性作用，提示 Sul 的正性频率作用可能不是通过 cAMP 机理所致，而 Sul 在 1 mmol/L 的高浓度时细胞内 cAMP 水平的显著升高却可能是其产生节律不齐等毒性作用的原因之一. 从 Sul 300 μmol/L 影响 cAMP 水平的时程研究结果看来也不说明 Sul 正性频率作用由 cAMP 升高所启动，因为 Sul 的正性频率作用在加药后 1–2 min 就产生，4–5 min 已达到峰值(稳定状态)，而 cAMP 的显著升高要在 10 min (至少是 > 5 min)后才出现，但这是否能说明 cAMP 升高在 Sul 正性频率作用的维持中起一定的作用，尚需证明.

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中国药理学报 *Acta Pharmacologica Sinica* 1990 May; **11** (3) : 220–225

Effects of dauricine and lidocaine alone or combined on electrophysiological properties of canine Purkinje fibers

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ABSTRACT Dauricine (Dau) 1 to 30 μmol/L produced the concentration–dependent depressions in the APA, V_{max}, MDP, and prolongations of APD_{50} and APD_{90} as well as ERP of the isolated canine cardiac Purkinje fibers (PF). The automaticity and excitation were significantly reduced at concentration of 30 μmol/L.The effects of Dau on all action potential parameters of PF were observed at all stimulation frequencies (60, 75, 100, 150 beats/min). Lidocaine (Lid) markedly shortened APD_{50} of PF at concentration of 30 μmol/L and also shortened APD_{90}, ERP and significantly depressed APA, V_{max} at 100 μmol/L. When perfused in combination with Dau, Lid appreciably shortened APD_{50} and APD_{90}, and lightly abbreviated ERP prolonged by Dau.

KEY WORDS dauricine/drug effects; lidocaine /drug effects; Purkinje fibers; action potentials;

Received 1989 May 23 Accepted 1990 Jan 3

electrophysiology

Dau has been found to be a potent anti-arrhythmic drug in experimental and clinical trials[1-3]. It has been shown that Dau could depress the action potential (AP) of guinea pig papillary muscles[4] and the cardiac conduction system[2,3,5]. Recently, we have confirmed the dose-dependent depressing effects of Dau on the atrial ventricular (A-V), His-Purkinje fiber system (HPFS) and intraventricular conduction in rabbits, among which the inhibition of Dau on the HPFS conduction was the greatest. Lid could considerably weakened the inhibition of Dau on the HPFS conduction (unpublished data). Up to date, the direct action of Dau on the electrophysiological characteristics of PF and interaction with Lid have not been studied.

Thus, the present study was designed to examine the action of Dau and Lid on the electrophysiological properties of canine PF when administered alone and in combination, and to study the anti-arrhythmic mechanism of Dau and its direct interaction with Lid.

MATERIALS AND METHODS

Adult mongrel dogs, weighing 11 ± SD 2 kg, were anesthetized with sodium pentobarbital (30 mg/kg iv). Free-running false tendons were excised from either ventricles and superfused with the modified Tyrode's solution according to the method stated by Varro[6]. The composition of the Tyrode's solution was (in mmol/L): NaCl 149, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, trihydroxymethyl- aminomethane (Tris) 10, glucose 10. For studying automaticity, KCl was reduced to 2.7 mmol/L. The solution was oxygenated with 100% O_2 (pH 7.2-7.4). The temperature was kept constant at 37 ± 0.5 ℃.

The preparations were stimulated with square pulses of twice threshold intensity and 2 ms duration provided by a 8303-stimulator and isolated by a stimulus isolator. The basic stimulation frequency was 60 beats/min. In the studies of the frequency-dependent effects, the preparations were paced at the 60, 75, 100, 150 beats/min. In order to determine the effective refractory period (ERP), premature stimuli (S_2) were introduced after every eighth basic stimuli (S_1). The ERP was determined as the approach mentioned by Nattel[7] and repeated to ensure its reproducibility.

Glass microelectrodes filled with KCl 3 mol/L were used to record transmembrane potentials. The microelectrode was connected to FW-2 microelectrode amplifier and an electronic differentiator. The output signals from both were displayed on a SBR-1 double beam oscilloscope for viewing and recording photographically.

In order to examine the effect of Dau on the excitability of PF, the diastolic excitable threshold was determined by stimulation of the square current pulse of 0.5, 1, 3, 5, 8, 10 ms duration and defined as the minimal diastolic threshold intensity required to elicit a propagated AP.

Dau used in present studies was provided by the Faculty of Pharmacy in Tongji Medical University and disolved in distilled water. pH was adjusted to about 6.7. Lid was kindly supplied by the Institute of Drug-Examination in Wuhan and dissolved in distilled water as required concentration. After the tissue had stabilized in Tyrode's solution for two hours, action potential amplitude (APA), maximal rate of rise of potentials (V_{max}), maximal diastolic potentials (MDP), action potential duration to 50% and 90% (APD_{50} and APD_{90}), ERP, spontaneous frequency (SF) of AP and time-intensity curve were determined. Then the preparations were superfused with Dau and Lid at concentrations of 1, 3, 10, 30 μmol/L for both, respectively, and additional 100 μmol/L for Lid. All data were taken 30 min after superfused with every concentration either Dau and Lid. The impalement was maintained in the same cell throughout the ex-

periment.

The statistical analysis of results were made by analysis of variance. All values were expressed as means ± standard deviation.

RESULTS

Effect of Dau on the SF of AP The spontaneous AP occurred in all preparations superfused with the Tyrode's solution containing KCl 2.7 mmol/L. The concentration-dependent effect of Dau on the SF of cardiac PF was seen in all preparations. The cumulative concentration of 3, 10, 30 μmol/L reduced the SF of AP from 48 ± 8 beats/min in the control condition to 45 ± 7, 40 ± 7, 33 ± 5 beats/min respectively. The significant reduction was seen at 30 μmol/L and complete depression (4/11 preparations) of spontaneous rhythm at Dau 100 μmol/L.

Effects of Dau and Lid on AP characteristics Table 1 shows the concentration- dependent effects of Dau and Lid on the AP characteristics of PF. Dau did not produce significant effects on the parameters of AP at low concentrations (1, 3 μmol/L) but considerably depressed the APA, V_{max} and MDP and prolonged APD_{90} at 10 μmol/L. APD_{50} also tended to be lengthened. During superfused with Dau 30 μmol/L, the APA, V_{max} and MDP were further suppressed with the decreases of 75 ± 6 %, 60 ± 26% and 17 ± 5 %, respectively, and APD_{50}, APD_{90} were prolonged with the alteration of 68 ± 31 %, 65 ± 26 %, respectively.

The effects of Lid on the parameters of AP were concentration-dependent. Lid produced insignificant effects on all parameters of AP at 1 and 3 μmol/L, and significantly shortened APD_{50} at 10 μmol/L and APD_{90} at 30 μmol/L. The superfusion with Lid 100 μmol/L markedly reduced APA and V_{max}, but failed in affecting MDP (Tab 1).

When Dau 10 μmol/L considerably decreased the APA, V_{max}, and prolonged

Tab 1. Effects of dauricine (Dau) and lidocaine (Lid) on the membrane potentials in canine Purkinje fibers. $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **baseline.**

Drugs (μmol/L)		*n*	APA (mV)	APD_{50} (ms)	APD_{90} (ms)	ERP (ms)	V_{max} (V/s)	MDP (mV)
Baseline		11	133 ± 7	256 ± 24	349 ± 37	312 ± 38	615 ± 43	90 ± 5
Dau	1	11	130 ± 8*	260 ± 28*	356 ± 42*	319 ± 40*	575 ± 50*	90 ± 5*
	3	11	124 ± 10*	272 ± 34*	372 ± 45*	340 ± 47*	524 ± 55*	88 ± 4*
	10	11	115 ± 8***	305 ± 59*	419 ± 44***	328 ± 48***	345 ± 102***	83 ± 4**
	30	10	100 ± 8***	429 ± 125***	581 ± 80***	490 ± 59***	241 ± 165***	74 ± 5**
Baseline		7	128 ± 10	312 ± 47	429 ± 47	397 ± 41	633 ± 67	89 ± 4
Lid	1	7	127 ± 10*	297 ± 43*	417 ± 50*	387 ± 45*	630 ± 67*	89 ± 4*
	3	7	125 ± 10*	276 ± 43*	394 ± 61*	371 ± 45*	617 ± 67*	89 ± 4*
	10	7	119 ± 11*	242 ± 55**	368 ± 72*	352 ± 56***	591 ± 73*	88 ± 4**
	30	7	113 ± 14*	224 ± 49***	357 ± 72*	348 ± 58*	540 ± 102*	88 ± 4***
	100	6	104 ± 15**	198 ± 24***	326 ± 54**	310 ± 49**	417 ± 87***	85 ± 4***
Dau +Lid	10	7	102 ± 14	427 ± 51	588 ± 68	505 ± 32	407 ± 134	73 ± 4
	1	7	100 ± 14*	413 ± 45*	578 ± 53*	502 ± 29*	390 ± 147*	72 ± 3*
	3	7	96 ± 15*	404 ± 42*	578 ± 53*	498 ± 28*	357 ± 147*	72 ± 3*
	10	7	88 ± 11*	368 ± 27**	538 ± 62*	488 ± 23*	307 ± 134*	72 ± 4*
	30	7	83 ± 15*	321 ± 38***	498 ± 69*	461 ± 56*	226 ± 115*	70 ± 4*
	100	7	80 ± 15*	305 ± 42***	473 ± 66***	440 ± 54*	215 ± 114*	69 ± 4*

APA : action potential amplitude; APD_{50} and APD_{90} : action potential duration of repolarization 50% and 90%; ERP: effective refractory period; V_{max} : maximal rate of rise of potentials ; MDP: maximal diastolic potential

Tab 2. Effects of Dau on action potentials characteristics of canine Purkinje fibers at various stimulation frequencies. $n=8$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* baseline.

	Drugs (μmol / L)		Stimulation frequencies (beats / min) 60	75	100	150
	Baseline		133 ± 9	134 ± 10	137 ± 10	139 ± 10
	Dau	3	123 ± 11*	123 ± 10*	126 ± 9*	124 ± 8**
APA		10	113 ± 9***	113 ± 9***	115 ± 8***	114 ± 6***
(mV)		30	98 ± 9***	99 ± 10***	100 ± 10***	
	Baseline		350 ± 44	343 ± 46	334 ± 49	294 ± 37
	Dau	3	369 ± 53*	368 ± 50*	343 ± 48*	315 ± 39*
APD_{50}		10	411 ± 50**	410 ± 54**	389 ± 50*	322 ± 19*
(ms)		30	545 ± 69***	501 ± 36***	467 ± 36***	
	Baseline		245 ± 17	239 ± 19	224 ± 15	204 ± 11
	Dau	3	254 ± 19*	253 ± 13*	238 ± 19*	214 ± 15*
APD_{90}		10	275 ± 34*	276 ± 34*	263 ± 31*	229 ± 33*
(ms)		30	377 ± 50***	328 ± 41***	312 ± 63***	
	Baseline		311 ± 46	303 ± 44	292 ± 48	269 ± 37
	Dau	3	340 ± 57*	333 ± 60*	324 ± 60*	286 ± 44*
ERP		10	378 ± 57**	347 ± 57**	364 ± 59**	307 ± 42*
(ms)		30	459 ± 38***	446 ± 35***	434 ± 28***	
	Baseline		620 ± 44	622 ± 43	633 ± 47	647 ± 45
	Dau	3	527 ± 65**	530 ± 65**	529 ± 67**	533 ± 65**
V_{max}		10	376 ± 103***	376 ± 103***	370 ± 113***	344 ± 141***
(V/s)		30	127 ± 59***	94 ± 42***	92 ± 49***	

APD_{50}, APD_{90} of PF, the preparations were superfused with Lid (from 1 to 100 μmol/L). Lid produced a further decreases of APA and V_{max} without statistical significance. Lid significantly shortened the APD_{50} and APD_{90} prolonged by Dau (Tab 1).

In all preparations, the changes in APD described above were accompanied by alteration of ERP. The low concentration of Dau produced insignificant changes in ERP and its high concentration considerably prolonged ERP. The results were summarized in Tab 1. In contrast, Lid progressively reduced the ERP in a concentration–dependent manner (Tab 1) and could partially antagonized the prolongation effect of Dau on ERP.

Frequency–dependent effect of Dau on AP and ERP All results were summarized in Tab 2. In control condition, there were no significant changes in APA and V_{max} as the increase in frequencies but APD_{50}, APD_{90} and ERP were markedly abbreviated in frequency–dependent manner. Dau suppressed APA, V_{max} and prolonged APD_{50}, APD_{90} as well as ERP at all stimulation frequencies in concentration–dependent manner.

Effect of Dau on excitability of PF

Fig 1 showed the concentration related effect of Dau on the PF excitability. Dau considerably shifted time–intensity curves to the right and upward direction in concentration–dependent manner, suggesting that the excitability was decreased.

DISCUSSION

The present studies confirmed the direct depressing effects of Dau and Lid on APA and V_{max} of AP in isolated canine PF. It has been reported that Lid depresses cardiac sodium channels[8] and decreases V_{max} of the cardiac AP[9]. Recently, it has also been shown that Dau could inhibit sodium current in canine PF (unpublished data). The results suggest that the Dau–induced inhibition of the HPFS con-

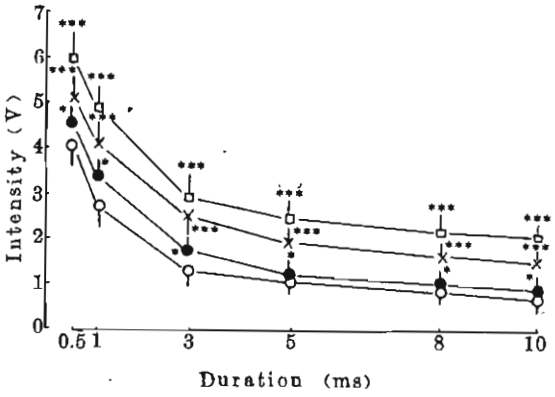

Fig 1. Effects of dauricine on excitability in canine Purkinje fibers. Dauricine 3 μmol / L (●), 10 (×), 30 (□). $n=6$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* baseline (O)

duction is related to its direct effect on PF.

The effect of Dau on characteristics of APD completely differs from that of Lid. It has been found that the shortening effect of Lid on APD of PF is mainly related to its suppression of " window" current[10]. Dau markedly diminished the shortening effects of Ach on APD in guinea pig atrial muscles [4] and depressed K^+ outward current in canine PF (unpublished data). It might be concluded that Dau lengthenes APD of PF by depressing the transportation of K^+ through the cellular membrane.

The mechanism of suppressing the automaticity by Dau is still unclear. There was evidence that those drugs suppressing automaticity may do so by altering either gK or gNa [11]. It is most possible that Dau suppresses automaticity of PF by depressing sodium influx.

The present results is in accordance with the findings in clinical electrophysiological studies in which Dau significantly prolonged the H–V interval of His–bundle electrogram and suppressed HPFS conduction[5]. We believe that Dau exerts its antiarrhythmic effect, especially on ventricular premature and tachycardia, through both of depressing conduction which might eventually render unidirectional block to bidirectional block leading to the discontinuing of reentrant pathway and prolonging ERP of cardiac PF and ventricular muscles, as a result reentrant activities are abolished. The effects of Dau on all parameters of PF at all stimulation frequencies are beneficial to terminate the clinical ventricular tachyarrhythmias. Additionally, the depressing effect of Dau on automaticity of PF might play an important role in inhibiting ectopic rhythm and controlling arrhythmias induced by the enhanced automaticity.

On the other hand, it has been reported that the toxic dose of Dau could result in cardiac conduction block and ventricular tachyarrhythmias accompanied with QRS and Q–T prolongation[12] and that Lid could shorten QRS width and Q–T interval prolonged by Dau[12]. This might be due to Lid–induced acceleration of the repolarization of AP, leading to the higher membrane potentials during next depolarization and conduction acceleration. These results efficaciously suggest that Lid might posses important clinical value in the treatment of Dau–induced His–PF conduction delay and ventricular tachyarrhythmias.

ACKNOWLEDGMENTS The technical assistances of ZONG Xian–Gang and WANG Gang, and the help of CHEN Bi–Yi and LENG Da–Mao in the experiments and Wuhan Institute for Drug Control for providing lidocaine.

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蝙蝠葛碱和利多卡因单用和联合用药对犬心浦氏纤维电生理特性的影响

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提要 用标准微电极法证明, 蝙蝠葛碱(Dau) 1-30 μmol/L 浓度依赖性降低犬浦氏纤维 APA, V_{max} 和 MDP, 延长 APD_{50} ,APD_{90} 及 ERP, 并降低其自律性和兴奋性. 利多卡因(Lid) 100 μmol/L 明显缩短 APD_{50}, APD_{90} 和 ERP, APA 和 V_{max} 亦显著降低, 并可缩短由 Dau 延长的 APD_{50} 和 APD_{90}, ERP 亦呈缩短趋势.表明 Lid 可改变 Dau 部分电生理特征, 可能构成其增强 Dau 药效学, 对抗其心脏毒性的基础.

关键词 蝙蝠葛碱; 利多卡因; 浦氏纤维; 动作电位; 电生理学

中国药理学报 *Acta Pharmacologica Sinica* 1990 May; **11** (3) :225-229

Rate-dependent depression of maximal rate of depolarization in guinea pig papillary muscle action potentials by changrolin

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ABSTRACT The rate-dependent block (RDB) of changrolin on the maximal rate of depolarization (V_{max}) of action potentials was studied in guinea pig right ventricular papillary muscles. The result was compared with that of class I A (quinidine), I B (mexiletine) and I C (lorcainide) drugs to approach the subclassification of changrolin, by using standard microelectrode techniques with computer. Mexiletin exhibited the fastest response in the onset rate of RDB. V_{max} reached 61% of its final value by the second beat during a train of stimuli. In response to a similar train of stimuli, quinidine, lorcainide and changrolin produced exponential falls of V_{max} with the constants of −0.143, −0.085 and −0.051 AP^{-1} (AP = action potentials), respectively. Time constants of re-

Received 1989 Apr 13 Accepted 1989 Dec 05