

Dauricine inhibited L-type calcium current in single cardiomyocyte of guinea pig¹

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

AIM: To study the effect of dauricine (Dau) on L-type calcium current in guinea pig ventricular myocytes. **METHODS:** Using whole-cell recording method to record L-type calcium current (I_{Ca}) in single ventricular cell of guinea pig. **RESULTS:** Dau 1, 10, and 100 $\mu\text{mol}\cdot\text{L}^{-1}$ markedly reduced I_{Ca} by 15.2% \pm 2.2%, 41% \pm 5%, and 82% \pm 8%, respectively. After washing out, I_{Ca} partially recovered. Dau inhibited I_{Ca} at 3 Hz and 1 Hz to a similar extent, its effect on I_{Ca} appeared to be not frequency-dependent. **CONCLUSION:** Dau had a calcium channel blocking effect.

Dauricine [2-hydroxy-5,4'-bis(2-methyl-6,7-dimethoxy-1,2,3,4-tetra-hydroisoguinolin-1-ylmethyl) diphenyl ether], was isolated from rhizome of *Memispermum dauricum* DC. Its anti-arrhythmic effect had been demonstrated in various animals^[1]. Our studies suggested that Dau play an important role to efficient antagonism to delayed afterdepolarizations (DAD) and triggered activity (TA) by preventing an increase of myocardial cytoplasmic Ca^{2+} activity^[2,3]. The present experiments were to reveal the effect of dauricine on L-type calcium current (I_{Ca}).

MATERIALS AND METHODS

Solutions and drugs The Ca^{2+} -free solution contained NaCl 100, KCl 10, KH_2PO_4 1.2, MgSO_4 5, glucose 20, taurine 20, and 3-(*N*-morpholino) propanesulfonic acid (MOP) 10 $\mu\text{mol}\cdot\text{L}^{-1}$, pH 7.2 - 7.4 with KOH. The enzyme for cell dispersion was collagenase (type II, Sigma, final concentration 300 $\text{kU}\cdot\text{L}^{-1}$) in the nominally Ca^{2+} -free solution. All solutions used during the cell isolation procedure were oxygenated

and maintained at 37 °C.

The external solution used to superfuse cells during recording of currents contained NaCl 137, KCl 51.4, MgCl_2 1.0, CaCl_2 1.8, HEPES 10, glucose 10, tetraethyl ammonium chloride (TEACl) 20 $\text{mmol}\cdot\text{L}^{-1}$, pH adjusted to 7.2 - 7.4 with NaOH at 24 - 28 °C. The pipette solution contained CsCl 80, CsOH 40, MgCl_2 2, egtazic acid 10, HEPES 10, Mg-ATP 5 $\text{mmol}\cdot\text{L}^{-1}$, pH adjusted to 7.2 - 7.4 with CsOH.

Dauricine supplied by Dr PAN Xi-Ping (Pharmaceutical College of Tongji Medical University), was a white powder, M_r 624, mp 103 - 104 °C, purity > 99%. It was dissolved in distilled water to 1 $\text{mmol}\cdot\text{L}^{-1}$.

Cell preparation Enzymatic dissociation method was used to prepare single ventricular cell^[4,5]. Guinea pig (250 \pm 25 g, supplied by Experimental Animal Center, Shanghai Institute of Physiology) hearts were perfused retrogradely through the aorta at a rate of 10 $\text{mL}\cdot\text{min}^{-1}$ with oxygenated solution first with the standard Ca^{2+} -free solution for 5 min; then with the same solution containing collagenase 300 $\text{kU}\cdot\text{L}^{-1}$, 1% bovine serum albumin (BSA), and CaCl_2 50 $\mu\text{mol}\cdot\text{L}^{-1}$ for 5 min. The enzyme-digested hearts were cut until single cells were dissociated. The cells were filtered from 200 μm nylon mesh, resuspended in Ca^{2+} -free solution containing CaCl_2 1.8 $\text{mmol}\cdot\text{L}^{-1}$, and stored at 24 - 26 °C for < 10 h.

Whole cell patch-clamp technique A small aliquot (0.1 mL) of dissociated cells was placed in a 0.5-mL chamber mounted on an inverted microscope (model CK2, Olympus). Cells were allowed to adhere to the coverslip and superfused at 24 - 28 °C. Complete replacement of external solution 2 $\text{mL}\cdot\text{min}^{-1}$ in the chamber was achieved within 2 - 3 min.

The currents were recorded by the whole-cell configuration of the patch-clamp technique with a CEZ 2300 amplifier (Nihon Kohden). Voltage-clamp command was generated by a 12-bit digital-to-analog converter (model TL/1, Axon Instruments Inc) controlled by the pCLAMP Software Package (version 5.6, Axon Instrument Inc). Heat-polished patch-clamp pipette electrodes had a tip resistance of 3 - 5 M Ω when filled the pipette solution. Pipette capacitance and series resistance were compensated to minimize the duration of capacitive currents.

Protocol Rod-shape cells with clear cross-striations and resting potentials of at least -78 mV were used. I_{Ca} was elicited in cells held at -40 mV (to inactivate I_{Na}) and depolarized by pulses (40 mV) lasting 300 ms, a voltage-step (-40 mV to +60 mV) was used to obtain the current-voltage

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(*I* - *V*) relation of *I*_{Ca}. To study the use-dependent effect of dauricine on *I*_{Ca}, *I*_{Ca} was elicited by a train of 20 depolarizing voltage step (250 ms in duration) from holding potential (*V*_h) of -40 mV to a test potential (*V*_t) of 0 mV delivered at a frequency of 1 or 3 Hz.

Data analysis Data were expressed as $\bar{x} \pm s$ and stored on hard disk for subsequent analysis with the pCLAMP software (version 5.6, Axon Instrument Inc). Statistical significance was determined by paired *t*-test.

RESULTS

Effect of dauricine on *I*_{Ca} When cell was held at -40 mV, and given a 40 mV, 300 ms 1 Hz depolarizing pulse, *I*_{Ca} reached its maximum. Under this condition the activities of Na⁺ channel and T-type Ca²⁺ current were completely inhibited, and L-type Ca²⁺ current was measured. Five min after perfusion with external solution containing Dau 1, 10, and 100 μmol·L⁻¹, the peak currents of *I*_{Ca} were reduced from 840 ± 97, 840 ± 96, 840 ± 93 pA in the absence of Dau to 710 ± 84 (*P* < 0.05, *n* = 6), 500 ± 61 (*P* < 0.01, *n* = 6), 150 ± 20 pA (*P* < 0.01, *n* = 6), respectively. Dau 1, 10, and 100 μmol·L⁻¹ markedly reduced *I*_{Ca} by 15.2 % ± 2.2 %, 41 % ± 5 %, and 82 % ± 8 %, respectively. After washing out Dau with external solution, the peak of *I*_{Ca} partially returned (Fig 1). These results suggested that Dau inhibited *I*_{Ca}.

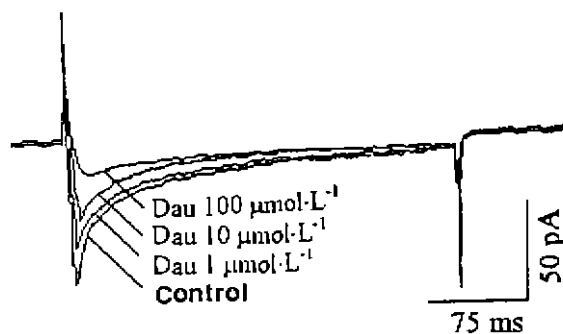


Fig 1. Effect of Dau on *I*_{Ca} of guinea pig ventricular single cell. Holding potential -40 mV; Command potential 40 mV; Stimulating duration 300 ms.

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Effect of Dau on current-voltage relationship of *I*_{Ca} Cells were depolarized from a holding potential of -40 mV to +60 mV in steps of 10 mV resulting in an activation and inactivation of *I*_{Ca}. The

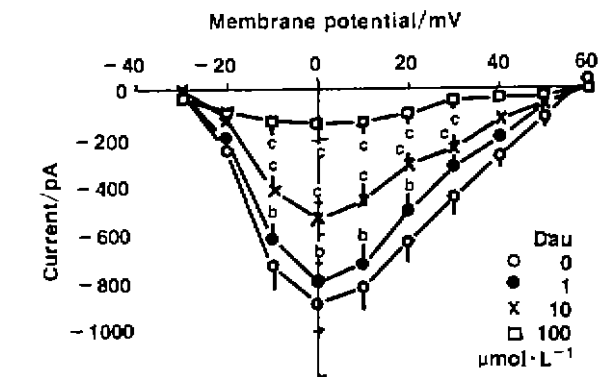


Fig 2. Superimposed current-voltage relations of *I*_{Ca} before and after exposure to Dau. *n* = 6, $\bar{x} \pm s$. **P* < 0.05, ***P* < 0.01 vs control.

Use-dependent effect of Dau on *I*_{Ca} *I*_{Ca} was elicited by a train of 20 depolarizing voltage steps from a *V*_h of -40 mV to a *V*_t 0 mV depolarizing in duration 250 ms at a frequency of 1 or 3 Hz, Dau 10 μmol·L⁻¹ inhibited *I*_{Ca} at 1 Hz and 3 Hz to a similar extent, appeared to be less frequency-dependent. Dau inhibited *I*_{Ca} in a normal use-dependent (not reverse) manner (Fig 3).

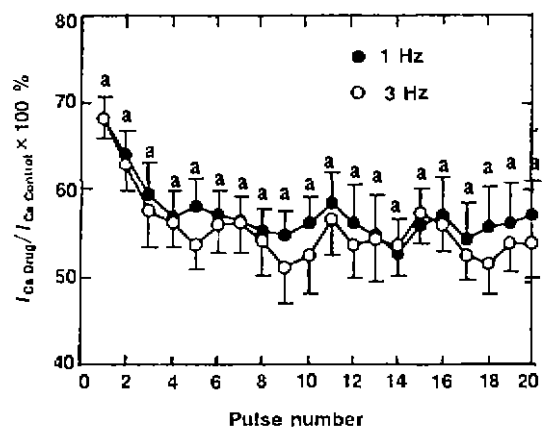


Fig 3. Frequency-independent block of *I*_{Ca} by Dau. Peak *I*_{Ca} was measured during each pulse under control conditions and Dau 10 μmol·L⁻¹. The ratio of *I*_{Ca} after drug application to the control is shown. *n* = 5 cells. **P* > 0.05 vs 3 Hz.

The inhibition during the last pulse of the train at

1 Hz was not significant than at 3 Hz (Tab 1).

Tab 1. Frequency-independent inhibition of I_{Ca} by Dau. $n=5$ cells, $\bar{x} \pm s$. $^*P > 0.05$ vs 1 Hz.

I_{Ca} /pA		Control	Dau	Block %
Pulse 1	1 Hz	670 ± 128	480 ± 89	31 ± 3
	3 Hz	580 ± 95	400 ± 79	32 ± 4 ^a
Pulse 20	1 Hz	760 ± 134	440 ± 78	42 ± 4
	3 Hz	590 ± 97	270 ± 45	44 ± 5 ^a

DISCUSSION

Rundown of ionic currents is always a concern in whole-cell voltage-clamp recording. We minimized time-dependent changes in I_{Ca} by using high resistance pipettes filled with MgATP $5 \mu\text{mol} \cdot \text{L}^{-1}$, and by performing the experiments ≤ 6 min after membrane rupture^[6]. Moreover, a strict compare of timecourse of I_{Ca} was used in the experiment, in the normal control group ($n=4$), after 5, 10, 15 min the I_{Ca} reduced only by 3.4%, 14.7%, 23.4%, respectively. Comparatively, in the experimental group, Dau 2 and $10 \mu\text{mol} \cdot \text{L}^{-1}$ for 10 min markedly reduced I_{Ca} by 28.4%, 59.3% respectively, and after washing out Dau, I_{Ca} were partially recovered, indicating the effect of Dau on I_{Ca} was not the consequence of the rundown of I_{Ca} .

Dau prolonged APD in a use-dependent manner in guinea pig papillary muscles^[7]. In canine Purkinje fibers, Dau prolonged APD at fast rate and at slow rate to a similar extent, the effect of Dau on APD like amiodarone^[8]. In this experiment, Dau appeared to be less frequency-dependent on inhibiting I_{Ca} . The results above also suggest that Dau block I_K in a use-dependent manner. We will further investigate the effect of Dau on I_K . Dau inhibited I_{Ca} in a concentration-dependent and frequency-independent manner (not reverse) suggesting that Dau might be promising in the treating of tachyarrhythmia.

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蝙蝠葛碱对豚鼠心室肌细胞 L 型钙电流阻断作用

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关键词 蝙蝠葛碱; 膜片钳技术; 钙通道; 心肌; 培养的细胞

目的: 研究蝙蝠葛碱对豚鼠心室肌细胞 L 型钙电流的阻断作用及其特性。 **方法:** 利用全细胞记录方法, 记录单个豚鼠心室肌细胞 L 型钙电流。 **结果:** 蝙蝠葛碱 1, 10, 100 $\mu\text{mol} \cdot \text{L}^{-1}$ 可使钙电流分别减少 15.2% ± 2.2%, 41% ± 5%, 82% ± 8%。冲洗后, 可使钙电流部分恢复, 蝙蝠葛碱具有浓度依赖性阻断钙电流的作用。在刺激频率 3 Hz 与 1 Hz, 其阻断钙电流的程度相似。 **结论:** 蝙蝠葛碱具有阻断 L 型钙电流的作用。