Adrenergic receptor antagonist prevents the left ventricle with chronic pressure-overload from electrical remodeling

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Abstract: Experiments were performed to investigate the effects of long-term treatment with adrenergic receptor antagonist on electrical remodeling of the left ventricle with chronic pressure-overload. New Zealand rabbits underwent subtotal banding of suprarenal abdominal aorta. At 10 weeks after surgery, echocardiography examination was performed, then action potential (AP), inward rectifier potassium current (I_K), delayed rectifier potassium current (I_K) and Na+/Ca2+ exchanger current (I_Na,K,Ca) were recorded in midmyocardial cells isolated from left ventricle of abdominal aorta banded group (banded group), abdominal aorta banding plus Carvedilol intervention group (Carvedilol group), and normal control group rabbits by using the whole-cell patch-clamp techniques. The results showed that left ventricular mass index in control, banded, and Carvedilol groups were 1.78±0.06 (n=7), 2.33±0.11 (n=7), and 1.87±0.08 (n=7), respectively (banded vs control and Carvedilol, P<0.01). At basic cycle length of 2 s, AP duration (measured at 90% repolarization, APD90, ms) in control, banded, and Carvedilol groups were 522.0±19.5 (n=6), 664.7±46.2 (n=7), 567.8±14.3 (n=8) respectively (banded vs control, P<0.01; Carvedilol vs banded, P<0.05). At test potential of –100 mV, inward I_K density (pA/pF) in control, banded, and Carvedilol groups were –11.8±0.50 (n=8), –8.07±0.28 (n=8), –10.69±0.35 (n=8) respectively (banded vs control and Carvedilol, P<0.01). At test potential of +50 mV, I_K tail current density (pA/pF) in control, banded, and Carvedilol groups were 0.59±0.04 (n=8), 0.40±0.02 (n=9), 0.51±0.02 (n=8) respectively (banded vs control, P<0.01; Carvedilol vs banded, P<0.05). At test potential of +60 mV, outward I_Na,K,Ca density (pA/pF) in control, banded, and Carvedilol groups were 1.06±0.11 (n=8), 1.54±0.10 (n=9), 1.24±0.07 (n=8), respectively (banded vs control and Carvedilol, P<0.01). At test potential of –120 mV, inward I_K density (pA/pF) in control, banded, and Carvedilol groups were –5.5±0.04 (n=12), –1.00±0.02 (n=12), –1.20±0.02 (n=12) respectively (banded vs control, P<0.01; Carvedilol vs banded, P<0.05). It is shown that long-term treatment with Carvedilol not only prevents development of cardiac hypertrophy, but also improves the electrophysiological alterations in rabbit hearts with chronic pressure-overload. This finding may add new electrophysiological evidence for the treatment of heart failure and hypertension with adrenergic receptor antagonist.

Key words: physiology; patch-clamp techniques; Carvedilol; ventricular remodeling; ionic channel

肾上腺素能受体阻断剂预防慢性压力超负荷左心室的电重构

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摘 要: 研究长期使用肾上腺素能受体阻断剂治疗对慢性压力超负荷左心室电重构的影响。新西兰兔通过肾上腹主动脉次全结扎诱发慢性压力超负荷，10周后行心脏超声检查，并采用单细胞膜片钳技术分别记录腹主动脉结扎组（简称结扎组）、腹主动脉结扎+Carvedilol干预组（简称Carvedilol组）及正常对照组中层细胞的动作电位（action potential，AP）、内向整流钾电流（inward rectifier potassium current，I_K）、延迟整流钾电流（delayed rectifier potassium current，I_K）及Na+/Ca2+交换体电流。结果表明，结扎组的左室质量指数较对照组明显升高，Carvedilol组较结扎组明显降低（P<0.01）。在2 s的基础周期下，动作电位持续时间（以90%的复极时间为界，简称APD90）在对照组、结扎组及Carvedilol组分别为522.0±19.5 ms（n=6）、664.7±19.5 ms（n=6）、567.8±14.3 ms（n=8）。

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46.2 ms (n=7), 567.8 ± 14.3 ms (n=8), 尾结扎组同对照组相比, P<0.01, Carvedilol组同结扎组相比, P<0.05。在测试电位为 -100 mV 时, \( I_k \) 电流密度 （pA/pF）在对照组、结扎组及 Carvedilol 组分别为 -11.8 ± 0.50 (n=8), -8.07 ± 0.28 (n=8), -10.69 ± 0.35 (n=8), 结扎组与对照组及 Carvedilol 组相比, P<0.01。在测试电位为 +50 mV 时, \( I_{Na}^{\text{p}} \) 电流密度 （pA/pF）在对照组、结扎组及 Carvedilol 组分别为 0.59 ± 0.40 (n=8), 0.40 ± 0.02 (n=9), 0.51 ± 0.01 (n=8), 结扎组与对照组相比, P<0.01, Carvedilol组与结扎组相比, P<0.05。在测试电位为 +60 mV 时, 外向 \( I_{Na}^{\text{p}} \) 电流密度 （pA/pF）在对照组、结扎组及 Carvedilol 组分别为 1.06 ± 0.11(n=8), 1.54 ± 0.10 (n=9), 1.24 ± 0.07(n=8), 结扎组同对照组及 Carvedilol 组相比, P均<0.01。在测试电位为 -120 mV 时, 内向 \( I_{Na}^{\text{p}} \) 电流密度 （pA/pF）在对照组、结扎组及 Carvedilol 组分别为 -0.54 ± 0.06(n=8), -0.75 ± 0.04(n=9), -0.60 ± 0.03(n=8), 结扎组同对照组相比, P<0.01, Carvedilol组与结扎组相比, P<0.05。结果显示, Carvedilol 长期干预不仅可以预防慢性压力超负荷兔左心室肥厚, 而且可以明显改善其电重构特征。这一发现为其临床用于高血压及心力衰竭的治疗提供新的电生理依据。

**Keywords:** physiology; patch clamp technique; Carvedilol; heart remodeling; ion channels

**Chinese Classification Number:** Q463

Myocardial hypertrophy and failure is a major risk factor predisposing to the occurrence of ventricular arrhythmia and sudden cardiac death, which is thought to be strongly associated to the alterations of electrophysiological characteristics in diseased hearts, including prolongation of action potential (AP), down-regulation of repolarization potassium current, and up-regulation of Na/Ca2+ exchanger current (\( I_{Na}\text{,Ca}^{2+} \)). So preventing or reversing electrical remodeling should also be a clinical therapeutic target. The COPERNICUS trial shown that Carvedilol, a new adrenergic receptor antagonist, reduced mortality of heart failure patients significantly. This favorable effect was thought to partly due to the lowering of the incidence of malignant ventricular arrhythmia, but its electrophysiological mechanism is unclear. Based on these observations, the present study was designed to examine the effect of long-term treatment with Carvedilol on the electrical remodeling of left ventricle (LV) with chronic pressure-overload, and to supply electrophysiological evidence for its clinical application.

**1 MATERIALS AND METHODS**

1.1 Animal models. Adult healthy New Zeland rabbits of either sex (2.0~2.5 kg, supplied by Experimental Animal Center, Xi'an Jiaotong University) underwent subtotal abdominal aorta banding as previously described. After anesthesia with intravenous administration of sodium pentobarbital (30~50 mg/kg), the superrenal abdominal aorta was surgically isolated. A 5 F or 6 F catheter and the isolated aorta portion were ligated together, and the catheter was immediately removed. This procedure induced an aortic diameter reduction of approximately 50%. After surgery, the animals were randomly devided into abdominal aorta banded group (banded group) and abdominal aorta banding plus Carvedilol intervention group (Carvedilol group).

Age and weight matched normal rabbits were selected as control group. Carvedilol (1 mg/kg·d, twice everyday) was administrated to Carvedilol group rabbits by oral gavage 1 d before sacrifice. Interventricular septal thickness (IVST), LV posterior wall thickness (PWT), LV end diastolic diameter (LVEDD), LV end systolic diameter (LVEDS), ejection fraction (EF), fractional shortening (FS) were measured by an experienced echocardiographer which was blinded to the animal group. LV mass (LVM) = [(LVEDD+PWT+IVST)×LVEDD] × 1.04, LVM index (LVMI) = LVM/body weight, LV end diastolic volume (LVEDV) = 1.047×LVEDD³, LVEDV index (LVEDVI) = LVEDV/body weight.

1.2 Echocardiography examination. Echocardiography examinations (HP Sonos 2500, 7.5/5.5 MHz) were performed 1 d before sacrifice. Interventricular septal thickness (IVST), LV posterior wall thickness (PWT), LV end diastolic diameter (LVEDD), LV end systolic diameter (LVEDS), ejection fraction (EF), fractional shortening (FS) were measured by an experienced echocardiographer which was blinded to the animal group. LV mass (LVM) = [(LVEDD+PWT+IVST)×LVEDD] × 1.04, LVM index (LVMI) = LVM/body weight, LV end diastolic volume (LVEDV) = 1.047×LVEDD³, LVEDV index (LVEDVI) = LVEDV/body weight.

1.3 Myocyte isolation. The rabbit hearts were quickly removed, cannulated on a Langendorff apparatus and first perfused with Ca2+ free Tyrode's solution for 5 min, then with Ca2+ free Tyrode's solution containing 0.4 mg/ml collagenase I (Sigma, USA), 0.1 mg/ml protease (Sigma, USA) and 1 mg/ml BSA for 20~25 min, and finally with modified KB solution for 5 min. The tissue samples were dissected from midmyocardial layers of left ventricle free wall, minced with fine surgical scissors in KB solution, then passed through a 200 µm stainless steel filters. The filtrate was incubated in a 37°C homoiothermic cradle for 10 min, then washed twice into KB solution. After 1 h, the KB solution was exchanged into Tyrode's solution to restore the normal Ca2+ concentration for further experiment. The cells suspension was stored at room temperature for
using.

1.4 Electrophysiological recordings. The whole-cell patch-clamp technique was used for recording the membrane currents and action potentials. An aliquot of cell was placed into the recording chamber on the stage of an inverted microscope. Five minutes were allowed for cell adhesion to the coverslip at the bottom of the chamber, and then the cells were superfused with extracellular solution at a rate of 2–3 ml/min for about 3–5 min. Only quiescent rod-shaped cells with clear cross-striations were used. The glass pipettes made by using horizontal microelectrodes puller (Sutter P-97, USA) had a resistance of 2–4 MΩ.

PETTES made by using horizontal microelectrodes puller cells with clear cross-striations were used. The glass pipettes were filled with the pipette solution. After a high-resistance seal had been attained (seal resistance >2 GΩ), the membrane was ruptured with gentle suction. Cell membrane capacitance was measured by applying a 5 mV hyperpolarization pulse from a holding potential of ~40 mV, then both cell membrane capacitance and series resistance were compensated. Test protocols were produced by a Axopatch 200 B amplifier and Clampex 8.1 software. Acquired signals were filtered at 2 kHz lowpass and digitized by A/D conversion (Digidata 1200A, Axon instrument, USA), then stored in a compatible computer.

1.5 Solutions. The composition of solutions employed is as follows (in mmol/L): Tyrode’s solution: NaCl 143, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.5, NaH₂PO₄ 0.25, HEPES 5.0, and glucose 5.6 (pH was adjusted to 7.35 with NaOH). Ca²⁺-free Tyrode’s solution was the same as Tyrode’s solution except that lacking CaCl₂. KB solution: L-glutamic acid 70, KCl 80, aspartate 42.0, MgCl₂ 13.0, HEPES 10.0, and glucose 10.0 (pH was adjusted to 7.35 with KOH). The extracellular solution for AP was Tyrode’s solution for AP: KOH 50, KCl 80, aspartate 40, HEPES 5.0, EGTA 10, MgATP 5.0, sodium creatinine phosphate 5.0, and CaCl₂ 0.65 (pH was adjusted to 7.35 with KOH). The extracellular solution for AP was Tyrode’s solution, the pipette solution for AP: KOH 50, KCl 80, aspartate 40, HEPES 5.0, EGTA 10, MgATP 5.0, sodium creatinine phosphate 5.0, and CaCl₂ 0.65 (pH was adjusted to 7.35 with KOH).

1.6 Statistics. Values were expressed as means ± SD, and statistical analysis was performed using one-way ANOVA, P<0.05 was considered significant.

2 RESULTS

2.1 Echocardiography assessment

At 10 weeks after surgery, both LVMI and LVEDVI in banded group increased significantly over control group. In Carvedilol group, both LVMI and LVEDVI decreased significantly over banded group. This indicates that ventricular remodeling occurred in banded group animals, and Carvedilol could prevent this change effectively (Table 1).

2.2 Action potential (AP)

APs were recorded in current-clamp mode and were elicited by 4 ms long rectangular pulses of depolarization current at basic cycle length (BCL) of 2 s, the current level was about 50% above threshold. At BCL of 2 s, AP duration (measured at 90% depolarization, APD₉₀) was prolonged significantly in myocytes from Carvedilol group compared with control group (664.7±46.2 ms, n=7 vs 522.0±19.5 ms, n=6, P<0.01), and was shortened significantly in myocytes from Carvedilol group compared with banded group (567.8±14.3 ms, n=8 vs 664.7±46.2 ms, n=7, P<0.01).

2.3 Na⁺/Ca²⁺ exchanger current (I_{Na⁺Ca²⁺})

I_{Na⁺Ca²⁺} was recorded in voltage-clamp mode and measured.

<table>
<thead>
<tr>
<th>Table 1. Echocardiography assessment (n=7)</th>
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<tr>
<td>LVMI (g/kg)</td>
</tr>
<tr>
<td>Control group</td>
</tr>
<tr>
<td>Banded group</td>
</tr>
<tr>
<td>Carvedilol group</td>
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LVMI, left ventricular mass index; LVEDVI, left ventricular end diastolic volume index. *P<0.01 vs control group, **P<0.01 vs banded group, P<0.05 vs banded group.
sured as Ni\(^{2+}\)-sensitive current. Myocytes were depolarized to \(-40\) mV from a holding potential of \(-90\) mV to inactivate sodium current. The voltage was then stepped to \(+80\) mV and ramped down to \(-140\) mV at a speed of 110 mV/s to induce remaining currents, both inward and outward currents were recorded simultaneously under this condition. After the currents reached a steady state, the protocol was repeated in the presence of 5 mmol/L NiCl\(^{2+}\). In this way, \(I_{Na^{+}/Ca^{2+}}\) was measured as the difference between currents in the absence and presence of 5 mmol/L NiCl\(^{2+}\) \(^{[5]}\). Figure 1 shows the representative \(I_{Na^{+}/Ca^{2+}}\) recording. Both inward and outward \(I_{Na^{+}/Ca^{2+}}\) density were up-regulated in ventricular myocytes from banded group compared with control group, Carvedilol could ameliorate this change (see details in Table 2).

### Table 2. \(I_{Na^{+}/Ca^{2+}}\) density in ventricular myocytes from different group animals

<table>
<thead>
<tr>
<th>Group</th>
<th>(I_{Na^{+}/Ca^{2+}}) (pA/pF)</th>
<th>-120 mV</th>
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</thead>
<tbody>
<tr>
<td>Control group (n=8)</td>
<td>1.06 ± 0.11</td>
<td>-0.54 ± 0.06</td>
</tr>
<tr>
<td>Banded group (n=9)</td>
<td>1.54 ± 0.10*</td>
<td>-0.75 ± 0.04*</td>
</tr>
<tr>
<td>Carvedilol group (n=8)</td>
<td>1.24 ± 0.07*</td>
<td>-0.60 ± 0.03*</td>
</tr>
</tbody>
</table>

\*P<0.01 vs control group; \^P<0.01, \#P<0.05 vs banded group.

### 2.4 Inward rectifier potassium current (\(I_{Ki}\))

\(I_{Ki}\) was recorded by applying voltage-clamp steps for 300 ms from a holding potential of \(-40\) mV to different depolarization levels between \(-100\) and \(+20\) mV in increments of 10 mV. The steady-state currents at the end of test pulses were measured as \(I_{Ki}\). At the test potential of \(-100\) mV, inward currents density of \(I_{Ki}\) (pA/pF) were \(-11.8 ± 0.50\) (n=8), \(-8.07 ± 0.28\) (n=8), \(-10.69 ± 0.35\) (n=8) in left ventricular myocytes from control, banded and Carvedilol group rabbits, respectively (banded vs control and Carvedilol, P<0.01). At the test potential of \(-50\) mV, outward currents density of \(I_{Ki}\) (pA/pF) was \(1.49±0.29\), \(1.01 ± 0.13\), \(1.41 ± 0.26\) in left ventricular myocytes from control, banded and Carvedilol group respectively, and there was no significant difference among them. Representative recording and current-voltage relationship curves of \(I_{Ki}\) are shown in Fig. 2.

Fig. 1. Representative recording of \(I_{Na^{+}/Ca^{2+}}\). A: Current in the absence of 5 mmol/L NiCl\(^{2+}\). B: Current in the presence of 5 mmol/L NiCl\(^{2+}\). A-B \(I_{Na^{+}/Ca^{2+}}\) (Ni\(^{2+}\) sensitive current).

Fig. 2. A: Representative recording of \(I_{Ki}\). B: Current-voltage relationship curves of \(I_{Ki}\) in different groups. \^P<0.01, \#P<0.05 vs control; \*P<0.01 vs banded.
2.5 Delayed rectifier potassium current (I_K)

I_K was recorded by applying voltage-clamp steps for 3000 ms from a holding potential of −40 mV to different depolarization levels between −40 and +50 mV in increments of 10 mV. The tail current density of I_K (I_K,tail) was measured as an index of I_K. At the test potential of +50 mV, I_K,tail density (pA/pF) in ventricular myocytes from control, banded and Carvedilol groups was 0.59±0.04 (n=8), 0.40±0.02 (n=9), 0.51±0.02 (n=8), respectively (banded vs control, P<0.01; Carvedilol vs banded, P<0.05). Representative recording of I_K and current-voltage relationships of I_K,tail are shown in Fig. 3.

3 DISCUSSION

Chronic pressure-overload is the common reason for myocardial hypertrophy and failure. In the present study, we successfully developed a chronic pressure-overload model in rabbit hearts by subtotal banding of abdominal aorta. The results showed that LVMII, LVEDVI in banded group animals increased significantly, which indicated that ventricular remodeling occurred; meanwhile, electrophysiological characteristics also altered, including APD_{90} prolongation, I_K and I_{Ks} down-regulation, and I_{Na+/Ca^{2+}} up-regulation, which is consistent with the other researches and is thought to be strongly associated with the high incidence of ventricular arrhythmia resulting from myocardial hypertrophy and failure[5-8]. Furthermore, recent evidence has indicated that the structural remodeling and electrical remodeling in hypertrophied heart are partly dissociable[9]. So preventing or reversing electrical remodeling should also be a target of clinical therapy.

It has been shown that excessive activation of sympathetic nervous system (SNS) plays a key role in the development of myocardial hypertrophy and failure, and the prognosis of patients is related to plasma concentration of norepinephrine. Furthermore, a strong link has been demonstrated between cardiac sympathetic nervous stimulation and occurrence of ventricular arrhythmias, both under experimental conditions and clinically[10], which may be associated with the effects of sympathetic nerve stimulation on the ventricular electrical remodeling. In ventricular myocytes isolated from human end-stage failing hearts, norepinephrine induces AP prolongation and after-depolarization[11]. In cultured adult guinea pig cardiomyocytes, sustained isoproterenol stimulation decreases the density of the I_{Ks} and L-type calcium current, increases both inward and outward I_{Na+/Ca^{2+}} currents, which resembles the changes in myocardial hypertrophy and failure and was prevented by propranolol[12,13]. All these results suggest that excessive activation of SNS is directly involved in the electrical remodeling in myocardial hypertrophy and failure. Theoretically, long-term blockage of overactive SNS should be beneficial in preventing or attenuating electrical remodeling, but both clinical and experimental evidence is lacking.

Carvedilol is a non-selective adrenergic receptor antagonist with potent antioxidant activity. Both clinical and experimental studies have shown that long-term treatment with Carvedilol produces beneficial effects on left ventricle structural remodeling and lowers mortality[2,14,15]. But its effect on electrical remodeling has not been reported. In the present study, we found that 10-weeks oral administration of Carvedilol in rabbits with pressure-overload not only prevented development of cardiac hypertrophy, but also affects the electrophysiological alteration. APD pro-
longation resulting from chronic pressure-overload was attenuated significantly in Carvedilol treated rabbits, which was due to $I_{K_C}$ and $I_C$ up-regulation, and $I_{Na^+/Ca^{2+}}$ down-regulation. This action maybe underlies the effect of Carvedilol on lowering the incidence of ventricular arrhythmia and sudden cardiac death in patients with myocardial hypertrophy and failure.

The mechanisms of Carvedilol's beneficial effects on electrical remodeling are not quite clear. Although Carvedilol itself is a kind of antiarrhythmic drug, the electrophysiological changes in APD, $I_{K_C}$, $I_C$, and $I_{Na^+/Ca^{2+}}$ observed in the present study are likely independent of its direct antiarrhythmic action. Previous studies on single rabbit myocytes showed that direct perfusion with bath solution containing Carvedilol prolonged APD, reduced $I_{K_C}$, $I_C$, and $I_P$ by a dose-dependent mode. Interestingly, this is contrary to the effects of long-term oral administration of Carvedilol in the present study. Furthermore, in the present study, the last oral administration of Carvedilol was given 48 h before myocyte isolation, the hearts and myocytes were perfused with a great quantity of solutions not containing Carvedilol, so its direct antiarrhythmic actions could be excluded. Previous study in cultured bovine adrenal medullary cells showed that short and prolonged exposure to Carvedilol produces contrary effects on voltage-dependent $Na^+$ channels, prolonged exposure is believed to influence the synthesis of channel protein. In the present study, we think that long-term treatment with Carvedilol probably produces beneficial effects on electrical remodeling by blocking excessive activation of SNS, and then influences the expression and synthesis of channel protein.

In summary, we observed increasement of LVMI, LVEDVI, prolongation of APD, down-regulation of $I_C$, and up-regulation of $I_{Na^+/Ca^{2+}}$ in rabbit hearts with chronic pressure-overload induced by subtotal banding of abdominal aorta. These abnormalities were attenuated significantly by the long-term treatment with Carvedilol. This may add new electrophysiological evidence for the treatment of heart failure and hypertension by means of adrenergic receptor antagonists.

**REFERENCES**