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Comparison between effects of AMP579 and adenosine on Na^+/Ca^{2+} exchanger in isolated rat ventricular myocytes

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Abstract: AIM To elucidate possible mechanisms underlying the differences between 1*S*-[1*a*,2*b*,3*b*,4*a*(*S**)]-4-[7-[[1-(3-chloro-2-thienyl) methylpropyl] propyl-amino]-3*H*-imidazo [4, 5-*b*] pyridyl-3-yl]-*N*-ethyl-2, 3-dihydroxycyclopentane carboxamide (AMP-579) and adenosine in pharmacological and clinical effects. **METHODS** Na^+/Ca^{2+} exchange current was recorded by patch-clamp technique in whole-cell configuration. **RESULTS** AMP579 significantly enhanced both outward and inward Na^+/Ca^{2+} exchange currents in a concentration dependent manner. Neither infusion of an adenosine A_1 receptor antagonist PD116948 30 $\mu mol \cdot L^{-1}$ or an adenosine A_2 receptor antagonist DMPX 10 $\mu mol \cdot L^{-1}$ nor a protein kinase A special blocker KT 5720 0.2 $\mu mol \cdot L^{-1}$ or a protein kinase C special blocker GF 109203X 0.4 $\mu mol \cdot L^{-1}$ had effect on Na^+/Ca^{2+} exchange current increased by AMP579, suggesting that AMP579 possess a direct activating effect on Na^+/Ca^{2+} exchange current. **CONCLUSION** AMP579 possibly possesses a direct activating effect on Na^+/Ca^{2+} exchange current.

Key words: AMP579; myocardium; adenosine; sodium/calcium exchange current; patch clamp technique, whole-cell

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A wide coverage about pharmacological action of adenosine had been given in *American Heart Journal* in 1991[1], its cardiac electrophysiologic mechanism had been studied thoroughly as well[2]. However the clinical applied extent of adenosine was limited owing to its short half life, bradycardia and hypotension during intravenous administration, the efficacy of adenosine to limit myocardial infarct size was especially controversial[3,4]. Recent studies showed that a novel adenosine A_1 and A_2 receptor agonist 1*S*-[1*a*, 2*b*,3*b*,4*a*(*S**)]-4-[7-[[1-(3-chloro-2-thienyl) methylpropyl] propyl-amino]-3*H*-imidazo[4,5-*b*] pyridyl-3-yl]-*N*-ethyl-2, 3-dihydroxycyclopentane carboxamide (AMP579) was more efficient than adenosine in attenuating polymorphonuclear neutrophic mediated inflammatory responses, dilating coronary artery, reducing myocardial contracture and limiting infarct size[5, 6]. ADMIRE is the study item of AMP579 for clinical observation[7].

Differences between AMP579 and adenosine in pharmacological and clinical effects reflected the differences in ionic mechanism of the drugs, studies about action of adenosine on main ionic channels and exchanger had been reported[8,9], but little was known about AMP579 on exchanger. This study is to investigate the effects of AMP579 and adenosine on Na^+/Ca^{2+} exchanger in rat ventricular myocytes and elucidate their action mechanisms.

1 MATERIALS AND METHODS

1.1 Rat myocardial cell isolation

Ventricular myocytes were obtained from Wistar male rats (250 – 300 g, provided by Experimental Animal Center of Shanxi Medical University) by rapid enzymatic isolation procedure. In brief, rats were sacrificed by cervical dislocation and the heart was then removed rapidly, cannulat-

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ed via aorta and perfused through the coronary artery with Ca^{2+}-free Tyrode′s solution (mmol·L^{-1}: NaCl 140, KCl 5.4, $MgCl_2$ 1.0, NaH_2PO_4 0.33, glucose 10, HEPES 5.0, pH adjusted to 7.4 with NaOH at room temperature) for 10 min. The hearts were then perfused with enzymatic solution which was the low Ca^{2+} ($CaCl_2$ 150 μmol·L^{-1}) Tyrode′s solution with collagenase P (0.3 g·L^{-1}) for about 8 – 10 min. Then, the left ventricle was removed. The cells were isolated by gentle agitation and kept in Krebs buffer (KB) solution (mmol·L^{-1}: KOH 85, *L*-glutamic acid 50, KCl 30, taurine 20, KH_2PO_4 30, $MgCl_2$ 1.0, HEPES 10, glucose 10 and EGTA 0.5, pH adjusted to 7.4 by KOH).

1.2 Electrophysiologic measurement

The whole-cell patch-clamp configuration was used to detect Na^+/Ca^{2+} exchange currents and to record the membrane potenials with Pclamp 5.51 software package (Axon Instruments). Patch electrodes were made from thin walled glass capillaries (1.5 mm outside diameter) using a two-stage vertical microelectrode puller (model PP-83, Narishige Scientific Instruments, Japan). The electrode resistance ranges 3 MΩ.

For the measurement of Na^+/Ca^{2+} exchange current, the extracellular solution contained (mmol·L^{-1}) NaCl 140, $CaCl_2$ 1.8, $MgCl_2$ 2.0, HEPES 5.0 and glucose 10, pH adjusted to 7.4 with CsOH. The Na^+-K^+ pump, K^+ channels and Ca^{2+} channels were blocked with ouabain 20 μmol·L^{-1}, $BaCl_2$ 1 mmol·L^{-1}, CsCl 2 mmol·L^{-1} and nicardipine 1 μmol·L^{-1}. The pipette solution contained (mmol·L^{-1}) EGTA 42, $CaCl_2$ 29, $MgCl_2$ 13, potassium aspartate 42, K_2ATP 10, Na_2-creatine phosphate 5.0, tetraethylammonium 20 and HEPES 5.0, pH adjusted to 7.4 with CsOH. The Na^+/Ca^{2+} exchange current was measured as the Ni^{2+}-sensitive current[9], that could be blocked by $NiCl_2$ 5.0 mmol·L^{-1}. Na^+/Ca^{2+} exchange current density was expressed as membrane current per cell capacitance. AMP579 was a gift from Department of Cardiothoracic Surgery, Emory University School of Medicine, USA. AMP579 was dissolved in DMSO, then diluted to the desired final concentration before each experiment. PD 116948, an adenosine A_1 receptor antagonist; DMPX, an adenosine A_2 receptor antagonist were from Sigma, USA; KT 5720, a protein kinase A (PKA) blocker; GF109203X, a protein kinase C (PKC) blocker were from Tocris Co., UK.

1.3 Statistic analysis

Data were expressed as $\bar{x} \pm s$, statistical significance was determined by Student′s *t* test.

2 RESULES

2.1 Measurement of Na^+/Ca^{2+} exchange current of rat ventricular myocytes

Ramp voltage-clamp pulses (−40 mV to +60 mV to −120 mV, 90 mV·s^{-1}) were applied from a holding potential of −40 mV. The current-voltage relationship was constructed from the declining slope of the ramp pulse (Fig 1A, a). After the application of $NiCl_2$ 5.0 mmol·L^{-1}, the current immediately decreased in positive and negative potentials (Fig 1A, b). The difference between current-voltage relationships in the absence and

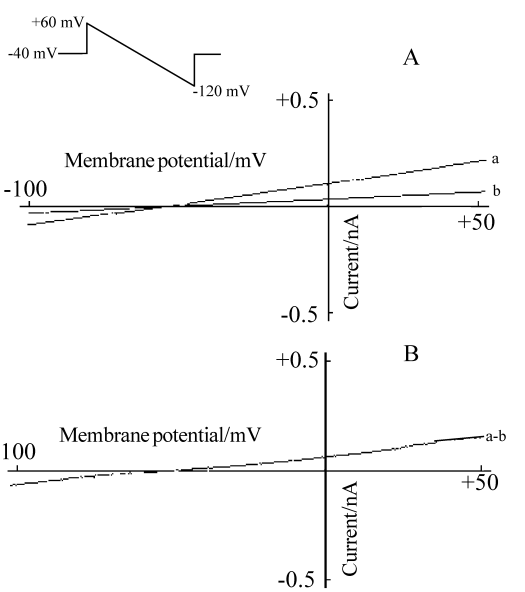

Fig 1. Measurement of Na^+/Ca^{2+} exchange current. A: Current-voltage curve before (a) and after (b) infusion of $NiCl_2$ 5.0 mmol·L^{-1}. B: Na^+/Ca^{2+} exchange current.

presence of $NiCl_2$ (Ni^{2+}-sensitive current) reflected the activity of the Na^+/Ca^{2+} exchange current (Fig 1B). Significant run down of the Ni^{2+}-sensitive current was not found during the experiment.

2.2 Effect of AMP579 and adenosine on Na^+/Ca^{2+} exchange current in isolated rat ventricular myocytes

Adenosine had no effect on Na^+/Ca^{2+} exchange current at the range from 10 nmol·L^{-1} to 50 μmol·L^{-1} (Tab 1, Fig 2 A). AMP579 significantly enhanced outward and inward Na^+/Ca^{2+} exchange currents with concentration dependence (Tab 1, Fig 2 B).

As shown in Tab 2, neither adenosine A_1 receptor antagonist PD 116948 30 μmol·L^{-1} and adenosine A_2 receptor antagonist DMPX 10 μmol·L^{-1} nor PKA special blocker KT 5720 0.2 μmol·L^{-1} and PKC special blocker GF 109203X 0.4 μmol·L^{-1} had effects on the Na^+/Ca^{2+} exchange current induced by AMP579 ($P > 0.05$), suggesting that AMP579 possibly possess a direct pharmacological effect in activating Na^+/Ca^{2+} exchange current.

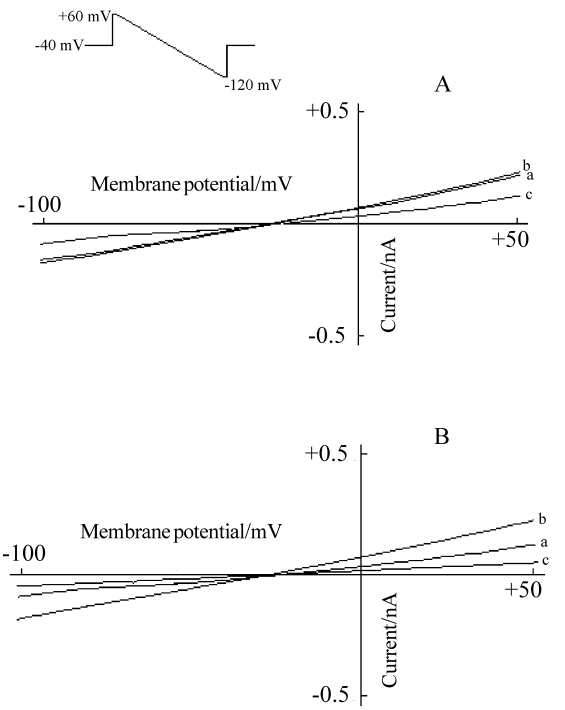

Fig 2. Effects of adenosine (A) and AMP579 (B) on Na^+/Ca^{2+} exchange current in isolated rat ventricular myocytes. See legend of Tab 1 for drug treatments. a: Control current; b: after 50 μmol·L^{-1} adenosine or 50 μmol·L^{-1} AMP579 was added; c: after 5.0 mmol·L^{-1} $NiCl_2$ was added.

Tab 1. Effects of AMP579 and adenosine on Na^+/Ca^{2+} exchange current in rat ventricular myocytes

Drug/ μmol·L^{-1}		Na^+/Ca^{2+} exchange current/pA·pF^{-1}			
		At +70 mV from V_{rev}	Increment	At −70 mV from V_{rev}	Increment
Adenosine	0	0.37 ± 0.30		1.16 ± 1.06	
	0.01	0.36 ± 0.27	−0.03 ± 0.01	1.13 ± 1.11	−0.03 ± 0.01
	1	0.38 ± 0.26	+0.01 ± 0.01	1.19 ± 1.13	+0.03 ± 0.01
	10	0.40 ± 0.26	+0.05 ± 0.02	1.10 ± 1.07	−0.06 ± 0.01
	50	0.40 ± 0.23	+0.05 ± 0.02	1.06 ± 1.01	−0.10 ± 0.02
AMP579	0	0.38 ± 0.28		1.40 ± 0.96	
	0.01	0.64 ± 0.36	0.26 ± 0.10	1.59 ± 1.00	0.19 ± 0.16
	1	0.80 ± 0.37**	0.42 ± 0.11	1.77 ± 0.99*	0.37 ± 0.22
	10	1.04 ± 0.29**	0.67 ± 0.10	2.09 ± 0.96*	0.69 ± 0.47
	50	1.14 ± 0.29**	0.76 ± 0.11	2.74 ± 1.24*	1.34 ± 1.07

Adenosine or AMP579 was added accumulatively, at the end, 5.0 mmol·L^{-1} $NiCl_2$ was added. The results given are differences between a given concentration and $NiCl_2$. V_{rev}: Reversal potential. $\bar{x} \pm s$, $n = 5$. * $P < 0.05$, ** $P < 0.01$, compared with corresponding 0 μmol·L^{-1}.

Tab 2. Effects of PD 116948, DMPX, KT 5720 and GF 109203X on Na^+/Ca^{2+} exchange current increased by AMP579 in rat ventricular myocytes

Drug/μmol·L^{-1}	n	Na^+/Ca^{2+} exchange current /pA·pF^{-1} At +70 mV from V_{rev}	At −70 mV from V_{rev}
Control	5	0.50 ± 0.27	1.38 ± 0.84
AMP579 10		1.10 ± 0.29*	2.04 ± 0.80*
PD 116948 30 + AMP579 10		1.12 ± 0.30*	2.06 ± 0.89*
Control	6	0.47 ± 0.28	1.41 ± 0.76
AMP579 10		1.14 ± 0.26*	1.98 ± 0.87*
DMPX 10 + AMP579 10		1.19 ± 0.32*	2.01 ± 0.82*
Control	5	0.52 ± 0.29	1.36 ± 0.72
AMP579 10		1.23 ± 0.27*	1.96 ± 0.79*
KT 5720 0.2 + AMP579 10		1.25 ± 0.31*	2.01 ± 0.80*
Control	4	0.42 ± 0.30	1.37 ± 0.74
AMP579 10		1.12 ± 0.29*	1.92 ± 0.79*
GF 109203X 0.4 + AMP579 10		1.17 ± 0.38*	1.93 ± 0.82*

After control current was recorded, AMP579 10 μmol·L^{-1} was added. Five minutes later, when the current was stable, the PD 116948, DMPX, KT 5720 and GF 109203X were added respectively in the presence of AMP579. At the end, 5.0 mmol·L^{-1} $NiCl_2$ was added. $\bar{x} \pm s$. * $P < 0.05$, compared with control.

3 DISCUSSION

Na^+/Ca^{2+} exchanger plays a pivotal roles in cellular Ca^{2+} homeostasis and ionic current generation[10]. Adenosine alone has been recently reported to show no effect on Na^+/Ca^{2+} exchanger. However, adenosine can significantly inhibit Na^+/Ca^{2+} exchange current increased by isoprenaline. A_1 adenosine receptor agonist CPA(N_6-cyclopentyladenosine) has the same effect as adenosine on the response of Na^+/Ca^{2+} exchange current to isoprenaline, whereas A_1 adenosine receptor antagonist DPCPX can inhibit the effect of adenosine on the response of Na^+/Ca^{2+} exchange current to isoprenaline. These data show that the A_1 adenosine receptor mediates the response. Our study showed that adenosine had no influence on Na^+/Ca^{2+} exchange current from 10 nmol·L^{-1} to 50 μmol·L^{-1}, manifesting that adenosine has no direct effect on Na^+/Ca^{2+} exchange current, consistent with previous report[11].

On the other hand, our experiment shows that AMP579 can markedly increase both outward and inward Na^+/Ca^{2+} exchange current, neither A_1 adenosine receptor antagonist PD116948 or A_2 adenosine receptor antagonist DMPX inhibits the Na^+/Ca^{2+} exchange current activated by AMP579, nor PKA blocker RT5720 or PKC blocker GF 109203X exert the effect, suggesting that AMP579 possibly possess a direct pharmacological effect in activating Na^+/Ca^{2+} exchange current, the unique effect could be favorable to improve heart failure[12,13]. However the activation of Na^+/Ca^{2+} exchanger by AMP579 is unfavorable to the anti-arrhythmia treatment, prevention of ischemia-reperfusion injury and reduction of myocardial infarct size, in the early ischemia and reperfusion period[14,15]. Recently a clinical investigation about AMP579 questioned the cardiac protective effect of AMP579 as well[7], the article dealed with AMP579 in a trail of 311 patients undergoing primary percutaneous transluminal coronary angioplasty after acute myocardial infarction. Patients were assigned to different doses of AMP579 continuously infused over 6 h. Infarct size did not differ among all the groups during follow up. But plasma concentration of AMP579 from 0.1 – 3.4 nmol·L^{-1} in the patients did not reach the minimum concentration of AMP579 which was required to activate Na^+/Ca^{2+} exchanger in our experiment, the author's conclusion is that AMP579 does not limit myocardial infarct size at usual doses. Whether the conclusion is related to the effect of increased Na^+/Ca^{2+} exchange current by AMP579 remains to be verified, further experiment and clinical observation need to be carried out.

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AMP579 与腺苷对大鼠心室肌细胞 Na^+/Ca^{2+} 交换电流作用的比较

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摘要: 目的 阐明 AMP579 与腺苷不同药理作用与临床效果的机制。**方法** 采用全细胞膜片钳记录 Na^+/Ca^{2+} 交换电流。**结果** AMP579 对 Na^+/Ca^{2+} 交换的外向和内向电流均呈浓度依赖性增强。灌流腺苷 A_1 受体阻断剂 PD 116948 30 $\mu mol \cdot L^{-1}$, A_2 受体阻断剂 DMPX 10 $\mu mol \cdot L^{-1}$, 蛋白激酶 A 特异阻断剂 KT 5720 0.2 $\mu mol \cdot L^{-1}$或蛋白激酶 C 特异阻断剂 GF 109203X 0.4 $\mu mol \cdot L^{-1}$对 AMP579 Na^+/Ca^{2+} 交换电流的激动作用均无影响, 提示 AMP579 对 Na^+/Ca^{2+} 交换电流具有直接的激动作用。**结论** AMP579 对 Na^+/Ca^{2+} 交换电流可能具有直接的激动作用。

关键词: AMP579; 心肌; 腺苷; 钠/钙交换电流; 膜片钳技术, 全细胞

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