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Effects of norepinephrine and isopentenyladenosine on Na^+/Ca^{2+} exchange currents in isolated guinea pig ventricular myocytes

DUN Wen, WU Bo-Wei, ZHAO Rong-Rui[1]
(*Department of Physiology, Shanxi Medical University, Taiyuan 030001, China*)

KEY WORDS Na^+/Ca^{2+} exchange; norepinephrine; isoproterenol; propranolol; phentolamine; patch-clamp techniques; myocardium; ion exchange

AIM: To study the effects of norepinephrine (NE) and isopentenyladenosine (Iso) on Na^+/Ca^{2+} exchange currents and the receptor mechanism. **METHODS:** The quasi-steady state current-voltage relationship from the isolated guinea pig ventricular myocytes was measured using whole-cell voltage-clamp techniques with a ramp pulse protocol. **RESULTS:** At potential of + 50 mV, NE 0.005, 0.05, and 5 $\mu mol \cdot L^{-1}$ increased the Ni^{2+}-sensitive current by 29 % ± 9 %, 72 % ± 11 %, and 124 % ± 31.4 %, respectively; Iso 1.5, 150, and 1500 $nmol \cdot L^{-1}$ caused increases in the Ni^{2+}-sensitive current by 2.8 % ± 2.8 %, 56 % ± 13 %, and 102 % ± 12 %, respectively. Propranolol 10 $\mu mol \cdot L^{-1}$ completely inhibited the current changes induced by NE and Iso while phentolamine 50 $\mu mol \cdot L^{-1}$ showed no effects. **CONCLUSION:** NE and Iso increased the Na^+/Ca^{2+} exchange currents *via* stimulation of cardiac β-adrenoceptor.

The Na^+/Ca^{2+} exchanger is the primary Ca^{2+} efflux mechanism of cardiac myocytes during excitation[1,2]. The exchanger is an important Ca^{2+} efflux mechanism even during periods of rest, when $[Ca^{2+}]_i$ is 100 $nmol \cdot L^{-1}$ or less[3]. Hence the exchanger can affect a transfer of Ca^{2+} from internal stores to the cell exterior during rest. During the initial phases of cardiac action potential, Ca^{2+} influx mediated by Na^+/Ca^{2+} exchange can initiate Ca^{2+} release from the sarcoplasmic reticulum (SR) by a "Ca-induced Ca^{2+} release process"[4]. The Na^+/Ca^{2+} exchange mechanism is involved in the regulation of cardiac inotropism[5]. With the single-cell voltage-clamp technique and appropriate channel blockers, the Na^+/Ca^{2+} exchange currents can be isolated from other membrane currents and measured accurately[6]. β-adrenergic agonists potentiate the force of cardiac contraction and accelerate the rate of its relaxation. The increase in the force of contraction results from enhanced Ca^{2+} current and Ca^{2+} release secondary to cAMP-dependent phosphorylation of the Ca^{2+} channel[7]. On the other hand, the phyosphorylation of phospholamban and the subsequent stimulation of Ca^{2+} pump[8], in addition to decreased myofilament Ca^{2+} sensitivity[9], are thought to mediate the relaxant properties of β-agonists. But, the regulatory effects of β-agonists on Na^+/Ca^{2+} exchange are not clarified yet. In this study we investigated the effects of NE and Iso on the Na^+/Ca^{2+} exchange currents and the involved receptor mechanism.

MATERIALS AND METHODS

Cell isolation Ventricular myocytes were obtained from Dunkin Hartley guinea pigs (250 ± 52 g) by a previously described rapid enzymatic isolation procedure[10]. Myocytes were dispersed and allowed to settle for at least 1 h at room temperature (20) before being used. Animals were provided by Experimental Animal Center of Shanxi Medical University.

Electrophysiologic measurements The whole-cell patch-clamp configuration[11] was used to evaluate Na^+/Ca^{2+} exchange currents with an AXOPATCH 200A patch-clamp amplifier connected to an AST computer with pCLAMP 5.5.1 software package (Axon Instruments). Patch electrodes were made from thin-walled glass capillaries (1.5 mm OD, Shanghai Brain Research Institute) using a two-stage vertical microelectrode puller (model PP-83, Narishige Scientific Instruments, Japan). The electrode resistances ranged between 1 and 3 MΩ. Because of the slow ramp protocol, no compensation was made for membrane capacitance or series resistance. The current signal was filtered at 2 kHz.

For the measurement of Na^+/Ca^{2+} exchange current, the extracellular (test) solution contained NaCl 140, $CaCl_2$ 2.0, $MgCl_2$ 2.0, HEPES 5.0, and glucose 10 $mmol \cdot L^{-1}$ (pH = 7.4 adjusted with CsOH). The Na^+-K^+ pump, background currents, and K^+ channel and Ca^{2+} channel were blocked with ouabain (Sigma) 20 $\mu mol \cdot L^{-1}$, $BaCl_2$ 1.0 $mmol \cdot L^{-1}$, $CsCl_2$

[1] Correspondence to Prof ZHAO Rong-Rui. Phn 86-351-413-5057. Fax 86-351-406-0031. E-mail sydw@shanxi.ihep.ac.cn
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2.0 mmol·L^{-1}, and nicardipine (Sigma) 1.0 μmol·L^{-1}. The pipette solution contained egtazic acid (EGTA) 42, $CaCl_2$ 29, $MgCl_2$ 13, potassium aspartate 42, K_2ATP 10, Na_2-creatine-phosphate 5.0, tetraethylammonium (TEA, Sigma) 20, HEPES 5.0 mmol·L^{-1} (pH = 7.4 adjusted with CsOH). In the internal solution, TEA 20 mmol·L^{-1} was used to block K^+ channel. The electrogenic Na^+/Ca^{2+} exchange current was measured as the (bi-directional) current that could be blocked by Ni^{2+} 5.0 mmol·L^{-1}[9].

Statistical analysis Results were expressed as $\bar{x} \pm s$. Paired t tests were made.

RESULTS

Measurement of the Na^+/Ca^{2+} exchange current Ramp voltage-clamp pulses (−40 to +60 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 Ni^{2+} 5.0 mmol·L^{-1}, the current immediately decreased, at both positive and negative potentials (Fig 1A, b). The difference between current-voltage relationships in the absence and presence of Ni^{2+} 5.0 mmol·L^{-1} (Ni^{2+}-sensitive current) reflected the activity of the electrogenic Na^+/Ca^{2+} exchange current (Fig 1B). We did not find significant run-down of the Ni^{2+}-sensitive current during the experiment.

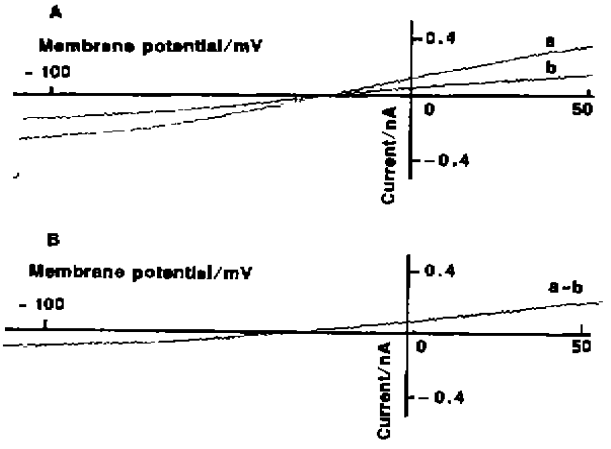

Fig 1. Ni^{2+}-sensitive electrogenic Na^+/Ca^{2+} exchange current of guinea pig ventricular myocytes. A: current-voltage relationships before (a) and after (b) application of Ni^{2+} 5.0 mmol·L^{-1}. B: Ni^{2+}-sensitive current (Na^+/Ca^{2+} exchange current).

Effects of NE and Iso on electrogenic Na^+/Ca^{2+} exchange current NE 5.0 μmol·L^{-1} or Iso 1.5 μmol·L^{-1} resulted in increases of membrane current (Fig 2A, b and Fig 2C, b). After 30-s application of Ni^{2+} 5.0 mmol·L^{-1}, the membrane current was decreased (Fig 2A, c and Fig 2C, c). The current-voltage relationships for Ni^{2+}-sensitive

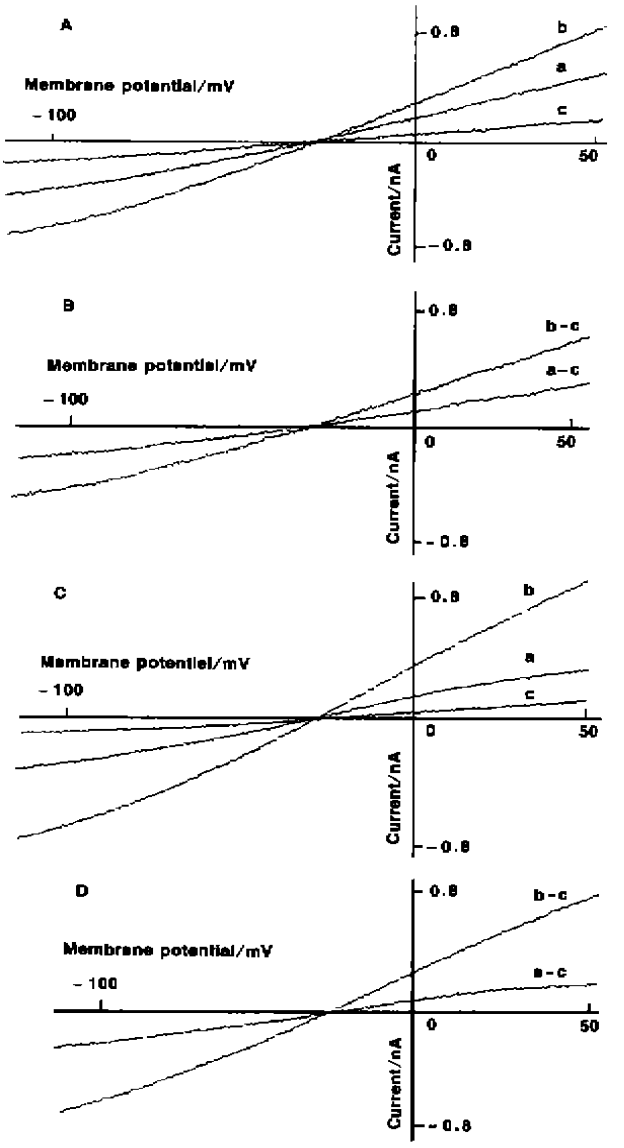

Fig 2. Effects of NE and Iso on electrogenic Na^+/Ca^{2+} exchange current of myocytes. A: current-voltage relationships before application of NE (a), after application of NE 5 μmol·L^{-1} (b) and Ni^{2+} 5.0 mmol·L^{-1} (c). B: Ni^{2+}-sensitive current in A before (a − c) and after application of NE (b − c). C: current-voltage relationships before application of NE (a), after application of Iso 1.5 μmol·L^{-1} (b) and Ni^{2+} 5.0 mmol·L^{-1} (c). D: Ni^{2+}-sensitive current in C before (a − c) and after application of Iso (b − c).

currents of the same experiment were shown in Fig 2B and 2D. NE and Iso increased the Ni^{2+}-sensitive current in a concentration-dependent manner (Tab 1).

Tab 1. Effects of NE and Iso on electrogenic Na^+/Ca^{2+} exchange current of guinea pig ventricular myocytes. Membrane current (pA) measured during a ramp pulse. $n = 4$ cells from 4 guinea pigs, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* before drugs.

Drugs	Before	After	Change %
At potential of +50 mV			
NE/μmol·L^{-1}			
0.005	243 ± 77	313 ± 99^b	29 ± 8
0.05	226 ± 88	383 ± 127^c	72 ± 11
5	190 ± 35	418 ± 38^c	120 ± 31
Iso/nmol·L^{-1}			
1.5	135 ± 89	138 ± 93^a	2.8 ± 2.8
150	199 ± 98	307 ± 148^b	56 ± 13
1500	214 ± 68	430 ± 141^b	102 ± 12
At potential of +100 mV			
NE/μmol·L^{-1}			
0.005	119 ± 95	164 ± 82^a	60 ± 50
0.05	166 ± 26	243 ± 43^b	47 ± 26
5	106 ± 34	178 ± 32^c	77 ± 34
Iso/nmol·L^{-1}			
1.5	104 ± 84	106 ± 85^a	1.1 ± 0.8
150	79 ± 4	170 ± 91^b	123 ± 44
1500	134 ± 74	304 ± 144^b	134 ± 21

Effects of propranolol and phentolamine on action of NE and Iso Propranolol 10 μmol·L^{-1} inhibited the changes of inward and outward membrane currents induced by both NE 5 μmol·L^{-1} and Iso 1.5 μmol·L^{-1} (Fig 3A and 3B). However, phentolamine 50 μmol·L^{-1} did not affect the enhancement of Na^+/Ca^{2+} exchange current induced by NE 5 μmol·L^{-1} (Fig 3C). Similar results to those shown in Fig 3 were obtained in 4 other cells.

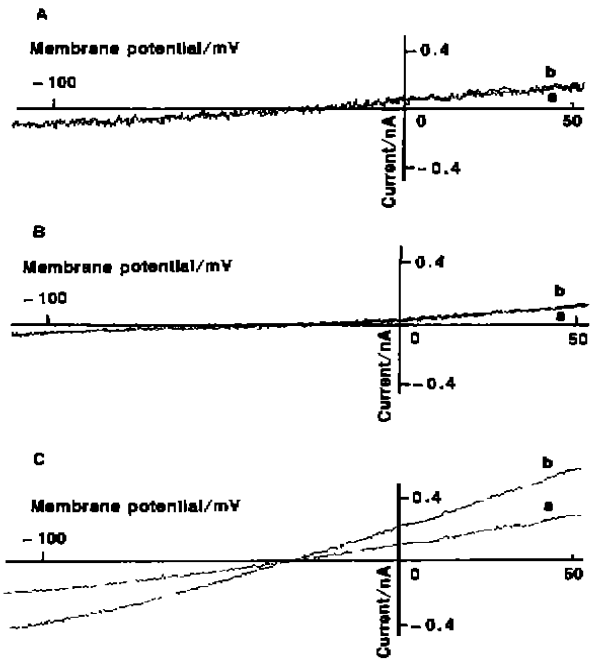

Fig 3. Effects of propranolol and phentolamine on action of NE and Iso. A: effects on Ni^{2+}-sensitive current before (a) and after application of propranolol 10 μmol·L^{-1} plus NE 5 μmol·L^{-1} (b), $n = 4$ cells from 4 guinea pigs, $P > 0.05$. B: effects on Ni^{2+}-sensitive current before (a) and after application of propranolol 10 μmol·L^{-1} plus Iso 1.5 μmol·L^{-1} (b), $n = 4$ cells from 4 guinea pigs, $P > 0.05$. C: effects on Ni^{2+}-sensitive current before (a) and after phentolamine 50 μmol·L^{-1} plus NE 1.5 μmol·L^{-1} (b), $n = 4$ cells from 4 guinea pigs, $P < 0.05$.

DISCUSSION

In the present study, the electrogenic Na^+/Ca^{2+} exchange currents were measured by using methods previously described[12]. The advantage of these methods is that high concentration of intracellular Ca^{2+} buffer (EGTA) are used, which should prevent any possible increases in cytosolic [Ca^{2+}]. The contribution of other membrane currents to the total recorded current was minimized using various blockers (nicardipine, Cs^{2+}, Ba^{2+}, TEA and uabain). The shape of the current-voltage relationship we obtained for the Na^+/Ca^{2+} exchange current is theoretically and practically compatible with the available literature regarding the voltage dependence of electrogenic Na^+/Ca^{2+} exchange current.

Our experimental results clearly showed that both NE and Iso could significantly increase the electrogenic Na^+/Ca^{2+} exchange current. Propranolol, a β-adrenoceptor blocker, inhibited the increase of Na^+/Ca^{2+} exchange current induced by NE and Iso, while phentolamine, a α-adrenoceptor blocker, showed no effects on the NE-induced increase of Na^+/Ca^{2+} exchange current. This suggests that β-adrenoceptor, but not α-adrenoceptor, is involved in the increasing effects of NE and Iso on the Na^+/Ca^{2+} exchange current. Our results also demonstrated that NE and

Iso increased not only Na^+/Ca^{2+} exchange inward currents, but also Na^+/Ca^{2+} exchange outward currents, indicating that β-adrenergic agonists are able to enhance the rate of transmembrane movement of Ca^{2+} by Na^+/Ca^{2+} exchanger. The enhancement of the Na^+/Ca^{2+} exchange rate can be one of the mechanisms responsible for the positive inotropism induced by β-adrenoceptor stimulation. However, further experiments are necessary to determine the molecular mechanisms of regulation of cardiac sodium/calcium exchanger by β-adrenergic agonists. It is concluded that the Na^+/Ca^{2+} exchange currents of guinea pig ventricular myocytes are enhanced by β-adrenoceptor stimulation.

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(11)

141 - 144

去甲肾上腺素和异丙肾上腺素对豚鼠离体心室肌细胞 Na^+/Ca^{2+} 交换电流的影响

顿 文, 吴博威, 赵荣瑞[1]

(山西医科大学生理教研室, 太原 030001, 中国)

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关键词 Na^+/Ca^{2+} 交换; 去甲肾上腺素; 异丙肾上腺素; 普萘洛尔; 酚妥拉明; 膜片箝技术; 心肌; 离子交换

目的: 研究去甲肾上腺素(NE)和异丙肾上腺素(Iso)对 Na^+/Ca^{2+} 交换电流的影响及受体调控机制. **方法**: 应用全细胞电压钳技术的斜坡脉冲程序, 测定离体豚鼠心肌细胞准稳态电流-电压关系曲线. **结果**: NE 0.005, 0.05 和 5 μmol·L^{-1}分别使膜电位 + 50 mV时的 Ni^{2+}敏感电流增加 29 % ± 9 %, 72 % ± 11 % 和 120 % ± 31 %; Iso 1.5, 150 和 1500 nmol·L^{-1}分别使该电流增加 2.8 % ± 2.8 %, 56 % ± 13 % 和 102 % ± 12 %. NE 和 Iso 的这种增强效应能被普萘洛尔 10 μmol·L^{-1}完全阻断, 而 酚妥拉明 50 μmol·L^{-1}无此作用. **结论**: NE 和 Iso 通过兴奋心脏 β-肾上腺素受体使 Na^+/Ca^{2+} 交换电流增加.