ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Aug; 22 (8): 701 – 705
http://www.chinaphar.com

Effect of dipfluzine on L-type calcium current in guinea pig ventricular myocytes

R96 A

ZHANG Yong-Jian, LI De-Pei, XUE Bao-Jian, WANG Yong-Li, HE Rui-Rong[1]
(*Department of Physiology, Hebei Medical University, Shijiazhuang 050017, China*)

KEY WORDS dipfluzine; patch-clamp techniques; myocardium; cultured cells; calcium channels

ABSTRACT

AIM: To study the effect of dipfluzine (Dip) on L-type calcium current in guinea pig ventricular myocytes. **METHODS**: Single myocytes were dissociated by enzymatic dissociation method. The current was recorded with the whole-cell configuration of the patch-clamp technique. **RESULTS**: Dip (0.3 – 30 μmol/L) reduced the voltage-dependently activated peak value of $I_{Ca\text{-}L}$ in a concentration-dependent manner. The characteristics of I-V relationship were not greatly altered by Dip, and the maximal activation voltage of $I_{Ca\text{-}L}$ in the presence of Dip was not different from that of control. Steady-state activation of $I_{Ca\text{-}L}$ was not affected markedly, and the half activation potential ($V_{0.5}$) and the slope factor (κ) in the presence of Dip 3 μmol/L were not markedly different from those of the control. $V_{0.5}$ value was (-12.8 ± 1.7) mV in the control and (-13.2 ± 2.4) mV in the presence of Dip 3 μmol/L. The κ value was (7.1 ± 0.4) mV in the control and (7.5 ± 0.5) mV in the presence of Dip 3 μmol/L ($n = 7$ cells from 3 hearts, $P > 0.05$). Dip 3 μmol/L markedly shifted the steady-state inactivation curve of $I_{Ca\text{-}L}$ to the left, and accelerated the voltage-dependent steady-state inactivation of calcium current. $V_{0.5}$ value was (-19.7 ± 2.4) mV in the control and (-31 ± 6) mV in the presence of Dip 3 μmol/L. The κ value was (3.6 ± 0.3) mV in the control and (1.8 ± 0.2) mV in the presence of Dip 3 μmol/L ($n = 4$ cells from 2 hearts, $P < 0.05$). Dip 3 μmol/L markedly delayed half-recovery time of Ca^{2+} channel from inactivation from (40 ± 11) to (288 ± 63) ms ($n = 4$, $P < 0.01$). **CONCLUSION**: Dip mainly acts on the inactivated state of L-type calcium channel, accelerates the inactivation of calcium channel, and slows the recovery of calcium channel from inactivated state in guinea pig ventricular myocytes, through which the $I_{Ca\text{-}L}$ is inhibited.

INTRODUCTION

Dipfluzine (Dip) decreased overshoot, action potential amplitude (APA), maximal rate of depolarization in phase 0 (V_{max}), duration of plateau phase, and durations of 50 % and 90 % repolarization in partially depolarized guinea pig papillary muscles[1], and reduced APA, V_{max}, velocity of diastolic (phase 4) depolarization (VDD), and rate of pacemaker firing (RPF) in rabbit sinoatrial node pacemaker cells, and human atrial fibers[2,3]. Dip antagonized high-Ca^{2+}- and Bay K 8644-induced increase in VDD and RPF[2], which may be attributed to its blocking effect on Ca^{2+} influx. The present experiment was undertaken to investigate the effect of Dip on L-type calcium current ($I_{Ca\text{-}L}$) of ventricular myocytes.

MATERIALS AND METHODS

Isolation of ventricular myocytes Single guinea pig ventricular myocytes were prepared by enzymatic dissociation technique[4]. Guinea pigs of either sex weighing 350 – 400 g, provided by Experimental Animal Center of Hebei Province (Grade Ⅱ, Certificate No 04064), were stunned by heavy blow on the heads and their hearts were rinsed in oxygenated ice-cold Ca^{2+}-free Tyrode's solution (NaCl 100, KCl 10, $MgSO_4$ 5, NaH_2PO_4 1.2, glucose 20, taurine 10, MOPS 10 mmol/L, pH was adjusted to 7.4 with KOH). The heart was rapidly excised and a Langendorff retrograde perfusion was performed through the aorta at a rate of 9 mL/min with Ca^{2+}-free Tyrode's solution for 5 min, and then with the same solution containing collagenase Ⅱ 270 mg/L, bovine serum albumin 570 mg/L, and $CaCl_2$

[1] Correspondence to Prof HE Rui-Rong.
Phn 86-311-604-4121, ext 5566. Fax 86-311-604-8177.
E-mail syho@263.net
Received 2000-06-09 Accepted 2001-04-02

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Aug; 22 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

34 μmol/L for 6 min at 37 ℃. The ventricles were cut, minced, and incubated for 10 min in Ca^{2+}-free Tyrode's solution containing 0.1 % bovine serum albumin. Myocytes were harvested after filtration through a 200 μm nylon mesh, and resuspended in Tyrode's solutions containing different concentrations of Ca^{2+}. The concentration of Ca^{2+} was gradually increased to 1 mmol/L.

Whole cell patch-clamp technique Glass pipettes (Shanghai Brain Research Institute) were made using a two-stage vertical microelectrode puller (PB-7, Narishige). Once filled with pipette solution (TEA-Cl 20, MgATP 5, CsCl 125, HEPES 10, egtazic acid 15 mmol/L, pH 7.2 adjusted with CsOH), the pipette resistance ranged from 2.0 to 4.0 MΩ.

Transmembrane currents were recorded using the single electrode (whole cell patch-clamp) technique[5] with an Axopatch amplifier (200B, Axon Instruments, Inc). The dissociated cells were transfered to a 0.4 mL chamber mounted on an inverted microscope (CK2, Olympus). After settling to the bottom of chamber, cells were perfused with external solution[6] (NaCl 137.6, KCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, glucose 10, HEPES 11.6, CsCl 20 mmol/L, gassed with 100 % O_2, pH 7.4 adjusted with NaOH) for 10 min at a rate of 2 – 3 mL/min. Only rod-shape cells with clear cross-striations and membrane capacitances about 140 pF were used. Liquid junction potential between the pipette solution and external solution was corrected after the pipette tip entered the external solution. After gigaseal formation, the membrane was ruptured with a gentle suction to obtain the whole cell voltage-clamp configuration. Membrane capacitance and series resistance were compensated after membrane rupture to minimize the duration of capacitive currents. Computer-generated voltage or current pulses were programmed using the pCLAMP 6.0 software (Axon Instruments, Inc). On-line acquired data were stored on a hard disk of the microcomputer.

Drug Dip (purity > 99.85 %) synthesized by Department of Chemistry, Peking University was dissolved in water-free ethanol as stock solution (10 mmol/L) and diluted in external solution to the desired final concentrations (0.3, 3, and 30 μmol/L) before experiment. The volume concentrations of ethanol in external solution were 0.03, 0.3, and 3 mL/L, respectively.

Statistics The values were expressed as $\bar{x} \pm s$. Statistical analysis was performed using t-test.

RESULTS

Identification of L-type calcium current A slowly inactivated inward current was recorded by depolarization of individual ventricular cells from a holding potential of – 50 mV to 0 mV. Under this condition, both sodium channel and T-type calcium channel were in inactivated state[7]. Potassium currents were eliminated by using TEA-Cl (20 mmol/L) and CsCl (125 mmol/L) in the pipette solution and CsCl (20 mmol/L) in the external solution. The slowly inactivated inward current was decreased by verapamil (1 μmol/L) and increased by Bay K 8644 (10 nmol/L) (data not shown). So, the inward current was L-type calcium current ($I_{Ca\text{-}L}$).

Effect of Dip on $I_{Ca\text{-}L}$ As cells were depolarized from a holding potential of – 50 mV to + 50 mV at 10-mV increment 5 min after whole cell formation, a Ca^{2+} current was progressively activated. After a 5-min period of external solution perfusion for control measurements, the external solution was changed to Dip-containing external solution, and data were collected again after 5 min. The concentrations of Dip in the external solution were 0.3, 3 and 30 μmol/L, respectively. Cells were perfused again with drug-free solution for 5 min to determine the reversibility of drug action. Dip 0.3 μmol/L had no marked effect on $I_{Ca\text{-}L}$, while Dip 3 and 30 μmol/L decreased $I_{Ca\text{-}L}$ markedly (Fig 1), and shifted I-V curve upwards (Fig 2). The V_{max} activation of $I_{Ca\text{-}L}$ appeared at 0 mV in the presence of Dip, which was not different from the control.

Effect of Dip on voltage-dependent inactivation of $I_{Ca\text{-}L}$ $I_{Ca\text{-}L}$ was activated with test pulses to + 10 mV for 400 ms, which were preceded by 1400 ms pre-pulses to – 10 mV from the holding potential of – 80 mV at 10-mV increment 5 min after whole cell formation. Double-pulse stimulation was repeated every 5 s. Increasing the potential of pre-pulses led to a decrease in $I_{Ca\text{-}L}$ amplitude during the test pulses, which indicated that the inactivating channel number of $I_{Ca\text{-}L}$ was increased. Inactivation reached V_{max} at 0 mV. Dip 3 μmol/L markedly decreased $I_{Ca\text{-}L}$. The V_{max} peak current in the presence of Dip 3 μmol/L was less than half of that in the absence of Dip.

Effects of Dip on activation and inactivation kinetics of $I_{Ca\text{-}L}$ The activation curves were fitted according to the Boltzmann equation: $g/g_{max} = 1/\{1 + EXP[-(V - V_{0.5})/\kappa]\}$. Dip 3 μmol/L did not markedly influence the activation kinetics, with half activation

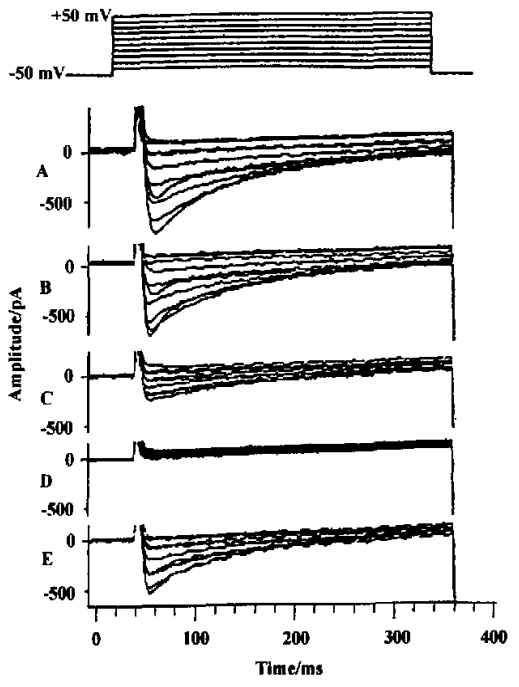

Fig 1. Effect of Dip on $I_{Ca\text{-}L}$ in isolated guinea pig ventricular myocytes. Currents were recorded during 320 ms depolarizations from a holding potential of -50 mV to test potentials between -40 mV and +50 mV in 10 mV increments. A: Control. B, C, D: Dip 0.3, 3, and 30 μmol/L, respectively. E: Washout of Dip 3 μmol/L.

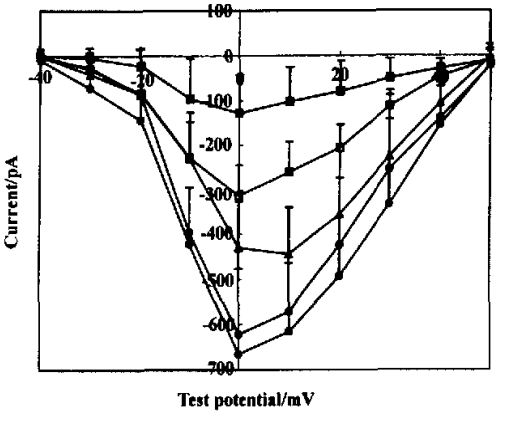

Fig 2. Effect of Dip on I-V curve of $I_{Ca\text{-}L}$ in isolated guinea pig ventricular myocytes. (○) Control. (●, □, ■) Dip 0.3, 3, and 30 μmol/L, respectively. (△) Washout of Dip 3 μmol/L. $n = 7$ cells from 3 hearts.

potential ($V_{0.5}$) from (-12.8 ± 1.7) mV to (-13.2 ± 2.4) mV, and slope parameter (κ) from (7.1 ± 0.4) mV to (7.5 ± 0.5) mV ($n = 7$ cells from 3 hearts, $P > 0.05$, Fig 3A). The inactivation curves were fitted according to the Boltzmann equation: $I/I_{max} = 1/\{1 + EXP[(V - V_{0.5})/\kappa]\}$. Dip 3 μmol/L shifted half inactivation potential ($V_{0.5}$) from (-19.7 ± 2.4) mV to (-31 ± 6) mV, and κ from (3.6 ± 0.3) mV to (1.8 ± 0.2) mV ($n = 4$ cells from 2 hearts, $P < 0.05$, Fig 3B).

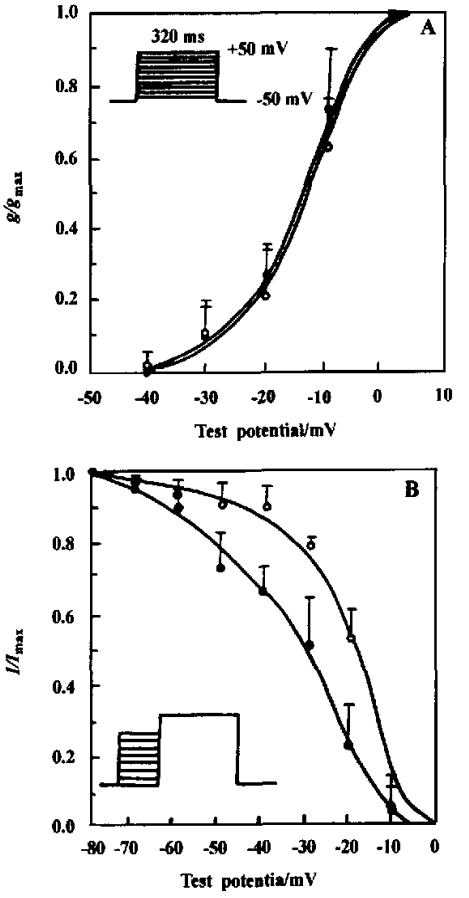

Fig 3. A) Activation curve of $I_{Ca\text{-}L}$. $n = 7$ from 3 hearts. $\bar{x} \pm s$. B) Inactivation curve of $I_{Ca\text{-}L}$. $n = 4$ cells from 2 hearts. $\bar{x} \pm s$. (○) Control, (●) Dip 3 μmol/L.

Effect of Dip on recovery of $I_{Ca\text{-}L}$ from inactivation The recovery of $I_{Ca\text{-}L}$ from inactivation was

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Aug; 22 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

studied using double-pulse protocol consisting of a 1000-ms pre-pulse to + 10 mV (P1) followed by a 1000-ms test pulse to + 10 mV (P2) after a variable P1-P2 coupling interval from 0 to 3000 ms at the holding potential of − 80 mV. Double-pulse stimulation was repeated every 6 s. Dip 3 μmol/L markedly delayed the half-recovery time of Ca^{2+} channel from inactivation from (40 ± 11) to (288 ± 63) ms ($n = 4$, $P < 0.01$, Fig 4).

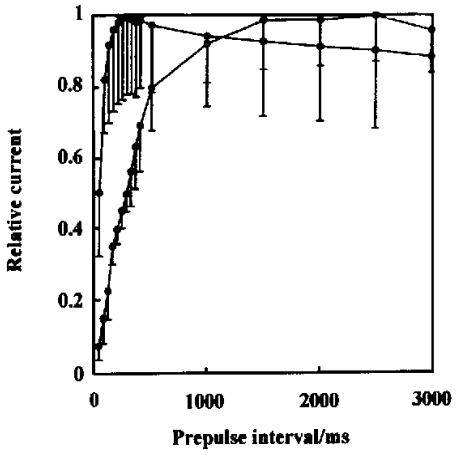

Fig 4. Time-dependent recovery of $I_{Ca\text{-}L}$ from the steady-state inactivation. (○) Control, (●) Dip 3 μmol/L. $n = 4$ cells from 2 hearts. $\bar{x} \pm s$.

DISCUSSION

In the present study, the slowly inactivated inward current recorded was L-type calcium current. Rundown of calcium current is always a concern in whole-cell patch-clamp recording. In this study, the rundown of I_{Ca} was minimized by adding MgATP (5 mmol/L) and egtazic acid (15 mmol/L) in the pipette solution[8]. In the normal control group ($n = 4$), the $I_{Ca\text{-}L}$ reduced only by 3 %, 6 %, and 10 %, respectively after 5, 10, and 15 min. Comparatively, in the experimental group, Dip 3 μmol/L reduced $I_{Ca\text{-}L}$ by 53 % after 5 min. In addition, the currents were partially recovered after washout of Dip for 5 min. These results indicated that the reduction of $I_{Ca\text{-}L}$ was the action of Dip, but not the consequence of rundown of $I_{Ca\text{-}L}$.

Dip reduced $I_{Ca\text{-}L}$ and shifted its $I\text{-}V$ curve upward, indicating that its effect of blocking L-type calcium channel in guinea pig ventricular myocytes, while having no marked effect on characteristics of voltage-dependence and activation kinetics of $I_{Ca\text{-}L}$.

Dip 3 μmol/L shifted steady state inactivation curve of calcium current to the left or accelerated the voltage-dependent steady state inactivation of calcium channel indicating that the blocking effect of Dip on inactivated state, like flunnarizine[9], was stronger than that on the activated state of L-type calcium channel. Dip 3 μmol/L also slowed the recovery of $I_{Ca\text{-}L}$ from inactivation. The results suggested that the inhibitory effect of Dip on $I_{Ca\text{-}L}$ was mainly determined by accelerating inactivation of calcium channel and slowing the recovery of $I_{Ca\text{-}L}$ from inactivation.

REFERENCES

1 Zhang YJ, Fan ZZ, Wang YL, He RR. Electrophysiologic effect of dipfluzine on guinea pig papillary muscles *in vitro*. Chin J Pharmacol Toxicol 1999; 13: 176 − 8.
2 Zhang YJ, Guo MF, Wang YL, He RR. Electrophysiologic effect of dipfluzine on pacemaker cells in sinoatrial node of rabbits. Chin J Pharmacol Toxicol 1999; 13: 179 − 82.
3 Zhang YJ, Duan HR, Wang YL, He RR, Liu S, Zhang WL, *et al*. Electrophysiologic effect of dipfluzine on human atrial fibers. Chin J Pharmacol Toxicol 2000; 14: 8 − 11.
4 Xu SZ, Zhang Y, Ren JY, Zhou ZN. Effects of berberine on L- and T-type calcium channels in guinea pig ventricular myocytes. Acta Pharmacol Sin 1997; 18: 515 − 8.
5 Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 1981; 391: 85 − 100.
6 Tytgat J, Vereecke J, Carmeliet E. Differential effects of verapamil and flunarizine in cardiac L-type and T-type Ca^{2+} channels. Naunyn-Schmiedeberg's Arch Pharmacol 1988; 337: 690 − 2.
7 Mitra R, Morad M. Two types of calcium channels in guinea pig ventricular myocytes. Proc Natl Acad Sci USA 1986; 83: 5340 − 4.
8 Belles B, Malecot CO, Hescheler J, Trautwein W. "Run-down" of the Ca^{2+} current during long whole-cell recordings in guinea pig hear cells: role of phosphorylation and intracellular calcium. Pflügers Arch 1988; 411: 353 − 60.
9 Sanguinetti MC, Kass RS. Voltage-dependent block of calcium channel current in the calf cardiac purkinje fiber by dihydropyridine calcium channel antagonists. Circ Res 1984; 55: 336 − 48.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Aug; 22 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

双苯氟嗪对豚鼠心室肌细胞 L-钙电流的影响

张永健，李德培，薛保健，王永利，何瑞荣[1]
(河北医科大学生理教研室，石家庄 050017，中国)

关键词 双苯氟嗪；膜片箝技术；心肌；培养的细胞；钙通道

目的：观察双苯氟嗪(Dip)对豚鼠心室肌细胞 L-型钙电流($I_{Ca\text{-}L}$)的影响. **方法**：酶解法制备单个心室肌细胞. 应用全细胞膜片箝技术记录豚鼠单个心室肌细胞钙电流. **结果**：在 0.3 – 30 μmol/L 范围内，Dip 可浓度依赖性地降低电压依赖性激活 $I_{Ca\text{-}L}$峰值，被 Dip 3 μmol/L 所抑制的 $I_{Ca\text{-}L}$在冲洗 5 min 后可得到部份恢复. 但 Dip 对 $I_{Ca\text{-}L}$的电压依赖特征，最大激活电压，以及 $I_{Ca\text{-}L}$稳态激活无明显影响. 在 Dip 3 μmol/L 存在下，半数激活电压($V_{0.5}$)和斜率参数(κ)与对照组相比，差异均无显著性. $V_{0.5}$分别为(−12.8±1.7) mV 和(−13.2±2.4) mV，κ 分别为(7.1±0.4) mV 和(7.5±0.5) mV ($P>0.05$). Dip 3 μmol/L 可明显使钙电流稳态失活曲线左移，加速钙通道电压依赖性稳态失活. $V_{0.5}$分别为(−19.7±2.4) mV 和(−31±6) mV，κ 分别为(3.6±0.3) mV 和(1.8±0.2) mV ($P<0.05$). Dip 3 μmol/L 还使 $I_{Ca\text{-}L}$从失活状态下的恢复明显减慢. **结论**：Dip 主要作用于 L-型钙通道的失活状态，加速钙通道失活，并使其从失活状态下恢复减慢，从而抑制 $I_{Ca\text{-}L}$.

(责任编辑 朱倩蓉)