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二氢吡啶类和维拉帕米对氯化钙及 5-羟色胺所致猪冠状动脉收缩的拮抗作用

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提要 Nif, Nim, Nic, Fel 和 Ver 对无 Ca^{2+}高 K^+ 去极化时 $CaCl_2$ 所致离体猪冠状动脉收缩的拮抗强度依次为：Nif > Fel > Nim > Nic > Ver，pD_2'分别为 9.1, 8.4, 7.9, 7.8, 7.2；对 5-HT 所致收缩的拮抗强度依次为：Nif > Nim > Fel > Ver > Nic，pD_2'分别为 8.3, 7.5, 6.8, 5.6, 5.3，以 Nif 最强. Ver 同时抑制 5-HT 所致的两种收缩成分. Fel 只抑制外 Ca^{2+}内流所致的收缩，提示两类钙拮抗剂的作用不同.

关键词 硝苯啶；尼莫地平；尼卡地平；非洛地平；维拉帕米；钾；钙；冠状血管

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Effects of glibenclamide and tolbutamide on ischemia- and ouabain- induced arrhythmias and membrane potentials of ventricular myocardium from rat and guinea pig

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ABSTRACT Glibenclamide (Gli) 0.3, 1, 3 mg · kg^{-1} and tolbutamide (Tol) 3, 10, 30 mg · kg^{-1} iv 10 min before ischemia or ouabain infusion prevented ventricular fibrillation induced by ischemia in rat and arrhythmias induced by ouabain in guinea pig. Gli 10 μmol · L^{-1} and Tol 1 mmol · L^{-1} increased APD and ERP in rat ventricular muscle. Gli 0.1, 1, 10 μmol · L^{-1} and Tol 0.01, 0.1, 1 mmol · L^{-1} prevented and reversed the shortening of APD and ERP induced by hypoxia in guinea pig ventricular muscle. These effects of Gli and Tol were dose-dependent. The results confirmed that Gli and Tol were effective on arrhythmias induced by ischemia and ouabain by blocking ATP-sensitive potassium channel.

KEY WORDS glibenclamide; tolbutamide; ischemia; ouabain; arrhythmia; membrane potentials; anoxia

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ATP-dependent potassium channels had been demonstrated in cardiac myocytes[1,2]. The channels opened when ATP concentration in myocardial cell was below normal, which may be responsible for K^+ loss and shortening of action potentials and consequently for

arrhythmias during myocardial ischemia[3,4].

Glibenclamide (Gli) and tolbutamide (Tol), two antidiabetic sulfonylureas, blocked ATP–dependent K^+ channels[3] and showed anti–arrhythmic effects in ischemic isolated rat hearts[4,5].

Because arrhythmias induced by ouabain were believed to be related to the decrease of ATP level in cardiac cells[6], we investigated the anti–arrhythmic effects of Gli and Tol as well as their effects on membrane potentials from rat and guinea pig ventricular myocardium.

MATERIALS AND METHODS

Drugs Gli, purchased from Tianjin institude of Medical and Pharmaceutical Industry, was dissolved in NaOH and alcohol to prepare a stock solution (10 mmol · L^{-1}). Tol, obtained from the Fifth Pharmaceutical Factory of Shijiazhuang, was dissolved in NaOH. The pH of solution was readjusted to 7.4 after adding the sulfonylureas from stock solution, and the concentration of alcohol was never greater than 0.05%. Ouabain, purchased from Sigma, was dissolved in saline.

Arrhythmias induced by ischemia in anesthetized rat Sprague–Dawley rats, ♂, weighing 295 ± SD 34 g, were anesthetized with pentobarbital 45 mg · kg^{-1} ip. Catheters were inserted into the carotid artery and external jugular vein for blood pressure monitoring and drug adminstration respectively. The rats were ventilated with room air at 50 cycles / min and 15–30 ml / cycle. The thorax was opened and the left coronary artery was ligated near its origin. Electrocardiograph was monitored before and after initial 20 min ligating. Onset of arrhythmias, incidence and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF), and mortality were recorded. Drugs or solvent 2 ml · kg^{-1} were injected 10 min before ligating.

Arrhythmias induced by ouabain in guinea pigs Guinea pig of both sex, weighing 350 ± 46 g were anesthetized by urethan 1 g · kg^{-1} ip. Catheters were inserted into carotid artery and external jugular veins for blood pressure monitoring and drug adminstration respectively. The electrocardiograph was monitored with oscilloscope. Drugs or solvent 2 ml · kg^{-1} were administrated on one side of vein 5 min before ouabain adminstration. Ouabain was infused by other side of the vein at 10 $\mu g \cdot ml^{-1} \cdot min^{-1}$. The doses of ouabain to induce ventricular extrasystole (VE), VT, VF, and hearts stopping (HS) were recorded.

Action potentials recordings in rat and guinea pig papillary muscles Isolated papillary muscle of rat left ventricle and guinea pig right ventricle were superfused with Tyrode solution containing: NaCl 149, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 0.5, Tris 10, glucose 10 mmol · L^{-1}. The preparation was stimulated electrically at 1 Hz and 2 ms rectangular pulse at two times threshold intensity. In the normoxic situation, the solution was equilibrated with 100% O_2. In the hypoxic situation, the solution was gassed with 100% N_2 and deprived of glucose. Action potentials were recored with standard microelectrodes filled with KCl 3 mol · L^{-1} (10–40 MΩ). The electric signal was amplified by an amplifier (MEZ–8201) and monitored with an oscil– loscope. The amplified signal was analyzed by microcomputer[8]. The preparation was allowed to recover in normoxic solution for at least 1 h.

RESULTS

Effects of Gli and Tol on ischemia–induced arrhythmia in anesthetized rats Gli and Tol significantly decreased, in a dose–dependent manner, incidence and duration of VF, and delayed the onset of arrhythmias (Tab 1). Gli

Tab 1. Effects of glibenclamide and tolbutamide (iv 10 min before ligating) on arrhythmias and mortality in anesthetized rats subjected to coronary artery ligation for 20 min. $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **solvent.**

Pretreatment, mg · kg^{-1}		*n*	Onset of arrhythmia, min	VT incidence, %	VT duration, s	VF incidence, %	VF duration, s	Mortality, %
Solvent		54	5.5 ± 2.4	98	43 ± 32	48	341 ± 175	37
Glibenclamide	0.3	8	9.2 ± 1.7**	100*	33 ± 37*	0***	0***	0*
	1	10	8.9 ± 2.2***	76*	27 ± 35*	0***	0***	0*
	3	11	9.9 ± 2.3***	50*	40 ± 29*	0***	0***	0*
Tolbutamide	3	10	8.0 ± 2.5**	71*	30 ± 39*	43***	345 ± 187*	30*
	10	11	9.3 ± 2.4***	55*	28 ± 34*	18*	23 ± 24***	0*
	30	13	9.0 ± 2.3***	69*	49 ± 45*	7***	2 ± 0***	0*

Tab 2. Effects of glibenclamide and tolbutamide (iv 10 min before ouabain infusion) on the doses of ouabain to induce ventricular arrhythmias and heart stopping in anesthetized guinea pig. $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **solvent.**

Pretreatment, mg · kg^{-1}		*n*	Dose of ouabain (μg · kg^{-1}) to produce VE	VT	VF	HS
Solvent		60	134 ± 21	164 ± 32	190 ± 32	256 ± 55
Glibenclamide	0.3	10	166 ± 19**	178 ± 33*	255 ± 38***	286 ± 46**
	1	9	169 ± 23***	223 ± 41***	266 ± 46***	295 ± 21***
	3	10	197 ± 44***	254 ± 34***	299 ± 43***	361 ± 26***
Tolbutamide	3	11	163 ± 32**	171 ± 17*	220 ± 4*	267 ± 43*
	10	10	175 ± 23***	196 ± 20**	230 ± 41*	276 ± 45**
	30	9	186 ± 16***	194 ± 14**	235 ± 33**	290 ± 26***

and Tol had no effect on VT. Gli 0.3, 1, 3 mg · kg^{-1} reduced mortality from 37% to 0%, although the differences were not siginificant because of low mortality of control (37%) in this model. But, if we treated all doses of Gli and Tol as one group ($n=63$, mortality = 5%), and compared with solvent ($n=54$, mortality = 37%), we found significant difference ($P<0.01$). Gli and Tol had no effects on the blood pressure and heart rates of anesthetized rats.

Effects of Gli and Tol on ouabain-induced arrhythmias in anesthetized guinea pigs Pretreatment of Gli and Tol significantly increased the dose of ouabain to induce arrhythmias. These effects of Gli and Tol were also dose-dependent. Gli and Tol had no effects on the blood pressure and heart rates of anesthetized guinea pigs (Tab 2).

Effects of Gli and Tol on membrane potentials from rat and guinea pig ventricular muscle Gli 10 μmol · L^{-1} and Tol 1 mmol · L^{-1} increased the duration of action potentials (Tab 3), but had no effects on other parameters. The effect of Tol was reversible, but that of Gli was not. Gli and Tol had no effect on membrane potentials recorded from guinea pig ventricular muscle.

Effects of Gli and Tol on changes of action membrane potentials induced by hypoxia in guinea pig ventricular muscle A 20-min hypoxic perfusion produced 70% shortening

Tab 3. Effects of glibenclamide (Gli) and tolbutamide (Tol) on membrane potential recorded from rat ventrivular muscle. $\bar{x} \pm$ SD. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* solvent.

Treatment, μmol · L^{-1}		n	RP, mV	OS, mV	APA, mV	V_{max}, V · s	APD, ms	APD_{50}, ms	APD_{90}, ms	ERP, ms
Control		6	79 ± 4	21 ± 5	102 ± 9	189 ± 23	69 ± 8	20 ± 1	55 ± 6	46 ± 7
Solvent		5	79 ± 4	21 ± 4	100 ± 8	176 ± 25	70 ± 6	20 ± 2	55 ± 6	49 ± 6
Gli	0.1	5	76 ± 7*	23 ± 4*	98 ± 5*	178 ± 16*	79 ± 8*	18 ± 7*	59 ± 3*	52 ± 9*
	1	5	78 ± 5*	20 ± 6*	98 ± 3*	182 ± 25*	84 ± 10*	23 ± 3*	70 ± 4*	71 ± 8*
	10	5	80 ± 4*	21 ± 2*	100 ± 2*	180 ± 18*	125 ± 12***	34 ± 6***	117 ± 9***	110 ± 11***
Tol	10	5	80 ± 2*	22 ± 5*	103 ± 6*	202 ± 14*	71 ± 7*	18 ± 3*	60 ± 8*	50 ± 6
	100	5	79 ± 7*	21 ± 4*	99 ± 6*	210 ± 26*	82 ± 7*	22 ± 5*	66 ± 8*	56 ± 3*
	1000	5	78 ± 6*	23 ± 7*	102 ± 4*	185 ± 20*	105 ± 10***	30 ± 4***	92 ± 5***	85 ± 9***

Tab 4. Effects of pretreatment of glibenclamide (Gli) and tolbutamide (Tol) on ADP and ERP induced by 20-min hypoxia in guinea pig ventricular muscle. $\bar{x} \pm$ SD. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* control. $^{+}P > 0.05$, $^{++}P < 0.05$, $^{+++}P < 0.01$ *vs* solvent.

Treatment		n	RP, mV	APD, ms	APD_{50}, ms	APD_{90}, ms	ERP, ms
Control		6	84 ± 7	165 ± 9	110 ± 9	130 ± 8	175 ± 8
Solvent		6	88 ± 8*	68 ± 10***	32 ± 5***	47 ± 9***	71 ± 10***
Gli, μmol · L^{-1}	0.1	5	79 ± 6*	97 ± 10^{+++}	64 ± 9^{+++}	66 ± 10^{+++}	107 ± 4^{+++}
	1	4	82 ± 8*	152 ± 14^{+++}	98 ± 10^{+++}	110 ± 14^{+++}	150 ± 11^{+++}
	10	5	84 ± 3*	160 ± 14^{+++}	101 ± 8^{+++}	128 ± 13^{+++}	172 ± 9^{+++}
Tol, mmol · L^{-1}	0.01	5	79 ± 6*	103 ± 12^{+++}	69 ± 8^{+++}	70 ± 13^{+++}	119 ± 14^{+++}
	0.1	5	82 ± 8*	142 ± 12^{+++}	80 ± 10^{+++}	105 ± 17^{+++}	117 ± 13^{+++}
	1	5	81 ± 7*	148 ± 11^{+++}	93 ± 11^{+++}	123 ± 15^{+++}	168 ± 9^{+++}

of APD at 50% repolarization and 60% reduction of ERP (Tab 4). Pretreatment the preparation with Gli and Tol for 20 min significantly prevented APD shortening of the preparation (Tab 4).

DISCUSSION

The potential effects of sulfonylureas on ischemic arrhythmias was suggested soon after they were identified as specific blockers of ATP-sensitive K^+ channel[3]. Recently, anti-arrhythmic effect of Gli and Tol on ischemic isolated rat hearts had been demonstrated[4,5]. This paper confirmed these effects of Gli and Tol by using different ischemic model.

Ouabain in toxic concentration caused significant decrease of ATP concentration in myocardial cell[6,8]. This effect of ouabain might play an important role in inducing arrhythmias[11]. Our results (Tab 2) showed that Gli and Tol dose-dependently increase the dose of ouabain to induced ventricular arrhythmias. So, we here hypothesized that opening of ATP-sensitive K^+ channel play an important role in ouabain-induced arrhythmias. Further investigations were needed to confirmed the hypothesis.

We had not expected that Gli and Tol would have any effects on APD in normoxic situation, since ATP-sensitive K^+ channel

only opened when ATP concentration was abnormaly low[1,2]. But, as it was showed in Tab 3, Gli and Tol increase APD of rat ventricular muscle at comparatively high concentration but had no effect on guinea pig ventricular muscle. It was well known that electrophysiologic properties of rat hearts were greatly different from other species of animals[9]. The same result had been gotten by other author[10].

Either pretreatment or adding Gli and Tol 20 min after hypoxia, both agents attenuated the effect of hypoxia on ERP and APD of guinea pig ventricular myocardium (Tab 4). During early ischemia, the action potentials shortening and K^+ loss were responsible for arrhythmias[11,12]. Gli and Tol, by blocking the ATP-sensitive K^+ channel, inhibited K^+ loss[4] and prevented the shortening of APD and ERP and consequently prevented ischemic arrhythmias.

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格列本脲和甲苯磺丁脲对大鼠缺血性和豚鼠哇巴因性心律失常及心室肌跨膜电位的作用

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提要 格列本脲(Gli) 0.3，1，3 mg·kg^{-1} 或甲苯磺丁脲(Tol) 3，10，30 mg·kg^{-1} iv 剂量依赖性减少大鼠结扎冠脉所致 VF 及豚鼠 iv 哇巴因诱导的心律失常. Gli 10 μmol·L^{-1} 和 Tol 1 mmol·L^{-1} 延长正常大鼠心室肌 ADP 和 ERP. Gli 0.1-10 μmol·L^{-1} 和 Tol 0.01-1 mmol·L^{-1} 浓度依赖性预防及翻转缺氧豚鼠心室肌 ADP 和 ERP 的缩短. 提示两药通过阻断 ATP 依赖性钾通道而抗心律失常.

关键词 格列本脲；甲苯磺丁脲；缺血；哇巴因；心律失常；膜电位；缺氧症