BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 May; 19 (3): 238 - 240

Effects of taurine on L-type voltage-dependent Ca^{2+} channel in rat cardiomyocytes infected with coxsackievirus B_3[1]

LIU Gong-Xin, YANG Ying-Zhen[2], GU Quan-Bao[3], LIU Yan-Hong[3], GUO Qi
(*Key Laboratory of Viral Heart Diseases of Ministry of Public Health, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Shanghai Medical University, Shanghai 200032*; [3]*Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS myocardium; calcium channels; coxsackieviruses B; taurine; patch-clamp techniques

AIM: To study the effects of taurine on L-type voltage-dependent Ca^{2+} channel (VDCC) in adult rat cardiomyocytes infected with coxsackievirus B_3 (CVB_3). **METHODS**: Whole-cell Ca^{2+} current of L-type VDCC was obtained by patch-clamp techniques. **RESULTS**: The density of L-type Ca^{2+} current was 4.1 ± 0.8 pA/pF in normal cardiomyocytes, but increased to 4.9 ± 1.4 pA/pF with CVB_3 infection. At 16 mmol·L^{-1}, taurine decreased the density to 3.5 ± 0.5 pA/pF in normal cardiomyocytes, and to 3.8 ± 0.8 pA/pF in CVB_3-infected cardiomyocytes. In addition, CVB_3 shifted the membrane potential depolarizing to peak current (V_p) from 8 ± 8 mV to 5 ± 3 mV which could also be reverted to 8 ± 4 mV by taurine. **CONCLUSION**: Taurine inhibited the increase of Ca^{2+} inflow through L-type VDCC and normalized the decreased V_p induced by CVB_3 infection. The effect of taurine on L-type VDCC was the mechanism of taurine attenuating the intracellular Ca^{2+} accumulation and abnormal electric activities induced by CVB_3 infection.

Taurine makes up 50 % of the total free amino acid pool in mammalian heart, and possesses a lot of cell protective actions such as modulating intracellular Ca^{2+} homeostasis[1], inhibiting lipoperoxide formation[2], reducing enzyme leakage, and maintaining membrane stability. Deficiency of taurine was implicated in many heart pathological states and could be reversed by taurine supplement[3]. We found that taurine was beneficial to clinical treatment for virus myocarditis[4] and to cultured rat cardiomyocytes infected with coxsackievirus B_3 (CVB_3)[5], and the reduction of transmembrane Ca^{2+} inflow was one of the functions of taurine's protection to cardiomyocytes[6]. However, the transmembrane Ca^{2+} influx involved in a lot of aspects including voltage-dependent Ca^{2+} channel (VDCC), Ca^{2+} leak channel, ATP-dependent Ca^{2+} pump, Na^+/Ca^{2+} exchange, and H^+/Ca^{2+} exchange, *et al*. This study was to investigate whether taurine affected Ca^{2+} current through L-type VDCC in cardiomyocytes infected with CVB_3 to provide a basis for the clinical treatment with taurine in viral myocarditis.

MATERIALS AND METHODS

Cardiomyocytes Cardiomyocytes were isolated from adult Sprague-Dawley rats (n = 29, 200 - 250 g, clean, from Experimental Animal Center, Shanghai Medical University) as previously described[7]. In brief, Hearts were quickly moved and mounted on a Langendorff apparatus for retrograde perfusion at 37 ℃, first with Ca^{2+} free Tyrode's solution for 5 - 7 min, then with the same solution containing 0.05 % collagenase (Type 1, Sigma), 40 - 60 mmol·L^{-1} $CaCl_2$, and 0.01 % BSA for 7 - 10 min. Afterwards, the hearts were incubated in KB solution for 10 - 15 min, then minced and dispersed with a pipette for 3 - 5 min. The suspension was filtered through a 200 μm nylon mesh, and kept at 15 - 25 ℃ for > 1 h before Ca^{2+} recovery with Eagle's minimum essential medium.

Groups (1) N: normal control group. (2) T: taurine control group; including T_1, T_2, and T_3 subgroups added with taurine 1, 8, and 16 mmol·L^{-1}, respectively. (3) CVB_3 control

[1] Project supported by the Key Projects for the Ninth-five Year Plan, No 969060213.
[2] Corespondence to Prof YANG Ying-Zhen.
Phn 86-21-6404-1990, ext 2509. Fax 86-21-6403-8472.
Received 1997-07-02 Accepted 1997-12-12

group, 1×10^5 cells/tube were inoculated with 200 $TCID_{50}$ (50 % tissue culture infection dose) CVB_3. (4) V + T: CVB_3-infected and taurine 16 mmol·L^{-1} treated group. All the cells were incubated at 37 ℃ for 2 h before use.

Recording Whole-cell Ca^{2+} current was recorded by Hamill patch-clamp methods[8] using a glass pipette (diameter 1 – 3 μm). The formation of whole-cell configuration was performed in Tyrode's solution. Then the cell was superfused with the external solution. The rest potential was monitored in current clamp mode, compensated the slow capacitance near rest potential. The data were acquired by a Macinntosh computer using an EPC-9 amplifier and Pulse + Pulsefit 8.0 software (HEAK, German). For L-type VDCC current recording, hold potential was kept at – 50 mV to inactivate Na^+ channel and T-type Ca^{2+} channel, test potential depolarizing from – 40 to + 60 mV, pulse width 100 ms, step + 10 mV, frequency 0.5 Hz. The mean peak Ca^{2+} current of L-type (I_{Ca}) was expressed as a function of membrane capacity (C_m).

Solution preparation (mmol·L^{-1}) (1) Tyrode's solution: NaCl 135, $CaCl_2$ 2, KCl 5.4, $MgCl_2$ 1, NaH_2PO_4 0.33, glucose 5, HEPES 5, pH adjusted to 7.2 with NaOH. (2) KB solution: KOH 80, KCl 40, KH_2PO_4 30, Mg_2SO_4 3, glutamic acid 50, taurine 20, glucose 10, HEPES 10, egtazic acid 1, pH adjusted to 7.4 with KOH. (3) Internal solution: CsCl 120, $MgCl_2$ 1, MgATP 4, egtazic acid 10, HEPES 10, pH adjusted to 7.2 with CsOH. (4) External solution: tetraethylammonium chloride (TEACl) 135, $CaCl_2$ 10, $MgCl_2$ 1, HEPES 10, glucose 10, pH adjusted to 7.4 with TEAOH.

RESULTS

In normal cardiomyocytes, the I_{Ca} was 4.1 ± 0.8 pA/pF, and the membrane potential depolarizing to peak current (V_p) was 8.3 ± 7.8 mV. Taurine 1, 8, and 16 mmol·L^{-1} decreased I_{Ca} to 3.9 ± 0.4, 3.7 ± 0.6, and 3.5 ± 0.5 pA/pF, and the corresponding V_p was 7 ± 7, 11 ± 3, and 9 ± 5 mV, respectively. Though taurine inhibited I_{Ca} in a concentration-dependent manner, only taurine 16 mmol·L^{-1} caused substantial effects on the basal I_{Ca} compared with normal group ($P < 0.05$). Taurine had no effect on V_p in normal cardiomyocytes. With the CVB_3 infection, the I_{Ca} increased from 4.1 ± 0.8 to 4.9 ± 1.4 pA/pF ($P < 0.01$, *vs* normal group), while taurine 16 mmol·L^{-1} counteracted the effects of CVB_3 on I_{Ca} and decreased the I_{Ca} to 3.8 ± 0.8 pA/pF ($P < 0.01$, *vs* virus group). The V_p in CVB_3-infected cardiomyocytes decreased to 5 ± 3 mV which was a little lower than V_p in normal group (Tab 1). Thus, the I_{Ca} trended to reach its peak value at lower membrane potential in CVB_3-infected cardiomyocytes (Fig 1). Similarly, taurine normalized the decreased V_p, and reverted it to 8 ± 4 mV.

Tab 1. C_m, V_p, and I_{Ca}. Cells from 29 rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* normal group. $^fP < 0.01$ *vs* virus group.

Group/ mmol·L^{-1}	n (cells)	C_m (pF)	V_p (mV)	I_{Ca} (pA/pF)
Normal	23	263 ± 64	8 ± 8	4.1 ± 0.8
Taurine 1	10	262 ± 58	7 ± 7	3.9 ± 0.4
Taurine 8	10	231 ± 38	11 ± 3	3.7 ± 0.6
Taurine 16	16	284 ± 53	9 ± 5	3.5 ± 0.5^b
Virus	14	260 ± 44	5 ± 3	4.9 ± 1.4^c
Virus + Taurine 16	14	289 ± 81	8 ± 4	3.8 ± 0.8^f

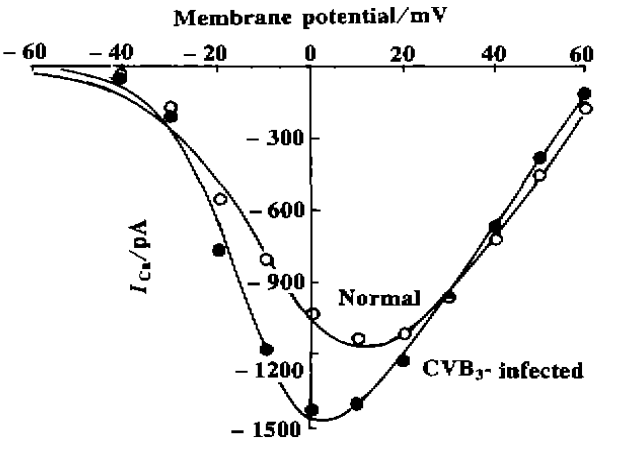

Fig 1. Relation between L type Ca^{2+} current and membrane voltage in normal and CVB_3-infected cardiomyocytes.

DISCUSSION

With CVB_3 infection the intracellular Ca^{2+}

was increased[6], and our study indicated that the increase was partly associated with the influx of Ca^{2+} through L-type VDCC. The enhanced L-type Ca^{2+} current induced by CVB_3 infection increased intracellular Ca^{2+} accumulation during action potential; besides, the Ca^{2+} inflow through VDCC was a promoter of Ca^{2+} releasing from sarcolemmal reticulum (SR). Except increasing calcium inflow, CVB_3 trended to decrease the V_p; in other words, the L-type VDCC could be activated at lower membrane potential after CVB_3 infection. The extra Ca^{2+} inflow, the intracellular free Ca^{2+} accumulation, and the shifted V_p resulted in intracellular Ca^{2+} overload and abnormal electric activities, which was the reason of cell damage in viral infection[9].

Taurine decreased Ca^{2+} entry both in normal cardiomyocytes and cardiomyocytes infected with CVB_3 and reversed the decreased V_p induced by CVB_3. Former study revealed that taurine affected the intracellular calcium by a dual effect that depended on calcium concentration[10]. It promoted Ca^{2+} inflow with low external calcium concentration, but exerted an opposite effect with high calcium concentration. In our experiment protocols, with Ca^{2+} 10 mmol·L^{-1} in external solution, taurine played a role of decreasing Ca^{2+} entry. By decreasing the Ca^{2+} entry through L-type VDCC and reversing the decreased V_p, taurine attenuated the intracellular Ca^{2+} accumulation and reduced abnormal electric activities induced by CVB_3.

REFERENCES

1 Satoh H. Cardioprotective action of taurine against intracellular and extracellular calcium-induced effects. Adv Exp Med Biol 1994; 359: 181 – 96.

2 Raschke P, Massoudy P, Becker BF. Taurine protects the heart from neutrophil-induced reperfusion injury. Free Radic Biol Med 1995; 19: 461 – 71.

3 Struman J. Review: taurine deficiency and the heart. Adv Exp Med Biol 1994; 315: 1 – 15.

4 Gu W, Yang YZ, He MX. A study on combination therapy of western and traditional Chinese medicine of acute viral myocarditis. Chin J Integ Trad West Med 1996; 16: 713 – 6

5 Su YG, Yang YZ, Gu Q, Gu QB, Zhao JX, Chen HZ. Effect of taurine on cultured rat cardiomyocytes infected with coxsackie B_3 virus. Chin Pharmacol Bull 1997; 13: 56 – 60.

6 Su YG, Yang YZ, Gu QB, Guo Q, Zhao JX, Chen HZ. Effects of taurine on calcium influx in rat cultured cardiomyocytes after coxsakie virus B_3 infection. Acta Acad Med Shanghai 1996; 23: 339 – 42.

7 Liu GX, Guo Q, Gu QB, Yang YZ. A simple, rapid method to isolate cardiomyocytes from rat and guinea pig. Ann Chin Appl Physiol 1997; 13: 361 – 2.

8 Hamill OP, Marty A, Neher L, Sakmann B, Sigworth FJ. Improved patch clamp technique for high resolution current recording from cells and cell free membrane patches. Pflüger Arch 1981; 391: 85 – 100.

9 Yang YZ, Guo Q, Zhou TS, Zhang J, Li L, Jin PY, *et al*. Electrophysiological and pathological observations on experimental coxsackie B-3 viral myocarditis in mice. Chin Med J 1993; 106: 100 – 104.

10 Sada H, Ban T, Speralakis N. Action potential duration stabilizing action of taurine in guinea pig ventricular myocytes. Jpn J Pharmacol 1996; 70: 303 – 12.

牛磺酸对感染柯萨奇 B_3 病毒大鼠心肌细胞 L 型电压依赖性钙通道的影响[1]

刘恭鑫, 杨英珍[2], 顾全保[3], 刘艳红[3], 郭 棋

(卫生部病毒性心脏病重点实验室, 上海医科大学附属中山医院, 上海市心血管病研究所, 上海 200032; [3] 中国科学院上海细胞生物学研究所, 上海 200031, 中国)

关键词 心肌; 钙通道; 柯萨奇 B 病毒; 牛磺酸; 膜片箝技术

目的: 观察牛磺酸对正常和感染柯萨奇 B_3 病毒大鼠心肌细胞 L 型钙通道的影响. **方法**: 用膜片箝技术记录经 L 型钙通道的 Ca^{2+} 电流. **结果**: 正常心肌细胞 L 型钙通道的 Ca^{2+} 电流密度为 4.1 ± 0.8 pA/pF, 柯萨奇 B_3 病毒感染后 Ca^{2+} 电流密度增加到 4.9 ± 1.4 pA/pF. 牛磺酸 16 mmol·L^{-1} 不仅使正常心肌细胞 L 型钙通道的 Ca^{2+} 电流密度降为 3.5 ± 0.5 pA/pF, 也使感染柯萨奇 B_3 病毒后心肌细胞的 Ca^{2+} 电流密度降为 3.8 ± 0.8 pA/pF. 柯萨奇 B_3 病毒感染使引起最大 Ca^{2+} 电流的膜电压 (V_p) 由 8 ± 8 mV 减为 5 ± 3 mV, 牛磺酸可使降低的 V_p 恢复到 8 ± 4 mV. **结论**: 牛磺酸抑制柯萨奇 B_3 病毒感染引起的 Ca^{2+} 电流的增加, 并使因感染而降低的引起最大 Ca^{2+} 电流的膜电压正常化. 牛磺酸对 L 型钙通道的影响是牛磺酸减轻病毒感染引起的细胞内 Ca^{2+} 增加和异常电活动的机制之一.