Class Ⅲ anti-arrhythmia drug E-4031 potentiates Na^+/Ca^{2+} exchange current in rat ventricular myocytes[1]

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KEY WORDS E-4031；anti-arrhythmia agents；tamoxifen；tetradecanoylphorbol acetate；protein kinase C；patch-clamp techniques；sodium-calcium exchanger

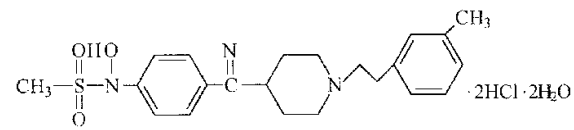
ABSTRACT

AIM：To study the effects of E-4031 on the Na^+/Ca^{2+} exchange currents ($I_{Na/Ca}$). METHODS：The quasi-steady state current-voltage relationship from the isolated rat ventricular myocytes was measured using whole-cell voltage-clamp techniques with a ramp pulse protocol. RESULTS：At potential of +50 mV，E-4031 5，10，and 20 μmol·L^{-1} increased Ni^{2+}-sensitive current from (0.48 ± 0.12)，to (0.78 ± 0.20)，(0.96 ± 0.16)，and (1.15 ± 0.13) pA/pF，respectively；tetradecanoylphorbol acetate (TPA) 50 nmol·L^{-1} increased Ni^{2+}-sensitive current from (0.60 ± 0.16) to (1.33 ± 0.25) pA/pF. Tamoxifen 20 μmol·L^{-1} completely prevented the current changes induced by E-4031 and TPA. CONCLUSION：E-4031 stimulates the Na^+/Ca^{2+} exchange via a protein kinase C-dependent pathway.

INTRODUCTION

The Na^+/Ca^{2+} exchanger which catalyses the electrogenic exchange of 3 Na^+ for 1 Ca^{2+} across the plasma membrane plays a major role in the excitation-contraction coupling of the heart[1]. The Na^+/Ca^{2+} exchange is particularly important for relaxation，by maintaining a balance between Ca^{2+} entry，via the Ca^{2+} channels，and Ca^{2+} extrusion from the cell via the exchanger[2]. On the other hand，the Na^+/Ca^{2+} exchanger may be involved in contraction，when Ca^{2+} entering the cell via reverse mode contributes to sarcoplasmic reticular (SR) Ca^{2+} loading and/or to triggering SR Ca^{2+} release[3]. So the Na^+/Ca^{2+} exchanger plays a central role in the regulation of cardiac ionotropism[4].

E-4031，{N-[4-[[1-[2-(6-methyl-2-pyridinyl) ethyl]-4-piperidinyl] carbonyl] phenyl] methanesulfonamide dihydrochloride dihydrate}[5] is representative of a group of new and potent methanesulfonanilide class Ⅲ anti-arrhythmia agents that are being developed for the prevention of ligant ventricular arrhythmia and sudden death[6]. E-4031 has shown effects of both delayed repolarization and positive ionotropy[7]. Electrophysiologic studies demonstrate that E-4031 does not affect the Na^+ or Ca^{2+} inward current while it prolongs both action potential duration (APD) and effective refractory period (ERP) in ventricular muscles from guinea pig，by specifically inhibiting cardiac delayed rectifying potassium channels (I_k channels)[7,8]. However the detailed mechanism of the E-4031-induced positive ionotropy remains unknown and the regulatory effects on Na^+/Ca^{2+} exchange are not clarified yet. The objective of the present study was to investigate the effects of E-4031 on the $I_{Na/Ca}$ and the possible signaling pathways involved.



E-4031
$C_{21}H_{27}N_3O_3 \cdot 2HCl \cdot 2H_2O$

MATERIALS AND METHODS

Cell isolation Single ventricular myocytes were isolated[9] from Wister rats (250 g ± 30 g) provided by Experimental Animal Center of Shanxi Medical University. In brief，cells were dissociated from Langendorff-perfused rat hearts by a protocol which consisted of a constant-flow

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perfusion 8 – 10 mL·min^{-1} with：1) 5 min of Ca^{2+}-free Tyrode's solution；2) 3 min of Ca^{2+}-free Tyrode's solution, containing collagenase (Type A, Boehringer Mannheim；0.3 g·L^{-1}) and protease (Type XIV, Sigma；0.12 g·L^{-1}；3) 8 – 10 min of Ca^{2+}-free, collagenase-containing Tyrode's solution；and 4) 9 min of 0.18 mmol·L^{-1} Ca^{2+} solution. The isolated myocytes were stored in normal Tyrode's solution at room temperature (22 ℃) for at least 1 h before use.

Electrophysiologic measurements Voltage-clamp recordings were performed in the whole-cell configuration of the patch-clamp method with patch clamp amplifier (Axopatch-ID, Axon Instruments, USA). 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 heat-polished electrode with resistances of 2 – 4 MΩ were made when filled with the pipette solution. Because of the slow ramp protocol, no compensation was made for membrane capacitance or series resistance. The current signal was filtered at 2 kHz. In all experiments, membrane current density was measured by expressing membrane current in terms of cell capacitance. Cell capacitance was measured using a fast (1.8 V·s^{-1}) ramp[10].

For the measurement of $I_{Na/Ca}$ the extracellular (test) solution contained (in mmol·L^{-1})：NaCl 140, $CaCl_2$ 2.0, $MgCl_2$ 2.0, HEPES 5.0, and glucose 10 (pH = 7.4 adjusted with CsOH). In addition, the Na^+-K^+ pump, background currents, and K^+ channel were blocked with ouabain (Sigma) 20 μmol·L^{-1}, $BaCl_2$ 1.0 mmol·L^{-1}, $CsCl_2$ 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-creatinephosphate 5.0, 4-aminopyridine (4-AP, Sigma) 20, HEPES 5.0 mmol·L^{-1} (pH = 7.4 adjusted with CsOH). The electrogenic $I_{Na/Ca}$ was measured as the (bi-directional) current that could be blocked by Ni^{2+} 5.0 mmol·L^{-1}.

Drugs E-4031 was obtained from Eisai Pharmaceuticals (Tokyo, Japan). It was dissolved in distilled water as stock solution and diluted in superfusates to the desired final concentrations immediately before each experiment. TPA (Sigma；1 mmol·L^{-1}) and tamoxifen (Sigma；5 mmol·L^{-1}) were made in dimethylsulphoxide and diluted to the desired concentration.

Statistical analysis All data were presented as $\bar{x} \pm s$, paired t-tests were made.

RESULTS

Measurement of the Na^+/Ca^{2+} exchange current In the experiments, ramp voltage-clamp pulses (from +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 $I_{Na/Ca}$. We did not observe significant run-down of the Ni^{2+}-sensitive current during the time course of a typical experiment.

Effects of E-4031 and TPA on electrogenic $I_{Na/Ca}$ E-4031 20 μmol·L^{-1} or TPA 50 nmol·L^{-1}

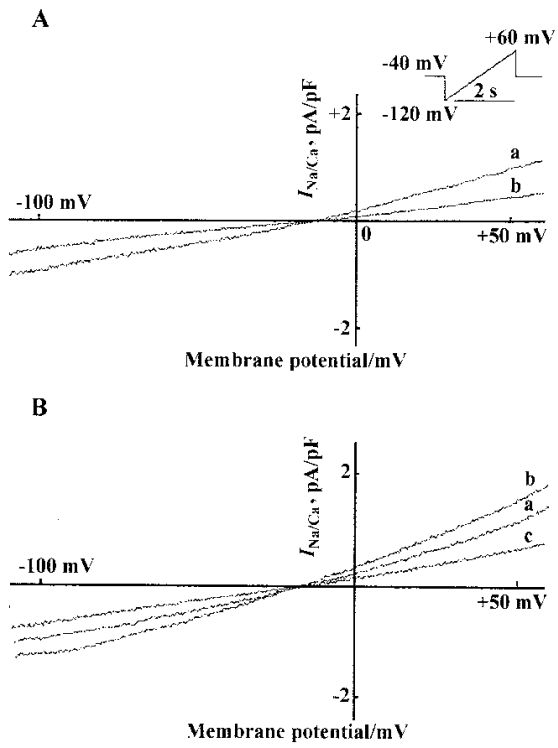

Fig 1. Measurement of Ni^{2+}-sensitive electrogenic $I_{Na/Ca}$ of rat ventricular myocytes. A: Current-voltage relationships (corrected for cell capacitance) before (trace a) and after (trace b) application of Ni^{2+} 5.0 mmol·L^{-1}, $n = 5$ cells from 5 rats, $P < 0.05$. B: Effects of E-4031 on $I_{Na/Ca}$ of myocytes. (a): Current-voltage relationships before application of E-4031, (b): after application of E-4031 20 μmol·L^{-1}, (c) Ni^{2+} 5.0 mmol·L^{-1}. $n = 6$ cells from 6 rats, $P < 0.05$.

resulted in increases of membrane current (Fig 1B, b and Fig 2b). After 30-s application of Ni^{2+} 5 mmol·L^{-1}, the membrane current was decreased (Fig 1B, c and Fig 2c). E-4031 increased the Ni^{2+}-sensitive current in a concentration-dependent manner. At membrane potential + 50 mV and − 100 mV, the coefficients of correlation were 0.98 and 0.96, respectively (Tab 1).

Effect of tamoxifen on action of E-4031 and TPA

Tamoxifen 20 μmol·L^{-1} prevented the changes of $I_{Na/Ca}$ induced by both E-4031 10 μmol·L^{-1} (Fig 3A) and TPA 50 nmol·L^{-1} (Fig 3B).

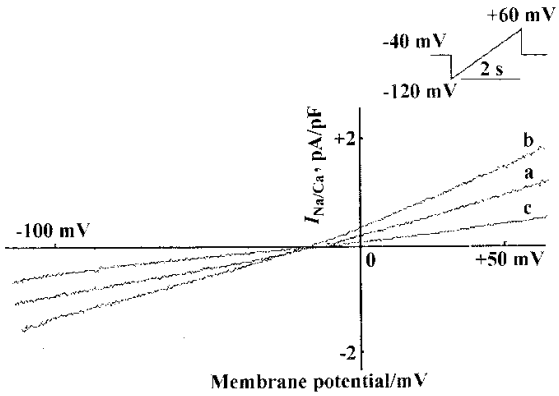

Fig 2. Effects of TPA on $I_{Na/Ca}$ of myocytes. (a): Current-voltage relationships before application of E-4031, (b) after application of E-4031 20 μmol·L^{-1}, and (c) Ni^{2+} 5.0 mmol·L^{-1}. $n = 6$ cells from 6 rats, $P < 0.05$.

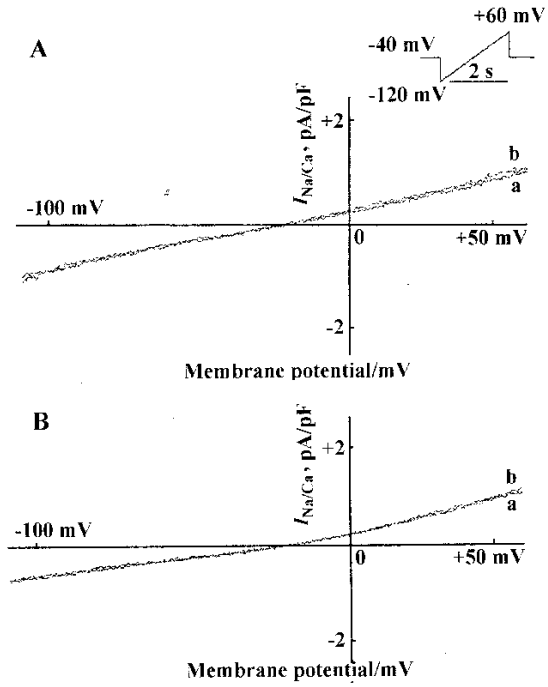

Fig 3. Effects of tamoxifen on action of E-4031 and TPA. A: Effects on Ni^{2+}-sensitive current before (a) and after application of tamoxifen 20 μmol·L^{-1} plus E-4031 10 μmol·L^{-1} (b), $n = 5$ cells from 5 rats. $P > 0.05$. B: Effects on Ni^{2+}-sensitive current before (a) and after application of tamoxifen 20 μmol·L^{-1} plus TPA 50 nmol·L^{-1} (b), $n = 4$ cells from 4 rats, $P > 0.05$.

DISCUSSION

The present study was performed to determine a direct effect of E-4031 on the $I_{Na/Ca}$ measured according to Kimural *et al*[10]. The advantage of these methods was that high concentration of an intracellular Ca^{2+} buffer (egtazic acid) was used, which prevented any change in the Ca^{2+} concentration caused by either Ca^{2+} entering the cell from extracellular space or from the SR. The contribution of other membrane currents to the total recorded current was minimized using various blockers (ouabain, 4-AP, Cs^+, Ba^{2+}, and nicardipine). The shape of the current-voltage relationship obtained from the

Tab 1. Effects of E-4031 and TPA on $I_{Na/Ca}$ in myocytes. $n = 6$ cells from 6 rats. $\bar{x} \pm s$.
$^bP < 0.05$, $^cP < 0.01$ *vs* control.

Drugs	Membrane current At + 50 mV (pA/pF) Before	After	Change %	Membrane current At − 100 mV (pA/pF) Before	After	Change %
E-4031/μmol·L^{-1}						
5	0.48 ± 0.12	0.78 ± 0.20^c	62 ± 10	0.27 ± 0.08	0.39 ± 0.09^b	58 ± 22
10	0.48 ± 0.12	0.96 ± 0.16^c	107 ± 26	0.27 ± 0.08	0.51 ± 0.17^c	96 ± 16
20	0.48 ± 0.12	1.15 ± 0.13^c	148 ± 29	0.27 ± 0.08	0.60 ± 0.18^c	128 ± 17
TPA/nmol·L^{-1}						
50	0.60 ± 0.16	1.33 ± 0.25^c	128 ± 32	0.42 ± 0.10	0.91 ± 0.20^c	115 ± 22

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$I_{Na/Ca}$ was theoretically and practically campatible with the available literature regarding the voltage dependence of the electrogenic $I_{Na/Ca}$.

Our results showed that E-4031 could significantly increase the electrogenic $I_{Na/Ca}$, both in the inward and outward directions, indicating that the new class antiarrhythmic agent, E-4031, was able to enhance the rate of transmembrane movement of Ca^{2+} by Na^+/Ca^{2+} exchanger. Stimulation of the Na^+-dependent $^{45}Ca^{2+}$ uptake by protein kinase C-dependent phosphorylation has been suggested in aortic smooth muscle[12] in rat neonatal cardiomyocytes CCL39 cells stably overexpressing canine cardiac Na^+/Ca^{2+} exchanger[13] has also been reported. Consistent with these findings, TPA, a protein kinase C activator, stimulated the $I_{Na/Ca}$, while tamoxifen, a selective antagonist of protein kinase C, prevented the effects of both E-4031 and TPA in our experiments.

In summary, our experimental results suggested that E-4031 stimulated the $I_{Na/Ca}$ of rat ventricular myocytes via protein kinase C-dependent pathway.

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Ⅲ类抗心律失常药 E-4031 增强大鼠心室肌细胞 Na^+/Ca^{2+} 交换电流

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关键词 E-4031；抗心律失常药；他莫昔芬；十四酰佛波醇乙酯；蛋白激酶 C；膜片箝技术；钠-钙交换

目的：研究 E-4031 对 Na^+/Ca^{2+} 交换电流的影响及其信号转导机制. 方法：应用全细胞电压箝技术的斜坡脉冲程序，测定离体大鼠心肌细胞准稳态电流-电压关系曲线. 结果：E-4031 5，10 和 20 $\mu mol \cdot L^{-1}$ 分别使膜电位 + 50 mV 时的 Ni^{2+} 敏感电流从对照组 (0.48 ± 0.12) 增加到 (0.78 ± 0.20)，(96 ± 0.16) 和 (1.15 ± 0.13) pA/pF. 蛋白激酶 C 激动剂-TPA 50 $nmol \cdot L^{-1}$ 使该电流从 (0.60 ± 0.16) 增加到 (1.33 ± 0.25) pA/pF. 蛋白激酶 C 拮抗剂 Tamoxifen 可完全阻断 E-4031 和 TPA 对该电流的刺激作用. 结论：E-4031 通过蛋白激酶 C 途径激动 Na^+/Ca^{2+} 交换系统.

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