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Inhibitory effects of serotonin on transient outward potassium current in rat ventricular myocytes[1]

ZHAO Ming-Gao[2], MEI Qi-Bing, ZHANG Yan-Feng, XING Bing, LI Xiao-Qiang, CUI Yi
(*Department of Pharmacology, The Fourth Military Medical University, Xi-an 710032, China*)

KEY WORDS serotonin; transient outward potassium current; patch-clamp techniques; cardiomegaly

ABSTRACT

AIM: To study the effects of serotonin (5-hydroxytryptamine, 5-HT) on transient outward potassium current (I_{to}) and elucidate its mechanism in rat ventricular myocytes. **METHODS**: I_{to} was recorded using the conventional whole cell patch-clamp techniques. **RESULTS**: I_{to} density in normal myocytes was similar to that in norepinephrine-induced hypertrophic myocytes. 5-HT depressed I_{to} in a concentration-dependent manner with the half-maximal inhibitory concentration of (40 ± 5) μmol/L and (38 ± 7) μmol/L in normal and hypertrophic ventricular myocytes respectively. Mianserin (5-HT_2 receptor antagonist), compound 48/80 (phospholipase C antagonist), and chelerythrine chloride (protein kinase C antagonist) reversed the inhibitory effects of 5-HT on I_{to}, while phorbol 12-myristate 13-acetate (protein kinase C agonist) enhanced the inhibitory effect of 5-HT on I_{to} in normal myocytes. **CONCLUSION**: 5-HT markedly inhibits I_{to} in rat ventricular myocytes. The putative signal pathway is that 5-HT activates phospholipase C, which causes inositol phospholipid hydrolysis. The activation of downstream signal molecule, protein kinase C, phosphorates substrate target proteins, which leads to inhibition of I_{to} in ventricular myocytes.

INTRODUCTION

It has been demonstrated that serotonin (5-hydroxytryptamine, 5-HT) causes positive chronotropic and inotropic effects in human and pig atrium[1,2]. The magnitude of transient outward potassium current (I_{to}) alters the time course of the initial phase of the action potential and determines the time course of other currents during the remainder of the action potential[3]. Thus, any effects of 5-HT on I_{to} would be expected to alter substantially the early phase of repolarization. Ketanserin, a 5-HT_2 receptor antagonist, prolongs the action potential duration and QT interval in rabbit ventricular myocytes[4]. However, the property of 5-HT on I_{to} is not known in ventricular myocytes. To explain 5-HT-related changes in heart rate and contraction, the present experiments were designed to investigate the effects of 5-HT on I_{to} in isolated rat normal and hypertrophic left ventricular cells using the conventional whole-cell patch-clamp technique.

MATERIALS AND METHODS

Animals Five-week-old male Sprague-Dawley rats weighing 170 - 190 g (provided by the Experimental Animal Center of Fourth Military Medical University, Grade Ⅱ, Certificate № C98008) were treated with norepinephrine (NE, Sigma) to produce left ventricular hypertrophy as described previously[5]. In brief, NE was injected ip twice daily at the dose of 1.5 mg/kg for 15 d. The study was carried out in the followed week, when the rats were 200 - 220 g. Age-matched normal rats weighing 210 - 230 g served as control.

Cell isolation The single myocytes were isolated from left ventricle of rats[6]. 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 Langendorff apparatus. It was perfused retrogradely *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, followed by perfusion for 5 min with Ca^{2+}-free Tyrode's solution. The heart was then perfused with 0.1 % collagenase (type Ⅰ, Sigma) dissolved in Ca^{2+}-free Tyrode's solution until the solution flowed freely (15

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[2] Correspondence to Dr ZHAO Ming-Gao. Phn 86-29-337-4555. Fax 86-29-337-4552. E-mail mgzhao@hotmail.com
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to 30 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 beaker containing KB solution. The tissue pieces were gently stirred, and isolated single cells were then filtered through a nylon mesh (180 μm). Isolated left ventricular cells were stored in the KB solution at 4 ℃ and studied 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 hydroxyethyl piperazing ethanesulfonic acid (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, ethylene glycol-bis (β-aminoethyl ether) egtazic acid (EGTA) 0.5, and HEPES 10 (pH 7.4 with KOH). For the recording of I_{to}, the external solution contained (in mmol/L): Choline chloride 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10, and glucose 10 (pH 7.4 with KOH). The pipette solution was composed of (in mmol/L) KCl 150, $MgCl_2$ 1, Na_2ATP 5, EGTA 10, and HEPES 5 (pH 7.2 with KOH).

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 cover slip and perfused at room temperature (18 – 22 ℃). Rod-shape cells with clear cross-striations and resting potential of at least – 75 mV were used. The currents were recorded by patch-clamp techniques with a CEZ 2300 amplifier (Nihon Kohden). The resistance of patch pipette ranged from 2 to 5 MΩ when filled with the pipette solution. Pipette capacitance and series resistance were compensated to minimize the duration of capacitive currents. I_{to} was recorded by applying 300-ms depolarizing pulses to a test potential ranging from – 40 mV to + 70 mV in 10 mV steps from a holding potential of – 60 mV at an interval of 5 s (0.2 Hz). The current amplitude of I_{to} was measured as the difference between the peak outward current and the current at the end of the pulse. The current signal was sampled directly into a computer and analyzed by pClamp software (version 7.0, AXON Instruments Inc).

Data analysis Data were expressed as $\bar{x} \pm s$ and differences were estimated by Student's *t*-test.

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 ($P < 0.05$) and the ratio of left heart weight to body weight (LHW/BW) was higher ($P < 0.05$) in NE-treated rats than those in control rats (Tab 1). The cell membrane capacitance was 134 pF ± 15 pF ($n = 36$) in normal cells and 182 pF ± 18 pF ($n = 24$) in hypertrophic cells ($P < 0.01$).

Tab 1. Characteristics of experimental animals. BW: body weight; LHW: left ventricular weight. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* normal.

Group	*n*	BW/g	LHW/g	LHW/BW
Normal	12	218 ± 11	0.41 ± 0.11	2.23 ± 0.21
Hypertrophy	8	212 ± 8	0.58 ± 0.16^b	2.92 ± 0.27^c

Effects of 5-HT on I_{to} in hypertrophic and normal cells The current traces were obtained before and after exposure to 5-HT 10 μmol/L (Fig 1A). In both normal ($n = 27$ cells from 7 rats) and hypertrophic cells ($n = 25$ cells from 5 rats), the threshold voltage for the activation of I_{to} was – 30 mV. I_{to} density in normal myocytes was similar to that in hypertrophic myocytes. I_{to} was inhibited by 36.0 % [from (25 ± 4) pA/pF to (16 ± 3) pA/pF] and 40.7 % [from (27 ± 4) pA/pF to (16 ± 3) pA/pF] in normal and hypertrophic myocytes, respectively (Fig 1B), showing no distinct difference between normal and hypertrophic myocytes. 5-HT depressed I_{to} in a concentration-dependent manner with the half-maximal inhibitory concentration of (40 ± 5) μmol/L and (38 ± 7) μmol/L in normal and hypertrophic ventricular myocytes respectively (Fig 2).

Roles of 5-HT_2 receptor, phospholipase C, and protein kinase C in the effect of 5-HT on I_{to} Mianserin (10 μmol/L), a selective 5-HT_2 receptor antagonist, partially abolished the inhibitory effects of 5-HT on I_{to}, increasing the peak current from (16.3 ± 2.0) pA/pF to (19.3 ± 2.2) pA/pF at test potential of + 70 mV ($P < 0.05$). The current-voltage relations of I_{to} were obtained in 9 cells from 3 normal rats (Fig 3A). Compound 48/80 (10 mg/L), a phospholipase C antagonist, reversed the inhibitory effect of 5-HT on I_{to} by 17.8 % (14.8 pA/pF ± 2.0 pA/pF *vs* 18.0 pA/pF

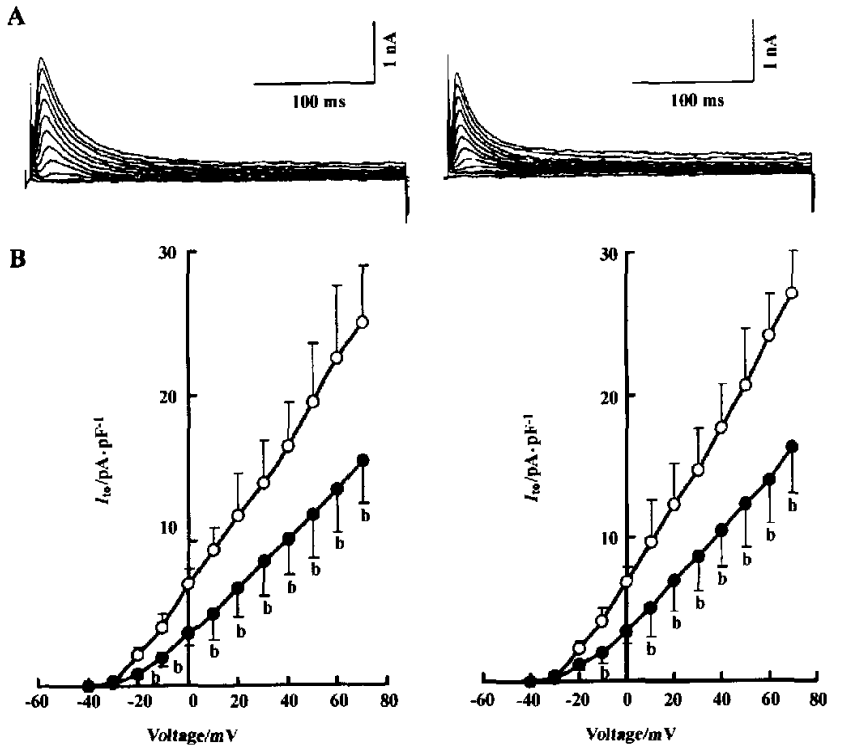

Fig 1. Effects of 5-HT on I_{to} in rat ventricular cells. A: representative traces of I_{to} recorded in normal ventricular myocytes of rats before (left) and after (right) perfusion of 5-HT 10 μmol/L. B: current-voltage relationships of I_{to} obtained in (left) normal and (right) hypertrophic cells before (○) and after (●) perfusion of 5-HT 10 μmol/L. $^bP < 0.05$ *vs* before perfusion of 5-HT. $\bar{x} \pm s$.

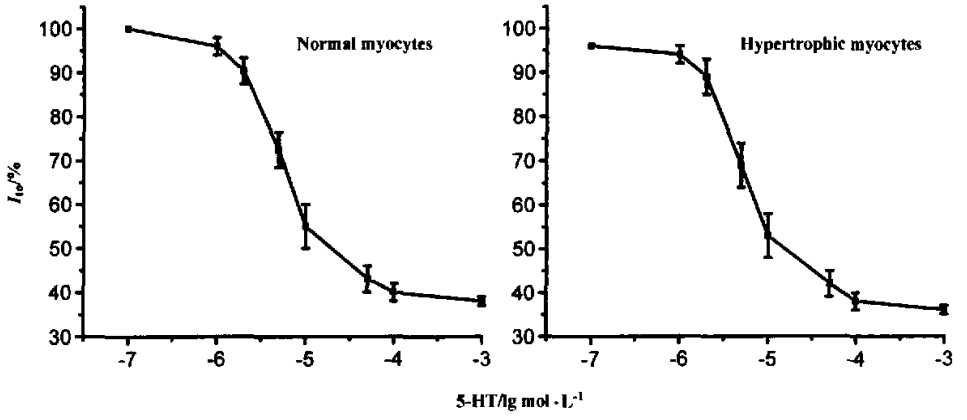

Fig 2. Dose-response curve for 5-HT inhibiting I_{to} in normal ($n = 7$) and hypertrophic ($n = 6$) myocytes. I_{to} was elicited at +70 mV from holding potential of −60 mV. $\bar{x} \pm s$.

± 2.5 pA/pF) at test potential of + 70 mV, showing that phospholipase C pathway is involved in 5-HT inhibiting I_{to}. The current-voltage relations of I_{to} were obtained in 6 cells (Fig 3B). Phorbol 12-myristate 13-acetate (PMA, 1 nmol/L), a potent protein kinase C agonist, enhanced the inhibitory effect of 5-HT on I_{to},

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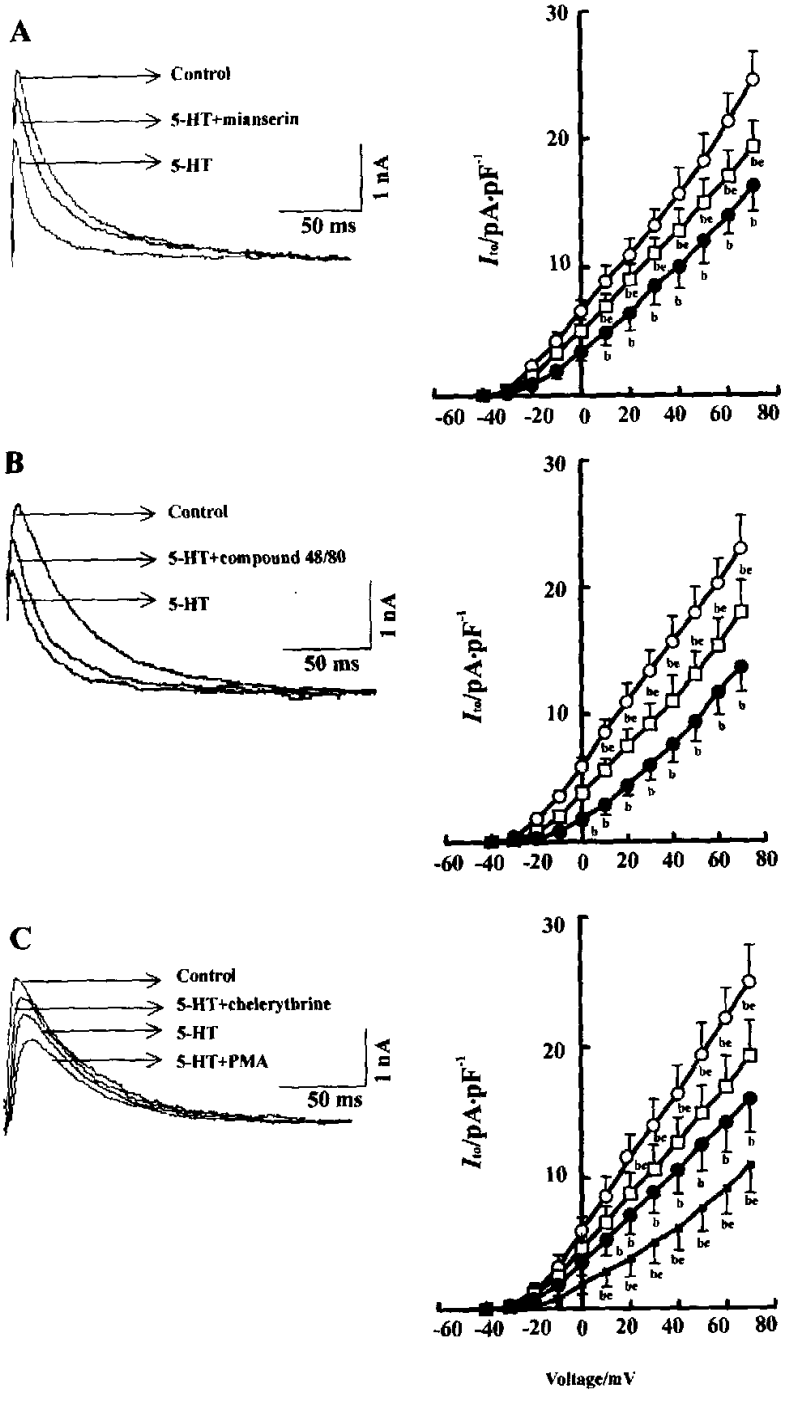

Fig 3. Effects of mianserin, compound 48/80, PMA, and CHE on I_{to}. Left column shows representative trace of I_{to} elicited in normal myocytes. A: mianserin (10 μmol/L) and B: compound 48/80 (10 μg/L) reversed the inhibitory effect of 5-HT on I_{to}. C: PMA enhanced while CHE (1 nmol/L) reversed the inhibitory effect of 5-HT. Right column shows the current-voltage relationship of I_{to} before (○) and after treated with 5-HT (●). A: 5-HT + mianserin (□); B: 5-HT + compound 48/80 (□); C: 5-HT + chelerythrine (□) and 5-HT + PMA (■). $\bar{x} \pm s$. $^bP < 0.05$ *vs* control. $^eP < 0.05$ *vs* 5-HT.

nearly increasing 31.2 % (from 16.0 pA/pF ± 2.6 pA/pF to 11.0 pA/pF ± 2.2pA/pF) at the test potential of + 70 mV. On the other hand, chelerythrine chloride (CHE, 1 nmol/L) reversed the inhibitory effect of 5-HT approximately by 20.6 % (from 16.0 pA/pF ± 2.6 pA/pF to 19.3 pA/pF ± 2.7 pA/pF). Results indicated that protein kinase C (PKC) played a key role in 5-HT inhibitory action. The current-voltage relations of I_{to} were obtained in 13 cells from 5 normal rats (Fig 3C).

DISCUSSION

According the literatures I_{to} density in hypertrophy models was shown to be increased, decreased, or unchanged in rats, cats, or guinea pigs[7-9]. In present study, I_{to} density unchanged in hypertrophic ventricular myocytes induced by NE compared with normal ones. 5-HT inhibited I_{to} in both hypertrophic and normal ventricular myocytes in rats, showing no distinctly difference in inhibition of I_{to} density before and after cardiac hypertrophy. I_{to} mainly contributes to repolarization at a slow heart rate because of its slow recovery from inactivation[10]. It suggests a possible role of 5-HT in regulating heart rate. It has been found 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptor subtypes distributing in mammal heart, mainly on the membrane of coronary vessel, heart sympathetic nerve terminal, or atrial myocytes[11-14]. There is no direct evidence to prove the existence of 5-HT receptor on the membrane of ventricular myocytes. Our study illuminates indirectly that there is 5-HT receptor distributing on the membrane of ventricular myocytes. The subtype of receptor might be 5-HT_2 for mianserin (selective 5-HT_2 receptor antagonist) partially abolishing the inhibitory effect of 5-HT on I_{to}. The further study should be carried out to find the direct proof of 5-HT_2 receptor distribution.

5-HT inhibited I_{to} in similar degree in normal and hypertrophic cells. This effect did not depend on the degree of cardiac hypertrophy. It is well known that 5-HT receptors are G-protein coupled ones except for 5-HT_3 receptor being ion channel coupled. In this study, we found that both selective 5-HT_2 receptor antagonist and phospholipase C inhibitor reversed the inhibitory effect of 5-HT on I_{to}. It suggests that phospholipase C signal pathway is involved in 5-HT inhibiting I_{to}. The putative signal pathway is that 5-HT activates phospholipase C, which causes inositol phospholipid hydrolysis. The activation of downstream signal molecule, protein kinase C, phosphorates substrate target proteins, which lead to inhibition of I_{to} in ventricular myocytes.

REFERENCES

1 Kaumann AJ, Sanders L, Brown AM, Murray KJ, Brown MJ. A 5-hydroxytryptamine receptor in human atrium. Br J Pharmacol 1990; 100: 879-85.
2 Kaumann AJ. 5-HT_4-like receptors in mammalian atria. J Neural Transm 1991; 34: 195-201.
3 Martinez ML, Delgado C. Methoxamine inhibits transient outward potassium current through α_{1A}-adrenoceptors in rat ventricular myocytes. J Cardiovasc Pharmacol 2000; 35: 212-8.
4 Le Grand B, Marty A, Colpaert FC, John GW. Ketanserin inhibits the transient outward current in rabbit ventricular myocytes. J Cardiovasc Pharmacol 1995; 25: 341-4.
5 Xiang Y, Huang J. Effect of valsartan and fosinopril on catecholamine-induced cardiac hypertrophy. Acta Pharmacol Sin 2000; 21: 850-4.
6 Ai J, Gao HH, He SZ, Wang L, Luo DL, Yang BF. Effects of matrine, artemisinin, tetrandrine on cytosolic $[Ca^{2+}]_i$ in guinea pig ventricular myocytes. Acta Pharmacol Sin 2001; 22: 512-5.
7 Tomita F, Basset AL, Myerburg RJ, Kimura S. Diminished transient outward currents in rat hypertrophic ventricular myocytes. Circ Res 1994; 75: 296-303.
8 Li Q, Keung EC. Effects of myocardial hypertrophy on transient outward current. Am J Physiol 1994; 266: H1738-45.
9 Ten Eick RE, Zhang K, Harvey RD, Bassett AL. Enhanced functional expression of transient outward current in hypertrophied feline myocytes. Cardiovasc Drugs Ther 1993; 7 Suppl 3: 611-9.
10 Kawano S, Hiraoka M. Transient outward currents and action potential alterations in rabbit ventricular myocytes. J Mol Cell Cardial 1991; 23: 681-93.
11 McFadden EP, Clarke JG, Davies GJ, Kaski JC, Haider AW, Maseri A. Effect of intracoronary serotonin on coronary vessels in patients with stable angina and patients with variant angina. N Engl J Med 1991; 324: 648-54.
12 Stassen FR, Fazzi GE, Leenders PJ, Smits JF, de Mey JG. Coronary arterial hyperreactivity and mesenteric arterial hyperreactivity after myocardial infarction in the rat. J Cardiovasc Pharmacol 1997; 29: 780-8.
13 Leal DM, Callera JC, Bonagamba LG, Nosjean A, Laguzzi R, Machado BH. Microinjection of a 5-HT_3 receptor agonist into the NTS of awake rats inhibits the bradycardic response to activation of the von Bezold-Jarisch reflex. Brain Res Bull 2001; 54: 7-11.
14 Pino R, Cerbai E, Calamai G, Alajmo F, Borgioli A, Braconi L, et al. Effect of 5-HT_4 receptor stimulation on the pacemaker current $I(f)$ in human isolated atrial myocytes. Cardiovasc Res 1998; 40: 516-22.

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血清素抑制大鼠心室肌细胞瞬时外向钾电流[1]

招明高[2]，梅其炳，张延凤，邢 斌，李小强，崔 毅 （第四军医大学药理学教研室，西安 710032，中国）

关键词 血清素；瞬时外向钾电流；膜片箝技术；心肥大

目的：观察血清素(5-HT)对大鼠心室肌细胞瞬时外向钾电流 I_{to} 的影响并探讨其作用机制．**方法**：全细胞膜片箝技术记录 I_{to}．**结果**：I_{to} 电流密度在正常心肌和肥厚心肌细胞无明显差异．在实验电压为 +70 mV 时，5-HT 浓度依赖性抑制 I_{to}，在正常和肥厚心肌细胞，其半数抑制浓度分别为(40 ± 5) μmol/L 和 (38 ± 7) μmol/L．5-HT_2 受体阻断剂米胺舍林和磷脂酶 C 抑制剂 Compound 48/80 均可逆转 5-HT 抑制 I_{to} 的作用；蛋白激酶激动剂醋酸佛波酯显著加强 5-HT 的抑制作用，而蛋白激酶抑制剂白屈菜季铵碱则逆转 5-HT 抑制 I_{to} 的作用．**结论**：5-HT 具有抑制心肌细胞 I_{to} 的作用．此作用是通过激动 5-HT_2 受体，启动磷脂酶 C 信号转导途径，进一步激活蛋白激酶从而抑制心肌 I_{to}．

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E-mail kwancy@mcmaster.ca