5-Hydroxytryptamine enhances L-type calcium current in norepinephrine-induced hypertrophic ventricular myocytes[1]

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KEY WORDS serotonin; cardiomegaly; calcium channels; patch-clamp techniques

ABSTRACT

AIM: To study the effects of 5-hydroxytryptamine (5-HT) on L-type calcium current (I_{Ca}) in norepinephrine (NE)-induced hypertrophic ventricular myocytes. **METHODS**: Left ventricular hypertrophy was induced by injecting NE intraperitoneally in rats. The single myocytes were isolated enzymatically from left ventricle. I_{Ca} was recorded with the whole-cell configuration of the patch-clamp technique. **RESULTS**: (1) The ratio of left heart weight to body weight (LHW/BW) was higher ($P < 0.01$) in the NE-treated rats compared with the control rats on d 15. LHW/BW was increased 31.8 % in NE-treated rats. (2) I_{Ca} was larger in hypertrophic cells than that in normal cells (4.5 pA/pF ± 0.5 pA/pF *vs* 3.5 pA/pF ± 0.3 pA/pF, respectively, at testing potential of 0 mV; $P<0.01$). (3) 5-HT (1, 10 μmol/L) increased I_{Ca} and decreased the peak current potential from 0 mV to -10 mV in both myocytes. The augmentation of I_{Ca} induced by 5-HT was larger in hypertrophic ones. (4) 5-HT did not markedly influence the steady-state activation kinetics. However, 5-HT shifted steady-state inactivation curve with half inactivation voltage $V_{1/2}$ changing from -39.5 mV ± 1.8 mV to -27.8 mV ± 1.7 mV ($P < 0.05$), while not changing the voltage responsiveness of calcium channel (slope factor k was not changed markedly). **CONCLUSION**: 5-HT increased I_{Ca} in ventricular myocytes by changing the kinetics of steady-state inactivation. A larger alteration of I_{Ca} induced by 5-HT in hypertrophic ventricular myocytes suggests that 5-HT be more prone to induce arrhythmia in hypertrophic heart than in normal one.

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INTRODUCTION

It has been demonstrated that 5-hydroxytryptamine (5-HT) shows positive inotropic and chronotropic effects in heart. These effects may result from direct action on cardiac tissue and indirect action mediated by the release of norepinephrine (NE) from sympathetic nerve terminals[1-3]. It has never been defined whether there is a 5-HT receptor on cardiac myocytes, and if any, which types of 5-HT receptors are involved in 5-HT actions in heart. In addition, 5-HT may be responsible for causing some of cardiovascular diseases, such as systemic hypertension, angina, and arrhythmia[4,5]. L-type calcium channels play a key role in excitation and contraction of cardiac myocytes. However, it is not known the action of 5-HT on I_{Ca} in ventricular myocytes. The present experiments were to reveal the effects of 5-HT on I_{Ca} in rat normal and hypertrophic ventricular myocytes with patch-clamp whole-cell technique.

MATERIALS AND METHODS

Animals Five-week-old male Sprague-Dawley rats (provided by the Experimental Animal Center of Fourth Military Medical University, Grade Ⅱ, Certificate № C98008) weighing 170 - 190 g were treated with norepinephrine (1.5 mg/kg, ip, twice daily for 15 d) to induce left ventricular hypertrophy as described previously[6]. The study was carried out within the following one week, when the rats were weighed 200 - 220 g. Age-matched normal rats weighing 210 - 230 g served as control.

Cell isolation The single myocyte was isolated from left ventricle of rats[7]. In brief, the rats were anesthetized with pentobarbital sodium (30 mg/kg, ip) and anticoagulated with heparin sodium (300 U/kg, iv). The heart was rapidly excised and mounted on a

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Langendorff apparatus. It was perfused conversely *via* the aorta for 5 min with a modified Tyrode's solution equilibrated with 100 % O_2 at 37 ℃ at a rate of 5 to 10 mL/min, then for 5 min with Ca^{2+}-free solution. The heart was then perfused with 0.1 % collagenase (type Ⅰ, Sigma) dissolved in Ca^{2+}-free Tyrode's solution until the solution flew freely (15 to 25 min). Then the heart was washed with 30 mL of a Kraftbrühe (KB) solution. After removing the atria, the left ventricular free wall was dissected and minced in a cup containing KB solution. The tissue pieces were gently stirred, and isolated single cells were then filtered through a nylon mesh (180 μm). The isolated left ventricular cells were stored in the KB solution at 4 ℃ and used within 12 h after isolation.

Solutions The composition of the modified Tyrode's solution was (in mmol/L) NaCl 144, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5, and HEPES 5.5 (pH 7.4 with NaOH). The Ca^{2+}-free Tyrode's solution was prepared by omitting $CaCl_2$ from the modified Tyrode's solution. The composition of the KB solution was (in mmol/L) KOH 70, KCl 40, *L*-glutamic acid 50, taurine 20, KH_2PO_4 10, $MgCl_2$ 0.5, glucose 11, egtazic acid 0.5, and HEPES 10 (pH 7.4 with KOH). For the recording of I_{Ca}, the external solution was (in mmol/L) choline chloride 137, $MgCl_2$ 0.5, $CaCl_2$ 1.8, HEPES 5, glucose 10, and CsCl 4.6 (pH 7.3 with NaOH). The pipette solution was composed of (in mmol/L) CsCl 140, $MgCl_2$ 0.5, Na_2ATP 4, egtazic acid 1, HEPES 5, and glucose 5.5 (pH 7.2 with CsOH). 5-HT (Sigma) was dissolved in the modified Tyrode's solution. Solutions were gased with 100 % O_2.

Whole-cell patch clamp recording A small aliquot (0.3 mL) of dissociated cells was placed in a 0.5 mL chamber mounted on an inverted microscope (Leica). Cells were allowed to adhere to the coverslip and perfused at room temperature (18 - 22 ℃). Currents were recorded by the gigaohm seal, patch-clamp technique in whole-cell configuration with a CEZ 2300 amplifier (Nihon Kohden). The resistance of patch pipette ranged from 2 to 5 MΩ when filled with the pipette solution. The cell membrane capacitance (Cm) was determined by applying a 10-mV hyperpolarizing voltage-clamp step from a holding potential (V_h) at -40 mV. Pipette capacitance and series resistance were compensated to minimize the duration of capacitive currents.

Protocol Rod-shape cells with clear cross-striations and resting potential of at least -75 mV were used. I_{Ca} was recorded by applying 300-ms depolarizing pulses to a test potential ranging from -40 mV to +50 mV in 10 mV steps from a V_h of -70 mV at an interval of 5 s (0.2 Hz). The current amplitude of I_{Ca} was measured as the difference between the peak inward current and the steady-state current during the depolarizing voltage-clamp pulses. The current amplitude of I_{Ca} decreases during the course of whole-cell clamp experiments. We found that this decline of I_{Ca} averaged 18 % over 15 min. All the recordings of I_{Ca} were, therefore, performed at 10 min after the establishment of a gigaohm seal. The current signal was sampled directly into a computer and analyzed by using pClamp software (version 7.0, AXON instruments Inc).

Data analysis Data were expressed as $x \pm s$ and the statistical significance of differences was estimated according to *t*-test for paired observations. To study steady-state activation of I_{Ca}, cells were maintained at a holding potential of -80 mV. I_{Ca} was evoked by depolarizing the cells to test potentials from -40 mV to +10 mV in 10-mV increments for 300 ms. To study steady-state inactivation of I_{Ca}, cells were clamped at a holding potential of -80 mV for 300 ms to a range of potentials from -80 mV to 0 mV. Steady-state activation or inactivation curves of I_{Ca} were fitted with Boltzman equation: $I/I_{max} = 1/\{1 + EXP[(V - V_{1/2})/k]\}$. I is the calcium current; I_{max} is the maximal amplitude of calcium current; V is the voltage of conditioning pulse; $V_{1/2}$ is the half activation or inactivation voltage; and k is the slope factor.

RESULTS

Animal and cell characteristics There was no difference in body weight between normal and NE-treated rats. However, the left heart weight was heavier ($P < 0.01$) and the ratio of left heart weight to body weight (LHW/BW) was higher ($P < 0.01$) in the NE-treated rats compared with the control rats. LHW/BW was increased by 31.8 % in NE-treated rats (Tab 1). The cell membrane capacitance (C_m) was 148 pF ± 17 pF ($n = 37$) in normal cells and 192 pF ± 21 pF ($n = 32$) in hypertrophic cells ($P < 0.01$).

I_{Ca} in normal and hypertrophic myocytes In both normal ($n = 27$ cells from 12 rats) and hypertrophic cells ($n = 25$ cells from 8 rats), the threshold for the

Tab 1. Characteristics of experimental animals. $\bar{x} \pm s$. [a]$P>0.05$, [b]$P<0.05$, [c]$P<0.01$ *vs* normal.

	Normal ($n=12$)	Hypertrophy ($n=8$)
Body weight (BW, g)	218±11	212±8[a]
Left heart weight (LHW, g)	0.41±0.06	0.63±0.17[b]
LHW/BW (mg·g^{-1})	2.24±0.23	2.9±0.3[c]

activation of I_{Ca} and the potential of peak current were −30 mV and 0 mV, respectively. However, the current density (the amplitude normalize to cell membrane capacitance) of I_{Ca} was significantly larger in hypertrophic cells than in normal cells at test potentials from −20 mV to +20 mV (Fig 1). At the test potential of 0 mV, the current density of I_{Ca} was 4.8 pA/pF±0.5 pA/pF in hypertrophic cells and 3.5 pA/pF ±0.3 pA/pF in normal cells ($P<0.01$).

Effects of 5-HT on the I_{Ca} in hypertrophic and normal cells 5-HT 1 and 10 μmol/L increased I_{Ca} in both hypertrophic and normal cells. It can be seen that the potential of peak current was shifted from 0 mV to −10 mV during application of 5-HT in both hypertrophic and normal cells (Fig 2). In addition, the augmentation of I_{Ca} was more significant in hypertrophic cells than in normal cells at the testing potential of −10 mV, 0 mV, +10 mV, and +20 mV (Tab 2).

Effects of 5-HT on activation and inactivation kinetics of I_{Ca} in normal cells 5-HT (10 μmol/L) did not markedly influence the steady-state activation kinetics of I_{Ca}, with half activation potential ($V_{1/2}$) changing from −12.8 mV±1.1 mV to −13.4 mV±1.3 mV and k from 4.4±0.4 to 4.5±0.6 ($n=5$ cells from 2 hearts, $P>0.05$, Fig 3A). However, 5-HT (10 μmol/L) shifted steady-state inactivation curve with $V_{1/2}$ changing from −39.5 mV±1.8 mV to −27.8 mV ±1.7 mV ($n=4$ cells from 2 hearts, $P<0.05$). The slope factor k was not changed markedly (−6.2±0.6 *vs* −6.4±0.5, $P>0.05$, Fig 3B).

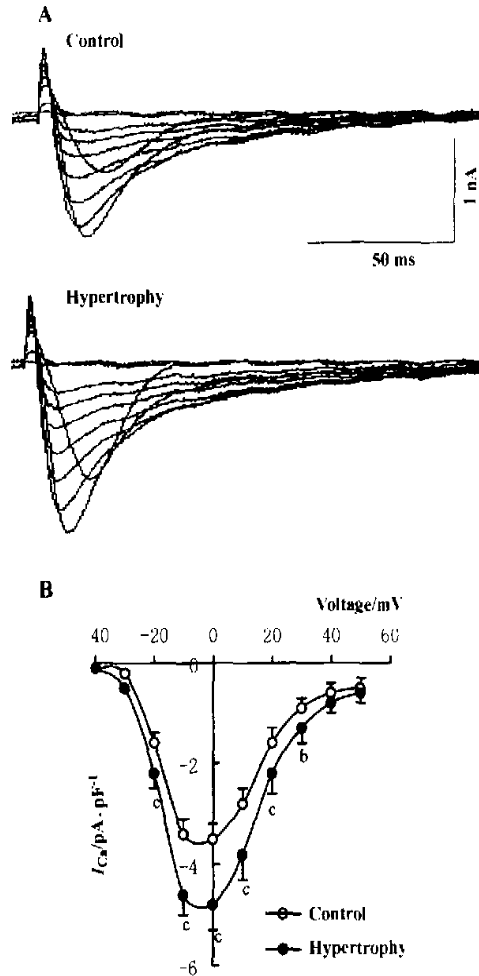

Fig 1. I_{Ca} in ventricular myocytes. A: The original currents recording in normal and hypertrophic cells. B: *I-V* relationship of I_{Ca} in normal ($n=27$ cells from 12 rats) and hypertrophic cells ($n=25$ cells from 8 rats). $\bar{x} \pm s$. [b]$P<0.05$, [c]$P<0.01$ *vs* control.

Tab 2. Increase of I_{Ca} density induced by 5-HT in both two-type cells at different test potential. $\bar{x} \pm s$. [a]$P>0.05$, [b]$P<0.05$, [c]$P<0.01$ *vs* normal cells.

	Dose of 5-HT /μmol·L^{-1}	I_{Ca} density/pA·pF^{-1} −20 mV	−10 mV	0 mV	+10 mV	+20 mV
Normal cells	1	0.42±0.11	0.69±0.17	0.53±0.15	0.39±0.12	0.34±0.09
($n=11$)	10	0.5±0.4	1.1±0.3	0.93±0.24	0.8±0.3	0.67±0.24
Hypertrophic cells	1	0.52±0.19[a]	1.3±0.4[b]	1.0±0.3[c]	0.91±0.20[c]	0.81±0.20[c]
($n=9$)	10	1.4±0.4[c]	2.6±0.5[c]	2.3±0.6[c]	1.8±0.7[c]	1.3±0.4[c]

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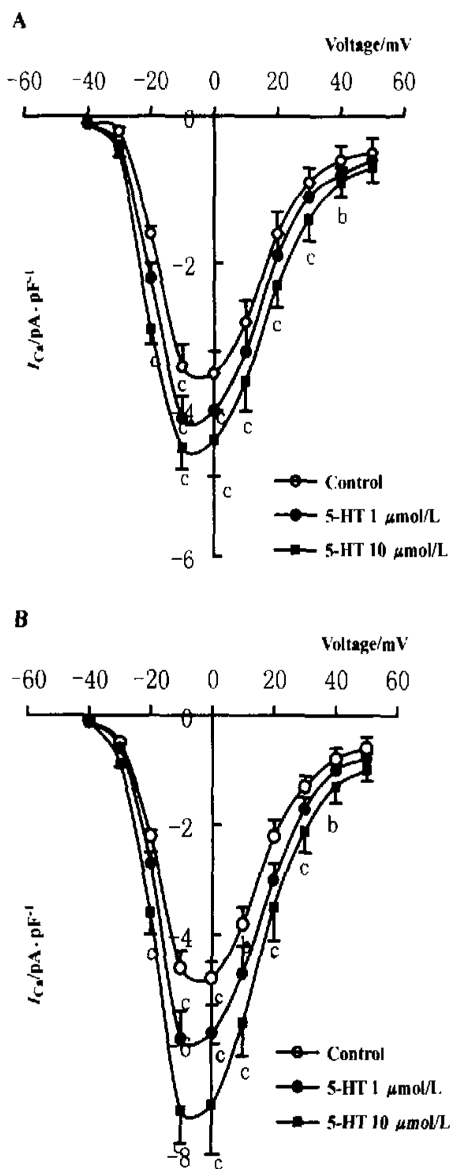

Fig 2. *I-V* relationship for I_{Ca} before and after treated with 5-HT in normal and hypertrophic cells. A: *I-V* relations for I_{Ca} in normal cells. $n = 18$ cells from 8 rats. B: *I-V* relationship for I_{Ca} in hypertrophic cells. $n = 14$ cells from 6 rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

DISCUSSION

It was shown in experiments on rat hearts with hypertrophy that the current density of I_{Ca} was diverse. I_{Ca} density was shown to be increased[8,9], decreased[10], or unchanged[11] in rats, cats, or guinea pigs. Different pathological process in different hypertrophy models might result in the diversity. Lee *et al*[12] found that membrane currents changed in stage-dependent manner in rats with monocrotaline-induced right ventricular hypertrophy. In early stage (< 14 d) I_{Ca} density was increased with mild hypertrophy; in moderate hypertrophy, I_{Ca} density was unchanged; and in late stage (> 28 d) I_{Ca} density was reduced with severe hypertrophy with failure. In the present study, I_{Ca} density increased in hypertrophic ventricular myocytes induced by NE on d 15 or so. This change was similar to previous reports on other models of rat ventricular hypertrophy.

5-HT showed diverse effects on I_{Ca} of various tissues. 5-HT decreased N- and P-type calcium currents in pyramidal neurons *via* a membrane-delimited pathway[13]. In human atrial myocytes 5-HT exerted a positive inotropic effect associated with an increase in I_{Ca}[14]. Now we found 5-HT increased I_{Ca} and decreased the potential of peak current from 0 mV to -10 mV in both hypertrophic and normal cells of rats. These results illuminate that there are 5-HT receptors distributing on the membrane of ventricular myocytes and 5-HT activate the L-calcium channels through an unknown mechanism. The observed changes in gating properties of the L-calcium channel by 5-HT are very similar to those which are known from isoprenaline-induced cAMP-dependent phosphorylation of the Ca^{2+} channel protein in human atrial myocytes[14].

In addition, we found the augmentation of I_{Ca} induced by 5-HT was more significant in hypertrophic cells than in normal cells. These alterations suggest that the larger augmentation of I_{Ca} induced by 5-HT in hypertrophic cells be due to (1) the increase in the number of functional L-calcium channel expression, or/and (2) the increase in the number of functional 5-HT receptor expression, or/and (3) the up-regulated response of L-calcium channel to 5-HT.

In this study, we found that 5-HT did not markedly influence the steady-state activation kinetics. However, 5-HT shifted steady-state inactivation curve with changed $V_{1/2}$ and unchanged slope factor k. The changed $V_{1/2}$ by 5-HT indicates that 5-HT acts on inactive calcium channel and accelerates its recovery from inactivation, which results in increased entry of calcium into cells with less negative resting potentials. The unchanged k shows that 5-HT dose not change the voltage responsiveness of the channel.

We conclude that 5-HT increases I_{Ca} in ventricular

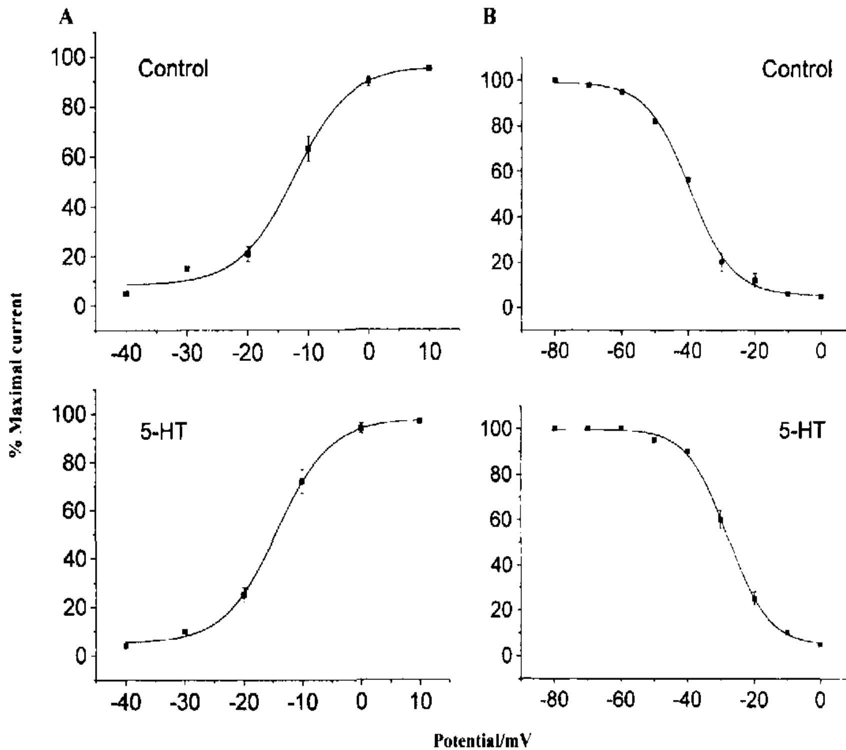

Fig 3. Effects of 5-HT on steady-state activation (A) and inactivation (B) kinetics of I_{Ca} in ventricular myocytes. Curves were derived from the Boltzmann equation using the average calculated values for $V_{1/2}$ and k. 5-HT (10 μmol/L) did not change the $V_{1/2}$ and k of I_{Ca} steady-state activation ($n=5$ cells from 2 hearts, $P>0.05$, Fig 3A). However, 5-HT (10 μmol/L) shifted the $V_{1/2}$ from -40.5 mV to -27.8 mV ($n=4$ cells from 2 hearts, $P<0.05$) and did not change k of I_{Ca} steady-state inactivation.

myocytes by changing the kinetics of steady-state inactivation. A larger alteration of I_{Ca} induced by 5-HT in hypertrophic ventricular myocytes suggests that 5-HT be more prone to induce arrhythmia in hypertrophic heart than in normal one.

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5-羟色胺增强去甲肾上腺素诱导的肥厚心肌 L-型钙电流[1]

R96 A

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关键词 血清素；心肥大；钙通道；膜片箝技术

目的：研究 5-羟色胺(5-HT)对去甲肾上腺素(NE)诱导的大鼠肥厚心肌 L-型钙电流(I_{Ca})的影响. **方法**：大鼠腹腔注射 NE 建立心肌肥厚模型；酶解分离单个心室肌细胞；全细胞膜片箝记录 I_{Ca}. **结果**：(1) 腹腔注射 NE 第 15 天，大鼠左心室与体重比增加 31.8 %. (2) 肥厚心肌细胞 I_{Ca}与正常心肌细胞相比，明显增加 0 mV 时分别为 4.5 pA/pF ± 0.5 pA/pF 和 3.5 pA/pF ± 0.3 pA/pF ($P < 0.01$). (3) 5-HT 可显著增加肥厚和正常心肌细胞 I_{Ca}，并使最大激活电流从 0 mV 降低至 – 10 mV；此外，5-HT 增加 I_{Ca}作用在肥厚心肌细胞更为显著. (4) 稳态激活和失活实验发现，5-HT 对稳态激活曲线无显著影响，而影响稳态失活曲线，使半失活电压从 – 39.5 mV ± 1.8 mV 升高至 – 27.8 mV ± 1.7 mV ($P < 0.05$)，而不改变钙通道电压依赖性(斜率因子 k 无显著变化). **结论**：5-HT 通过改变 L-型钙通道稳态失活特征而显著增加 I_{Ca}，此作用在肥厚心肌细胞更显著，提示在肥厚心肌 5-HT 更易于诱导心律失常发生.

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