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Dauricine-induced changes in monophasic action potentials and effective refractory period of rabbit left ventricle *in situ*[1]

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KEY WORDS dauricine; sotalol; rabbits; action potentials

ABSTRACT

AIM: To study the effects of dauricine and sotalol on monophasic action potentials and effective refractory period of the rabbit heart *in situ*. **METHODS**: Monophasic action potentials recording and programmed electrical stimulation techniques. **RESULTS**: Iv injection of dauricine 0.5 mg·kg^{-1}·min^{-1} for 24 min produced a decrease of the amplitude of monophasic action potentials from (17 ± 6) mV to (7.1 ± 1.5) mV ($P < 0.05$) and increased the duration of 50 % and 90 % repolarization of monophasic action potentials and effective refractory period from (130 ± 26), (167 ± 25), (128 ± 12) ms to (198 ± 33), (235 ± 34), (185 ± 25) ms ($P < 0.05$), respectively. Sotalol had the similar effects on monophasic action potentials and effective refractory period to that of dauricine. Dauricine and sotalol did not change the ratio between effective refractory period and the duration of 90 % repolarization. **CONCLUSION**: Dauricine and sotalol decreased the amplitude of monophasic action potentials and increased the duration of 50 % and 90 % repolarization and effective refractory period.

INTRODUCTION

Dauricine, a bisbenzylisoquinoline alkaloid derivative isolated from rhizome of *Menispermum dauricum* DC, has been found the antagonistic effect on experimental arrhythmias[1], and also been shown to be effective in the treatment of ventricular and supraventricular arrhythmias[2]. The previous studies on the properties of electrophysiology of dauricine and its antiarrhythmic mechanisms were mostly conducted *in vitro*. In this present study, the effects of dauricine and sotalol on the monophasic action potentials (MAP), and effective refractory period (ERP) were investigated in the rabbit heart *in situ* with the MAP recording technique.

MATERIALS AND METHODS

Drugs Dauricine used in this experiment was a white powder supplied by Dr PAN Xi-Ping in Division of Plant Chemistry of our institute. Its M_r was 624, mp 103 - 104 ℃, purity > 98 %. It was dissolved in normal saline to 0.5 g·L^{-1} with a pH ranging from 6.5 to 6.8. Sotalol was purchased from Sigma Co and dissolved in normal saline to 0.25 g·L^{-1}.

MAP recording[3] Sixteen adult Japanese White rabbits of either sex weighing (2.4 ± 0.5) kg, provided by the Experimental Animals Center of Tongji Medical University, were anesthetized iv with sodium pentobarbital (36 mg/kg). Ventilation was maintained by ventilator (DH-140, Zhejiang Medical Experiment Instrument Factory) through a tracheal cannula. The tidal volume was 10 mL·kg^{-1} and the respiratory rate was 40/min. The femoral vein was cannulated for injection of drugs. After left sternotomy, the heart was exposed and suspended in a pericardial cradle. A quadripolar contact electrode catheter (size 7F) for pacing and MAP recording (Franz MAP/pacing combination catheter EP Technologies, Sunnyvale, California, USA) was introduced into the left ventricle through a tiny stab wound made in the free wall. The electrode tip was placed against the left ventricular anterior endocardium close to the apex. MAP signals were amplified by high input impedance, MAP direct current (DC)-coupled

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isolated, differential preamplifier (model 300, EP Technologies). Together with surface electrocardiogram lead Ⅱ, amplified MAP signals were simultaneously displayed on oscilloscope and printed out on 8 channel physiologic recorder (model RM 6000, Nihon Kohden) at paper speeds of 25 – 100 mm·s^{-1}. The frequency response of the MAP recording system was DC to 5000 Hz.

ERP measurement[4,5] The determination of ERP was performed from the same left ventricular site with a programmed stimulator (model DXT-5, Suzhou Medical Electronic Instrument Factory). Pacing stimuli were supplied through the same catheter that had been used for MAP recording. The heart was driven by basic electrical stimuli (S_1) of twice diastolic threshold strength and 2 ms duration. An extra stimulus (S_2) of the same current strength and duration was delivered repeatedly after every 8 steady basic drive stimuli, with the extra stimulus coupling interval ($S_1 - S_2$) being shortened in 5 ms decrease until refractoriness occurred (Fig 1).

Experimental protocols The rabbits were randomly divided into three groups: dauricine (0.5 mg·kg^{-1}·min^{-1}, iv for 24 min, $n = 8$) treated group, sotalol (0.25 mg·kg^{-1}·min^{-1}, iv for 24 min, $n = 4$) treated group, and normal saline group ($n = 4$). The MAP and ERP were recorded before drug treatement and at 3, 6, 12, 18, 24 min after iv infusion of sotalol or dauricine.

Data analysis and statistics Only stable MAP with a constant amplitude (> 10 mV), configuration, and an isoelectric phase 4 were accepted for analysis. The amplitude of MAP (MAPA) was defined as the distance from the diastolic baseline to the crest of the MAP plateau phase. The duration of MAP was measured manually at

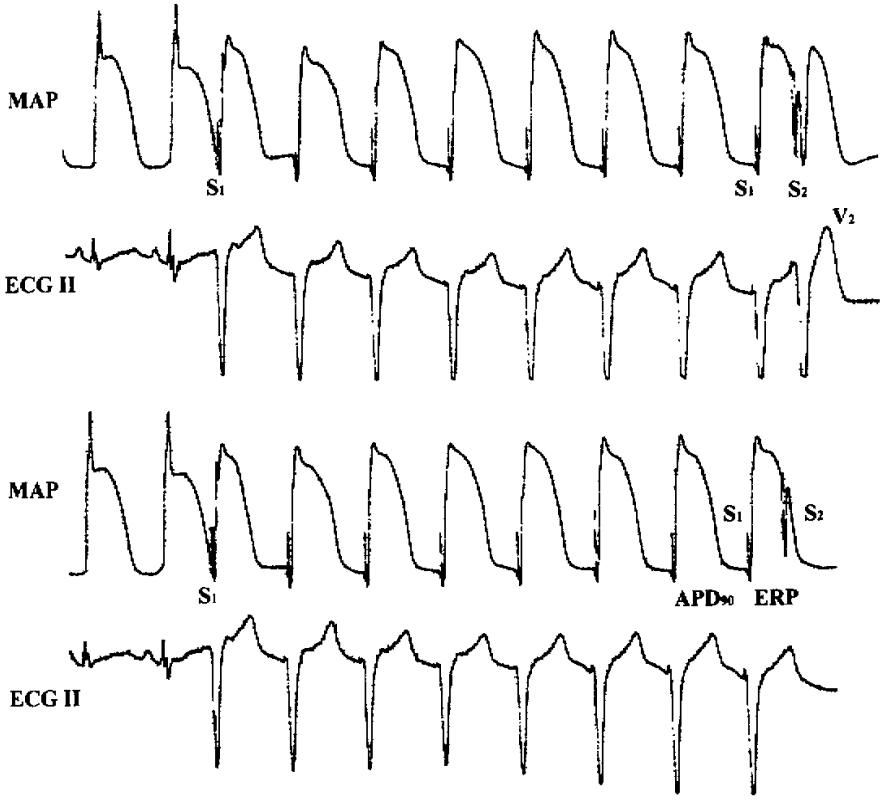

Fig 1. Simutaneous determination of action potential duration (APD) and effective refractory period (ERP) *in vivo*. S_1 and S_2 denote the basic and extrastimulus artifacts. The upper tracing shows the shortest S_1 S_2 coupling interval that elicits a propagated response (V_2). The lower tracing shows failure to capture at a 5 ms shorter coupling interval.

the time of 50 % ($MAPD_{50}$) and 90 % ($MAPD_{90}$) repolarization in three consecutive signals and the mean value was calculated. The ratio between ERP and $MAPD_{90}$ was determined from recordings made at the same ventricular site. Data were expressed as $\bar{x} \pm s$. Comparisons of results were performed by t-test. Values of $P < 0.05$ were considered significant.

RESULTS

Monophasic action potentials Before administration of drugs there were no significant differences in MAPA, $MAPD_{50}$, and $MAPD_{90}$ ($P > 0.05$) between dauricine and sotalol group. Both dauricine and sotalol produced a decrease of MAPA and a prolongation of $MAPD_{50}$ and $MAPD_{90}$. Iv injection of dauricine 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$ for 24 min produced a decrease of the amplitude of monophasic action potentials from (17 ± 6) mV to (7.1 ± 1.5) mV ($P < 0.05$) and increased the duration of 50 % and 90 % repolarization of monophasic action potentials from (130 ± 26), (167 ± 25) ms to (198 ± 33), (235 ± 34) ms ($P < 0.05$), respectively. After 12 min iv infusion of dauricine or sotalol, MAPA decreased remarkably. $MAPD_{50}$ and $MAPD_{90}$ were prolonged after 3 min of infusion of dauricine or sotalol.

The changes of these parameters were statistically significant compared with predrug. The results were summarized in Tab 1 and graphically illustrated in Fig 2.

ERP and $ERP/MAPD_{90}$ ratio There were no significant differences of ERP and $ERP/MAPD_{90}$ ($P > 0.05$) between dauricine and sotalol group before drugs infusion. Iv infusion of dauricine or sotalol caused increase of ERP, from (128 ± 12), (115 ± 18) ms to (185 ± 25), (170 ± 26) ms ($P < 0.05$), respectively. There were no significant differences of the $ERP/MAPD_{90}$ ratio before and after infusion of dauricine or sotalol.

DISCUSSION

In contrast to intracellular action potential (IAP) recordings, MAP can be recorded from the endocardium and epicardium of the beating heart *in situ*. The development of MAP recording technique, especially new contact electrode, provides a safety and reliable method for studying the electrophysiological properties of the heart *in situ*, the mechanisms of arrhythmias, and the action mechanisms of antiarrhythmic drugs. Similar to IAP recordings, the MAP signal produces precise information for the entire course of repolarization[6,7]. Therefore, the MAP recording technique is suitable for studying the characteristics of local myocardial repolarization in the experimental and clinical settings.

Tab 1. Effects of dauricine (Dar) and sotalol (Sot) on MAP and ERP of the left ventricle of the rabbit heart *in situ*. $n = 4$ (NS), $n = 8$ (*Dau*), $n = 4$ (Sot). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* Before. $^eP < 0.05$ *vs* Sot. MAPA = MAP amplitude. $MAPD_{50}$ = MAP duration at 50 % repolarization. $MAPD_{90}$ = MAP duration at 90 % repolarization. ERP = effective refractory period.

	Group	Before	After/min 3	6	12	18	24
MAPA/mV	NS	18 ± 3	17 ± 7[a]	17 ± 4[a]	16 ± 3[a]	17 ± 5[a]	16 ± 7[a]
	Dau	*17 ± 6*	*13 ± 6*[a]	*13 ± 5*[a]	*11 ± 6*[b]	*9 ± 4*[ce]	*7.5 ± 1.8*[ce]
	Sot	20 ± 6	17 ± 4[a]	17 ± 4[a]	16 ± 3[b]	14.3 ± 2.4[b]	14 ± 3[b]
$MAPD_{50}$/ms	NS	135 ± 23	145 ± 25[a]	141 ± 23[a]	139 ± 18[a]	140 ± 21[a]	138 ± 22[a]
	Dau	130 ± 26	158 ± 16[b]	158 ± 20[b]	167 ± 17[c]	180 ± 12[c]	198 ± 33[c]
	Sot	126 ± 10	143 ± 2[b]	*149 ± 5*[c]	159 ± 17[c]	177 ± 16[c]	188 ± 16[c]
$MAPD_{90}$/ms	NS	159 ± 19	166 ± 27[a]	164 ± 21[a]	160 ± 14[a]	158 ± 34[a]	156 ± 30[a]
	Dau	167 ± 25	188 ± 17[b]	192 ± 17[b]	201 ± 16[c]	218 ± 29[c]	235 ± 34[c]
	Sot	155 ± 15	171 ± 11[a]	185 ± 10[c]	208 ± 23[c]	221 ± 27[c]	231 ± 25[c]
ERP/ms	NS	129 ± 23	*132 ± 24*[a]	*130 ± 26*[a]	133 ± 19[a]	131 ± 25[a]	127 ± 31[a]
	Dau	128 ± 12	138 ± 15[a]	158 ± 13[c]	164 ± 19[c]	176 ± 24[c]	185 ± 25[c]
	Sot	115 ± 18	132 ± 10[b]	140 ± 13[b]	145 ± 15[b]	160 ± 23[b]	170 ± 26[b]
$ERP/MAPD_{90}$	NS	0.75 ± 0.16	0.72 ± 0.05[a]	0.74 ± 0.11[a]	0.74 ± 0.12[a]	0.79 ± 0.08[a]	0.80 ± 0.15[a]
	Dau	*0.75 ± 0.06*	*0.70 ± 0.07*[a]	*0.79 ± 0.05*[a]	*0.82 ± 0.10*[a]	*0.80 ± 0.05*[a]	*0.83 ± 0.18*[a]
	Sot	0.74 ± 0.10	0.77 ± 0.09[a]	0.74 ± 0.09[a]	0.70 ± 0.10[a]	0.73 ± 0.11[a]	0.72 ± 0.13[a]

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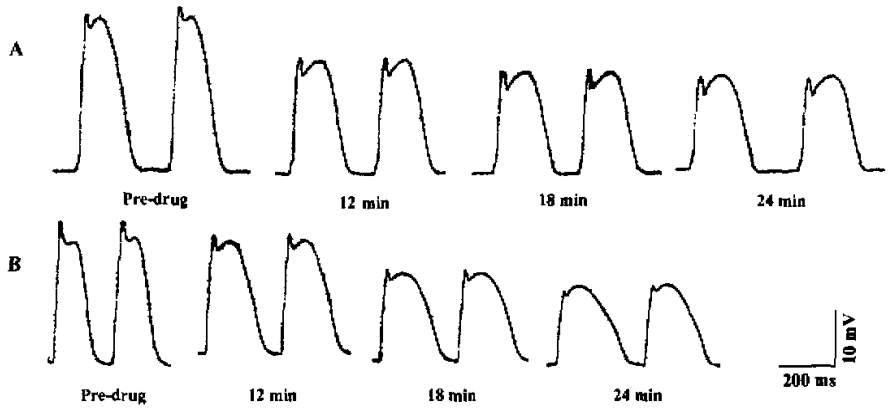

Fig 2. Effects of dauricine and sotalol on monophasic action potentials of the rabbit heart *in situ*. Dauricine (A) and sotalol (B) infused singly induced a decrease of MAPA and lengthening of $MAPD_{50}$ and $MAPD_{90}$.

In the MAP recordings, the contact electrode catheters are usually advanced into the heart through artery or vein with the aid of fluoroscopy. In the present study, the catheter was introduced through a tiny stab wound made in free wall of the left ventricle because of the small rabbit heart and its minute blood vessels. Through this method MAP could be recorded from the same endocardial site for up to 60 min. The configuration and amplitude (10 – 25 mV) of MAP were in agreement with the previous recordings in the rabbit heart *in situ*[7,8].

Sotalol, now classified as class Ⅲ antiarrhythmic drugs, is non-selective β-adrenergic receptor antagonist prolonging APD in reverse use-dependent manner by inhibiting delayed rectifier K^+ current. Dauricine has the similar myocardial electrophysiological properties to that of class Ⅰ/Ⅲ antiarrhythmic drugs since it delayed V_{max}, decreased conductivity within ventricles, and prolonged action potential duration in use-dependent manner. We compared the effects of dauricine on MAP and ERP with that of sotalol. Similar to the effects of dauricine, sotalol decreased MAPA and prolonged $MAPD_{50}$, $MAPD_{90}$ and ERP, but did not change the $ERP/MAPD_{90}$ ratio in our experiment. The present results are in agreement with the previous reports[9,10].

This study demonstrated that dauricine decreased the MAPA and prolonged $MAPD_{50}$ and $MAPD_{90}$ of the rabbit heart *in situ*. The results are in accordance with the previous study in the cat heart *in situ* with suction electrode recording technique[11]. MAPA is dependent on the state of sodium channel in the phase 0 of action potential. $MAPD_{50}$ and $MAPD_{90}$ reflects the changes of phase 2 and the entire duration of action potential, respectively. A recent study in the papillary muscles of guinea pig found that dauricine could inhibit sodium, potassium, and calcium currents[12,13]. It suggests that dauricine-induced changes of MAPA may be related to its inhibitory effects on sodium, and the comprehensive effects of Dau on calcium and potassium currents are related to its prolongation of action potential duration.

The effect of dauricine on the relationship between $MAPD_{90}$ and ERP was determined with a new quadripolar combination catheter. The results showed that dauricine increased the ERP of the left ventricle to the same extent as increase of $MAPD_{90}$, but did not change the $ERP/MAPD_{90}$ ratio. Drugs with pure prolongation of APD produce a parallel, voltage dependent prolongation of refractoriness. Thus, the prolonging effect of dauricine on ERP is predominantly mediated through its prolongation of APD. Franz and Costard found that quinidine increased the ratio between ERP and APD as the cycle length shortened since its sodium blocking effect was use-dependent[14]. In this experiment, the parallel prolongation effects of dauricine on $MAPD_{90}$ and ERP were observed at the sinus rhythm, which could explain partly its effects of antiarrhythmia. However, whether the dauricine has the different effects on ERP and the ratio of ERP/APD at different frequency remains to be further studied.

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蝙蝠葛碱对在体家兔左心室单相动作电位和有效不应期的影响[1]

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关键词　蝙蝠葛碱；索他洛尔；兔；动作电位

目的：研究蝙蝠葛碱(Dau)和索他洛尔对在体家兔左心室单相动作电位和有效不应期的作用．**方法**：采用单相动作电位测定技术记录家兔在体心脏单相动作电位，用程序电刺激法测定左心室有效不应期．**结果**：Dau 0.5 mg·kg^{-1}·min^{-1}恒速静脉灌注明显降低单相动作电位幅度，由用药前的(17 ± 6) mV 降至给药后 24 min 的(7.1 ± 1.5) mV．Dau 显著延长单相动作电位复极 50 % 和 90 % 时程及有效不应期，给药前分别为(130 ± 26)，(167 ± 25)，(128 ± 12)ms，给药后 24 min 分别延长至(198 ± 33)，(235 ± 34)，(185 ± 25) ms．但对有效不应期与单相动作电位时程($MAPD_{90}$)之比值无明显影响．索他洛尔对上述各指标的作用与 Dau 相似．**结论**：研究结果表明，Dau 和索他洛尔降低单相动作电位幅度，延长单相动作电位时程和有效不应期．

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