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Inhibitory effects of dauricine on potassium currents in guinea pig ventricular myocytes[1]

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KEY WORDS dauricine; myocardium; potassium channels; patch-clamp techniques

ABSTRACT

AIM: To study the effects of dauricine (Dau) on the rapidly activating component (I_{Kr}), the slowly activating component (I_{Ks}) of the delayed rectifier potassium current, and the inward rectifier potassium current (I_{K1}) in guinea pig ventricular myocytes. **METHODS**: Single myocytes were dissociated by enzymatic dissociation method. The currents were recorded with the whole-cell configuration of the patch-clamp technique. **RESULTS**: (1) Dau 1, 3, 10, 30, and 100 $\mu mol \cdot L^{-1}$ blocked I_{Kr} and tail current ($I_{Kr\text{-}tail}$) in a concentration-dependent manner. The IC_{50} for block of $I_{Kr\text{-}tail}$ was 16 (95 % confidence limits: 13 - 22) $\mu mol \cdot L^{-1}$. The time constant of $I_{Kr\text{-}tail}$ deactivation was (140 ± 38) ms in the control and (130 ± 26) ms in the presence of Dau 30 $\mu mol \cdot L^{-1}$ ($n = 6$ cells from 3 animals, $P > 0.05$). (2) Dau 1 - 100 $\mu mol \cdot L^{-1}$ produced concentration-dependent blocks of I_{Ks} and tail current ($I_{Ks\text{-}tail}$). The IC_{50} value for block of $I_{Ks\text{-}tail}$ was 33 (95 % confidence limits: 24 - 46) $\mu mol \cdot L^{-1}$. The time constant of $I_{Ks\text{-}tail}$ deactivation was (92 ± 18) ms in the control and (84 ± 16) ms in the presence of Dau 30 $\mu mol \cdot L^{-1}$ ($n = 8$ cells from 4 animals, $P > 0.05$). (3) Addition of Dau 30 $\mu mol \cdot L^{-1}$ induced block of I_{Ks} and $I_{Ks\text{-}tail}$ ($n = 7$ cells from 3 animals). The degree of block of I_{Ks} and $I_{Ks\text{-}tail}$ depended on test potentials, increasing with more positive depolarizations. (4) Dau 20 $\mu mol \cdot L^{-1}$ blocked mainly inward component of I_{K1} and reduced the reversal potential from - 72 mV (control) to - 78 mV ($n = 6$ cells from 3 animals). **CONCLUSION**: (1) Dau inhibited I_{Ks}, but not the process of I_{Ks} deactivation. (2) Dau blocked I_{Kr}, but not the process of deactivation. (3) Dau had a blocking effect on I_{K1}.

INTRODUCTION

Prolongation of cardiac repolarization was proposed as a mechanism of antiarrhythmic drug action. Action potential prolongation by antiarrhythmic drugs was a result of either an increase in inward currents or, more commonly, a decrease in outward currents. Multiple outward currents phenotypes had been identified in mammalian tissues, including time-independent current such as inward rectifier potassium current (I_{K1}), chloride current (I_{CL}), and the delayed rectifier K^+ current (I_K). Sanguinetti and Jurkiewicz[1] had identified two components of I_K in guinea pig ventricular myocytes: a rapidly activating component termed I_{Kr} and a slowly activating component termed I_{Ks}. Some antiarrhythmic agents, for example, dofetilide, increase APD by selectively blocking I_{Kr}. Other agents such as amiodarone and azimilide inhibit both I_{Kr} and I_{Ks} in guinea pigs. However, in numerous tissues, most class Ⅲ antiarrhythmic agents such as sotalol, E-4031, and dofetilide produce greater prolongation of APD at slow rates of stimulation than at rapid rates. This effect has been termed reverse use dependence or reverse rate dependence, which is an undesirable feature of class Ⅲ antiarrhythmic agents.

Dauricine (Dau) [2-hydroxy-5,4-bis(2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-1-ylmethyl) diphenyl · ether], a bisbenzyl tetrahydroisoquinoline derivative, was isolated from Rhizome of Menispermum

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dauricum DC. The previous studies had found that dauricine prolonged APD in a use-dependent manner[2]. The main purpose of the present study was to examine the effects of dauricine on I_{Kr}, I_{Ks}, and I_{K1}. It may be helpful for a better understanding of the ionic mechanisms underlying actions of dauricine on APD.

MATERIALS AND METHODS

Solutions and drugs The Ca^{2+}-free solution contained (mmol·L^{-1}) NaCl 100, KCl 10, KH_2PO_4 1.2, $MgSO_4$ 5, glucose 20, taurine 20, and 3-(*N*-morpholino) propanesulfonic acid (MOP) 10, adjusted pH 7.2 with KOH. The enzyme for cell dispersion was collagenase (type Ⅱ, Sigma, final concentration 300 kU·L^{-1}) in the normally Ca^{2+}-free solution. All solutions used during the cell isolation procedure were oxygenated and maintained at 37 ℃.

The external solution used to superfuse cells during recording of currents contained (mmol·L^{-1}) NaCl 145, KCl 4, $MgCl_2$ 1.0, $CaCl_2$ 0.1, $CdCl_2$ 0.1, HEPES 10, glucose 10, pH adjusted to 7.4 with NaOH at 24 – 28 ℃. The pipette solution contained (mmol·L^{-1}) KCl 140, $MgCl_2$ 0.5, egtazic acid 10, HEPES 10, K_2-ATP 5, pH adjusted to 7.2 with KOH.

Dauricine supplied by Dr PAN Xi-Ping (Division of Pharmacy in the Institute of Clinical Pharmacology in Tongji Medical University), was a white powder, M_r 624, mp 103 – 104 ℃, purity >99 %. It was dissolved in distilled water, pH being adjusted to 6.5 – 6.8. The stock solution was 1 mmol·L^{-1}.

Cell preparation Single ventricular myocytes were obtained from adult guinea pig hearts, provided by the Experimental Animal Center of Tongji Medical University (Certificate № 19 – 025), using enzymatic dissociation method similar to those previously described[3,4].

Whole cell patch-clamp technique Dissociated cells were 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 ℃. Complete replacement of external solution 2 mL·min^{-1} in the chamber was achieved within 2 – 3 min.

The currents were recorded with the whole-cell configuration of the patch-clamp technique by using 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). Pipette electrodes had a tip resistance of 3 – 5 MΩ 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. After the assessment of membrane currents in the absence of drug (at least 5 min), the perfusate was changed to one containing various concentration of dauricine. Data were collected in the subsequent 5 – 10 min, and baseline external solution was restarted to assess the reversal of drug effects. Holding potentials of – 40 mV were used to inactivate Na^+ and T-typed Ca^{2+} currents, and the external solution contained Cd^{2+} 0.1 mmol·L^{-1} and d-sotalol 0.1 mmol·L^{-1} to block $I_{Ca\text{-}L}$ and I_{Kr} (when measured I_{Ks}), respectively.

Data analysis Data were expressed as $\bar{x} \pm s$ and stored on hard disk for subsequent analysis with the pclamp software. Statistical significance was determined by *t*-test.

Concentration-response curves were fitted to a modified Hill equation, $\hat{Y} = (a-d)/[1+(X/c)^b] + d$, using a Marquardt-Levenberg method of nonlinear regression analysis, as in previous studies[5,6]. To study the kinetics of deactivation of the drug-sensitive currents, I_{Ks} tail current ($I_{Ks\text{-}tail}$) was fit by a single exponential function according to the following equation: $I(t) = A_0 + A_1 \exp(-t/\tau)$[1].

RESULTS

Concentration-dependent block of I_{Kr} I_{Kr} was recorded by applying 225 ms depolarizing pulses from a holding potential of – 40 to – 10 mV, followed by repolarizing to – 50 mV. The recordings of I_{Kr} in the control and in the presence of Dau 10 μmol·L^{-1} was shown in Fig 1.

Dau 1, 3, 10, 30, and 100 μmol·L^{-1} blocked I_{Kr} and tail current ($I_{Kr\text{-}tail}$) in a concentration-dependent manner. The IC_{50} for block of $I_{Kr\text{-}tail}$ was 16 (95 % confidence limits: 13 – 22) μmol·L^{-1}. The resulting concentration-response curve was shown in Fig 2. The time constant of $I_{Kr\text{-}tail}$ deactivation was

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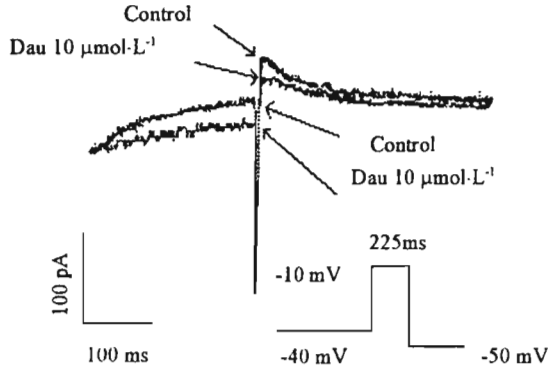

Fig 1. Inhibition of I_{Kr} and $I_{Kr\text{-}tail}$ by Dau 10 μmol·L^{-1} in guinea pig myocytes.

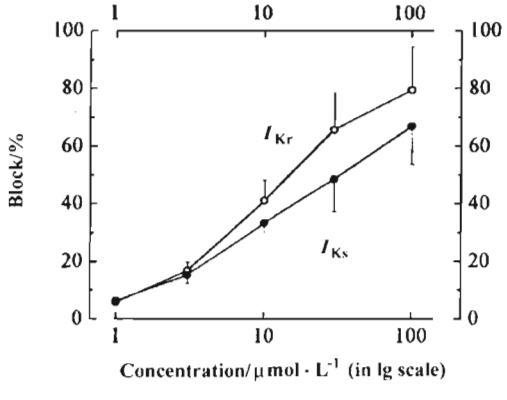

Fig 2. Concertration-dependent block of I_{Kr} (n = 6 cells from 3 animals) and I_{Ks} (n = 8 cells from 4 animals) by Dau. I_{Kr} and I_{Ks} were measured as $I_{Kr\text{-}tail}$ and $I_{Ks\text{-}tail}$, respectively.

(140 ± 38) ms in the control and (130 ± 26) ms in the presence of Dau 30 μmol·L^{-1} (n = 6 cells from 3 animals, $P > 0.05$).

Concentration-dependent block of I_{Ks} I_{Ks} was elicited in cells held at − 40 mV and depolarized by pulses (+ 50 mV) lasting 5000 ms. Dau 1 − 100 μmol·L^{-1} produced concentration-dependent block of I_{Ks} and $I_{Ks\text{-}tail}$. The IC_{50} value for $I_{Ks\text{-}tail}$ was 33 (95 % confidence limits: 24 − 46) μmol·L^{-1}), (Tab 1, Fig 2). The time constant of $I_{Ks\text{-}tail}$ deactivation was (92 ± 18) ms in the control and (84 ± 16) ms in the presence of Dau 30 μmol·L^{-1} (n = 8 cells from 4 animals, $P > 0.05$).

***I-V* relations of I_{Ks}** To study the effects of Dau on steady-state *I-V* relations for I_{Ks} in guinea pig myocytes, Current-voltage relations (*I-V* curve) of

Tab 1. Concentration-dependent block of I_{Ks} and $I_{Ks\text{-}tail}$ in guinea pig myocytes. n = 8 cells from 4 animals. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

		I_{Ks}/pA	$I_{Ks\text{-}tail}$/pA
Control		1050 ± 172	330 ± 71
Dau/μmol·L^{-1}	1	960 ± 129^a	310 ± 66^a
	3	760 ± 119^b	280 ± 60^b
	10	640 ± 108^c	220 ± 54^c
	30	510 ± 102^c	170 ± 42^c
	100	380 ± 91^c	110 ± 36^c

I_{Ks} was assessed by clamping from − 40 mV to depolarizing test potentials between − 30 and + 50 mV for 5000 ms (n = 7 cells from 3 animals). With this protocol, time-dependent I_{Ks} and $I_{Ks\text{-}tail}$, which developed slowly during these long depolarizations were, observed. Addition of 30 μmol·L^{-1} Dau induced block of I_{Ks} and $I_{Ks\text{-}tail}$. The degree of block of I_{Ks} and $I_{Ks\text{-}tail}$ depended on test potentials, increasing with more positive depolarizations (Fig 3). I_{Ks} and $I_{Ks\text{-}tail}$ were partially reversible after washout of drug (data not shown).

Effect of Dau on I_{K1} I_{K1} was measured during 300 ms test pulses to potentials between − 100 mV to + 40 mV from a holding potential of − 40 mV without (control) and with Dau (n = 6 cells from 3 animals). Dau 20 μmol·L^{-1} blocked mainly inward component of I_{K1} and reduced the reversal potential from − 72 mV (control) to − 78 mV (Fig 4).

DISCUSSION

In this study, Dau blocked the rapidly activating component (I_{Kr}) and the slowly activating component (I_{Ks}) of the delayed rectifier, as well as reducing I_{Kr} and I_{Ks} tail currents. It also inhibited the inward rectifier potassium current. Importantly, Dau has different characteristics of block of I_{Kr} and I_{Ks} from those of quindine and dofetilide[7,5]. Dau did not affect the process of I_{Kr} and I_{Ks} deactivation.

Most class Ⅲ antiarrhythmic agents (eg, sotalol, E-4031, dofetilide) increase APD by selectively blocking I_{Kr}[8,7,3]. In contrast, NE-10064 (azimilide), amiodarone, propafenone inhibit both I_{Kr} and I_{Ks} in guinea pig or canine ventricular myocytes[9,10,5,6]. Our results showed that the effects of Dau on I_{Kr} and

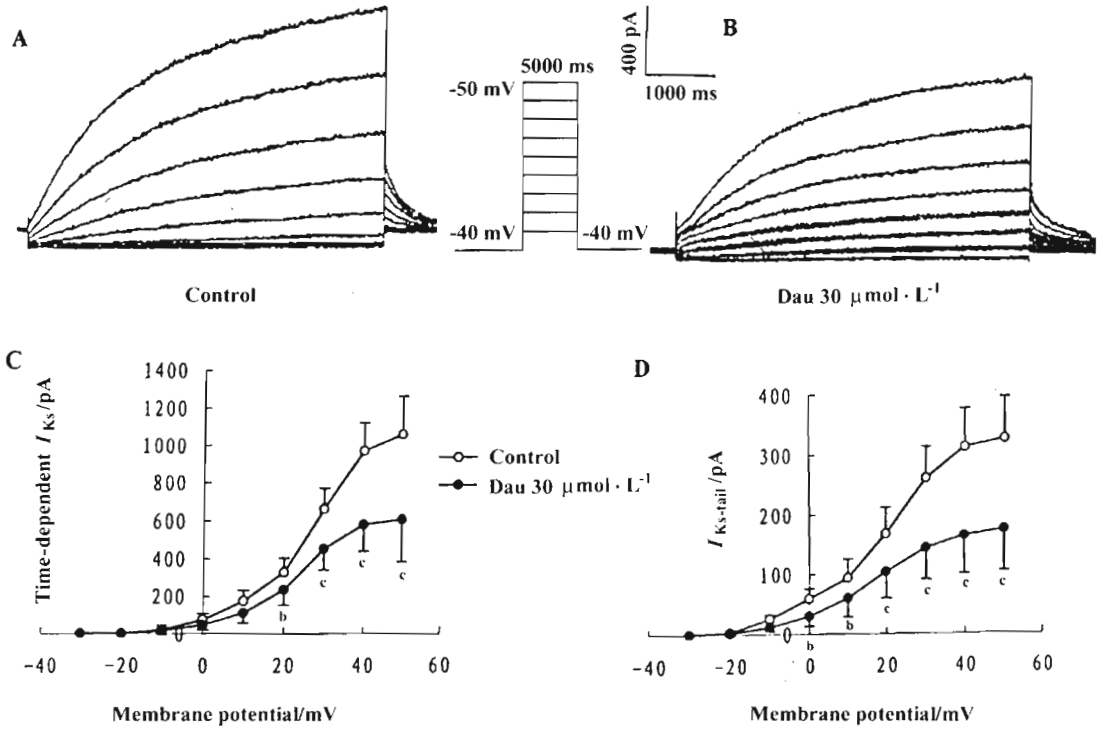

Fig 3. Voltage-dependent block of I_{Ks} of guinea pig myocytes by Dau. A and B: the original recording (A: control, B: Dau 30 μmol·L^{-1}). C and D: *I-V* relations for I_{Ks} and $I_{Ks\text{-}tail}$ during the pulse and repolarizing tail current, respectively. $n = 7$ cells from 3 animals. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

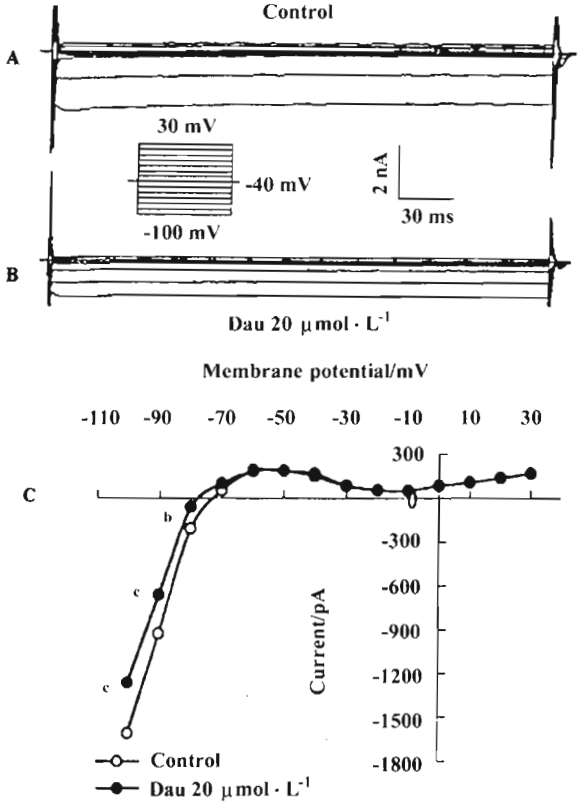

Fig 4. *I-V* relations for I_{Kl} before and after Dau 20 μmol·L^{-1}). A: Currents in one myocyte in predrug conditions. B: Currents with Dau 20 μmol·L^{-1}). C: *I-V* relations for I_{Kl}. $n = 6$ cells from 3 animals. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

I_{Ks} were similar to the latter three drugs.

In numerous tissues, class Ⅲ agents increase APD and effective refractory period (ERP) in a reverse use-dependent manner. In guinea pigs, this effect has been attributed to the accumulation of I_{Ks} at rapid stimulative rates (due to the slow deactivation kinetics of I_{Ks} in this species), which mitigates the effects of I_{Kr} blockade at rapid rates[7]. Unlike these antiarrhythmics, Dau prolonged APD in a use-dependent manner in guinea pigs[2]. In the present study, Dau inhibited both I_{Kr} and I_{Ks} in guinea pig ventricular myocytes and the changes in deactivation kinetics with Dau were different from those obseved with other blockers such as quinidine and dofetilide. These differences may account, at least in part, for the use-dependent prologation of APD by Dau.

These characteristics of Dau, along with its block

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of L-type calcium current[11], raises the possibility that this compound will provide effective antiarrhythmic therapy, while minimizing the risk of Tosades de Pointes. Certainly, the presence and characteristics of I_{Kr} and I_{Ks} in working ventricular myocardium are species-dependent[12]. Further studies are necessary to identity the effects of Dau on I_{Kr} and I_{Ks} and its use-dependent effect on APD in other species.

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蝙蝠葛碱对豚鼠心室肌细胞钾电流的阻断作用[1]

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关键词 蝙蝠葛碱; 心肌; 钾通道; 膜片箝技术

目的: 研究蝙蝠葛碱对豚鼠心室肌细胞快激活(I_{Kr})和慢激活(I_{Ks})延迟整流钾电流及内向整流钾电流(I_{K1})的作用. **方法**: 酶解法制备单个心室肌细胞. 电压箝制方式下全细胞记录豚鼠单个心室肌细胞钾通道电流. **结果**: 蝙蝠葛碱 1 – 100 $\mu mol \cdot L^{-1}$浓度依赖性阻断 I_{Ks}, $I_{Ks\text{-}tail}$ [IC_{50} = 33 (95 %可信限: 24 – 46) $\mu mol \cdot L^{-1}$]及 I_{Kr}, $I_{Kr\text{-}tail}$ [IC_{50} = 16 (95 %可信限: 13 – 22) $\mu mol \cdot L^{-1}$]. 对 $I_{Ks\text{-}tail}$, $I_{Kr\text{-}tail}$的去激活过程无明显影响, 给药前的时间常数分别为(92 ± 18) ms 和(140 ± 38) ms, 给药后分别为(84 ± 16) ms 和(130 ± 26) ms ($P > 0.05$). 蝙蝠葛碱对 I_{Ks}的抑制作用具有电压依赖性. 蝙蝠葛碱 20 $\mu mol \cdot L^{-1}$对 I_{K1}的内向部分具有阻断作用. **结论**: 蝙蝠葛碱对 I_{Kr}和 I_{Ks}具有阻断作用, 但不影响此两种成分的去激活过程. 蝙蝠葛碱同时具有阻断 I_{K1}的作用.

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