slightly different electrically from true nodal cells, might have been included in some experiments, and this might have caused the variability in the effects of carvedilol on

the ionic currents in the SA and AV nodal cells. This may be an important limitation in the quantitative analysis of the drug in this study. Further studies using other agents such as I_{K_r} blockers or I_{K_s} blockers will be needed to clarify this point.

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