并用高压液相电化学检测法和荧光法测定端脑、间脑、脑干的单胺类递质含量 结果：TS 注入侧脑室后，血压由 11.59 ± 0 84 升至 14 59 ±0.69 kPa，心率由 411 ± 21 增至 465 ± 14 次/分；外周血中 NE，E 的含量分别由 6 ± 3 和 6 ± 2 升至 64 ± 44 和 38 ± 34 nmol/L 血浆；脑干内的 NE 由 33 ± 7 升至 45 ± 8 nmol/g 湿组织；端脑、间脑及脑干内的 5-HT 分别由 9 ± 1，14 ± 2 及 14 ± 3 降至 5 ± 1，7 ± 2 和 6 ± 1 nmol/g 湿组织。结论：中枢内的 TS 的心血管效应与单胺类递质有关。

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Electrophysiological effects of felodipine on guinea pig papillary muscles

ZHENG Ping, SHI Nian-Ci, GONG Qin-Yan, YANG Zao-Chen (*Department of Pharmacology, School of Basic Medical Sciences, Shanghai Medical University, Shanghai 200032, China*)

KEY WORDS felodipine; papillary muscles; action potentials; patch-clamp techniques; nifedipine; verapamil

AIM: To determine whether felodipine (Fel) has Ca^{2+} channel blocking effect in mammalian myocardium in comparison with those of nifedipine (Nif) and verapamil (Ver). **METHODS**: The action potentials (AP), the slow AP and the inward slow Ca^{2+} currents of guinea pig papillary muscles were studied using intracellular microelectrodes and voltage-clamp techniques. **RESULTS**: Fel 1, 3, and 10 μmol·L^{-1} concentration-dependently shortened APD_{30}, APD_{50}, and APD_{90} of the AP, while V_{max} and APA were not affected. The effect of Fel was not reversible on washout. At 0.1, 1, 3, and 10 μmol·L^{-1}, Fel depressed V_{max}, APA, APD_{30}, APD_{50}, and APD_{90} of the slow AP in a dose-dependent manner. The inward slow Ca^{2+} currents were reduced by Fel 3 μmol·L^{-1}. APD_{30}, APD_{50}, and APD_{90} of the first AP after rest were still shortened by Fel. When the stimulation frequency was elevated, the effect of Fel on the AP and slow AP decreased. The effect of Fel 3 μmol·L^{-1} on the slow AP was abolished in preparation pretreated with trifluoperazine. The threshold concentrations of Nif and Ver for the inhibition of APD_{50} of the slow AP ($P < 0.05$) were 0.1 and 1 μmol·L^{-1}, respectively. The effect of Ver 3 μmol·L^{-1} on the fast AP was not reversible on washout, but that of Nif 3 μmol·L^{-1} was. When the stimulation frequency was elevated from 0.5 to 2 Hz, the effect of Nif 3 μmol·L^{-1} on the fast AP was reduced, but that of Ver 3 μmol·L^{-1} was increased. **CONCLUSION**: Fel inhibited mainly the resting state of the cardiac Ca^{2+} channel. The potency of Fel was about the same as that of Nif and about 10 times more potent than that of Ver.

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Felodipine (Fel) is a calcium antagonist in vascular muscles[1,2]. Unlike verapamil (Ver) and nifedipine (Nif), Fel was an intracellular Ca^{2+} blocker rather than Ca^{2+} channel blocker[3]. In vascular muscle Fel was a Ca^{2+} channel blocker[4]. A WHO Committee proposed that demonstration with electrophysiological techniques of its ability to block Ca^{2+} entry into myocardial cells was considered mandatory for a Ca^{2+} channel blocker[5]. However, we have not seen the reports about the electrophysiological effect of Fel on cardiac tissues. This paper was to determine whether Fel had Ca^{2+} blocking effect in mammalian myocardium in comparison with those of Nif and Ver.

MATERIALS AND METHODS

Guinea pigs (weighing 250 ± *s* 31 g) of both sexes were stunned, and the papillary muscle from right ventricle was perfused with Tyrode solution 8 mL·min^{-1} at 35 ℃ gassed with 95 % O_2 + 5 % CO_2. The muscle was stimulated at 1 Hz by square pulse (duration: 1 ms; intensity: 2 ·

threshold). Action potentials (AP) were recorded by microelectrodes filled with KCl 3 mol·L^{-1} (resistance of 10 - 30 MΩ). AP and the maximal rate of upstroke (V_{max}) were photographed from a storage oscilloscope. Slow AP was induced by isoprenaline 0.2 mg·L^{-1} in K^+ 16 mmol·L^{-1} Tyrode solution.

In voltage-clamp experiment, papillary muscle less than 0.5 mm in diameter was mounted in a 3-compartment chamber. The length of the preparation in the test compartment was limited to less than 0.5 mm to obtain a homogeneous potential distribution. The sucrose gap was established by perfusing the middle compartment with the sucrose solution (mmol·L^{-1}: sucrose 275; glucose 5; $CaCl_2$ 0.05). At the same time, the Tyrode solution perfusing the KCl compartment was replaced by K^+ 137 mmol·L^{-1}-Tyrode solution (NaCl was replaced by KCl). Voltages clamped by an amplifier (CEZ-1100, Nihon Kohden) and membrane currents were recorded by a storage oscilloscope. When the membrane potential of guinea pig papillary muscle was clamped from the holding potential of -40 to +10 mV, an inward current with low amplitude, slow activation, and slow inactivation was seen. This inward current was sensitive to changes in Ca^{2+} concentration and to verapamil. These results indicated that the inward current was the slow Ca^{2+} current. In addition, we used the method[6] to observe the effect of Fel on the resting state of the Ca^{2+} channel.

Drug Fel and Nif were provided by Prof ZHANG Di-Qun, Division of Medicinal Chemistry, Hebei Medical College. A stock solution of Fel or Nif in ethanol and acetone was used diluted in normal Tyrode solution to obtain the final concentration. The solvent in the final test solution had no direct effect on the parameters observed in this experiment. Ver was purchased from Tianjing Central Pharmaceutical Factory and dissolved with distilled water.

Data analyses The analyses of data for significance were performed by t test for paired data.

RESULTS

Effect of Fel on AP In 6 papillary muscles, Fel 1, 3, 10 μmol·L^{-1} reduced APD_{30}, APD_{50}, and APD_{90} in a concentration-dependent manner, but had no effect on V_{max} and APA (Tab 1).

At 10 min after administration of Fel 3 μmol·L^{-1}, APD_{50}/ms was shortened from 146 ± 30 (control) to 127 ± 26 (n = 4, $P < 0.05$). Between 10 and 60 min, APD_{50} was reduced to (94 ± 27) ms (n = 4, $P < 0.05$).

In 4 muscles, the shortening effect of Fel 3 μmol·L^{-1} on APD_{50}/ms (from 130 ± 40 to 110 ± 30, $P < 0.05$) was not reversed by washing out for 45 min.

Tab 1. Effects of Fel on AP of guinea pig papillary muscles. Resting potentials were about -80 mV. n = 6, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs control.

Fel/ μmol·L^{-1}	V_{max}/ V·s^{-1}	APA/ mV	APD_{30}/ ms	APD_{50}/ ms	APD_{90}/ ms
0	213 ± 38	116 ± 6	105 ± 16	142 ± 16	173 ± 16
1	210 ± 42^a	115 ± 4^a	102 ± 17^a	137 ± 21^a	173 ± 21^a
3	197 ± 24^a	114 ± 3^a	85 ± 13^b	119 ± 17^b	157 ± 18^b
10	218 ± 41^a	109 ± 8^a	68 ± 14^b	98 ± 19^c	136 ± 20^c

Effect of Fel on slow AP In 6 muscles, Fel 0.1, 1, 3, and 10 μmol·L^{-1} concentration-dependently inhibited V_{max}, APA, APD_{30}, APD_{50}, and APD_{90} of the slow AP (Tab 2). The threshold concentration was 0.1 μmol·L^{-1}.

Tab 2. Effect of Fel on slow AP of papillary muscles. Resting potentials were about -50 mV. n = 6, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs control.

Fel/ μmol·L^{-1}	V_{max}/ V·s^{-1}	APA/ mV	APD_{30}/ ms	APD_{50}/ ms	APD_{90}/ ms
0	12.5 ± 2.7	89 ± 10	125 ± 24	149 ± 31	165 ± 28
0.01	12.4 ± 2.4^a	88 ± 10^a	125 ± 26^a	147 ± 30^a	166 ± 26^a
0.1	9.4 ± 2.9^b	84 ± 10^b	118 ± 23^c	140 ± 30^b	155 ± 29^c
1	8.5 ± 2.7^b	82 ± 8^b	108 ± 26^b	127 ± 32^b	142 ± 34^b
3	7 ± 4^b	81 ± 11^b	97 ± 29^b	113 ± 40^b	130 ± 41^b
10	6 ± 4^b	77 ± 13^b	84 ± 30^b	98 ± 42^b	116 ± 45^b

Effect of Fel on inward slow Ca^{2+} currents Fel 3 μmol·L^{-1} decreased the slow Ca^{2+} currents/μA from 12 ± 4 (control) to 7.3 ± 2.7 (n = 6, $P < 0.01$).

Under control conditions, APD_{30}, APD_{50}, and APD_{90} of the first AP after 10 min of rest were longer than those of the pre-rest AP during regular stimulation. Fel 3 μmol·L^{-1} shortened not only APD_{30}, APD_{50}, and APD_{90} of the pre-rest AP but also those of the first AP after rest (Tab 3).

At a stimulation frequency of 0.5 Hz, Fel 3 μmol·L^{-1} reduced APD_{50} by (26 ± 8) ms (n = 4). When stimulation frequency/Hz increased from 0.5 to 1 and 2, Fel 3 μmol·L^{-1} reduced APD_{50}/ms by 19 ± 9 and 17 ± 6 ms, respectively. In the slow AP experiment, at stimulation frequencies/ms 0.2,

Tab 3. Effect of Fel 3 μmol·L^{-1} on AP durations during regular stimulation of 1 Hz and the first AP durations aftr 10 min of rest. $n = 4$, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Fel μmol·L^{-1}	APD/ms					
	Regular stimulation			1 st beat after rest		
	APD_{30}	APD_{50}	APD_{90}	APD_{30}	APD_{50}	APD_{90}
0	107 ± 31	131 ± 36	159 ± 37	119 ± 25	146 ± 25	174 ± 26
3	90 ± 27^b	115 ± 30^b	144 ± 34^b	90 ± 14^c	116 ± 14^c	143 ± 12^c

0.5, and 1, Fel 3 μmol·L^{-1} reduced APD_{50}/ms by 11 ± 4, 8 ± 4, and 5 ± 4 ($n = 3$), respectively.

In the slow AP experiment, the effect of Fel 3 μmol·L^{-1} on V_{max}, APA, APA_{30}, APD_{50}, and APD_{90} were abolished in preparation pretreated with trifluoperazine 10 μmol·L^{-1} (Tab 4).

Comparison with Nif and Ver In the slow AP experiments, the threshold concentrations of Fel, Nif and Ver for the inhibition of APD_{50} ($n = 3 - 6$, $P < 0.05$) were 0.1, 0.1, and 1 μmol·L^{-1}, respectively. The effect of Nif 3 μmol·L^{-1} on APD_{50} of the AP was reversed on washout, but Fel and Ver 3 μmol·L^{-1} was not. When the stimulation frequency/Hz was elevated from 0.5 to 1 and 2, Fel 3 μmol·L^{-1} reduced APD_{50}/ms by 26 ± 8, 19 ± 9, and 17 ± 6, Nif 3 μmol·L^{-1} reduced it by 27 ± 15, 25 ± 15, and 16 ± 11, and Ver 3 μmol·L^{-1} reduced it by 9 ± 4, 14 ± 4, and 15.6 ± 0.8 ($n = 3 - 4$), respectively.

DISCUSSION

Fel did not affect V_{max} and APA of the AP, but significantly shortened APD_{30}, APD_{50}, and APD_{90}, suggesting that the effect of Fel was not associated with the fast Na^+ channels, but with the currents participating in the plateau phase and phase 3. In the slow AP and voltage studies, Fel had a blocking effect on the cardiac Ca^{2+} channels.

The cardiac calcium channel has three different states: resting state, open state, and inactivated state. The results showed that Fel had an apparent effect on the resting state of the channel. In frequency-dependent experiment, when the stimulation frequency was elevated, the effect of Fel on the AP and slow AP decreased. With regard to the implication of the effect, this partialy explained the reason of the high selectivity of Fel for the vascular muscles. *In vivo*, the frequency of the pulse in the vasculer muscle was apparently lower than that in myocardial muscle. The lower the stimulation frequency was, the stronger the effect of Fel was. So Fel had a high selectivity for the vascular muscle.

Calmodulin plays an important role in regulating the inward slow Ca^{2+} currents in heart cells[7]. Trifluoperazine is one of the most potent inhibitors of calmodulin. In this paper, the effect of Fel was mediated by its inhibition of calmodulin. The binding of Fel to calmodulin with a binding constant of 1 - 10 μmol·L^{-1} has been demonstrated[3,8]. This provided a basis for Fel to inhibit

Tab 4. Effects of Fel 3 μmol·L^{-1} on slow AP in papillary muscles pretreated with trifluoperazine (TFP). Resting potentials were about − 50 mV. $n = 4$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control or TFP group.

Parameters	Control	Fel/ 3 μmol·L^{-1}	Control	TFP/ 10 μmol·L^{-1}	TFP + Fel
APD_{30}/ms	83 ± 5	72 ± 3^b	80 ± 16	83 ± 15^a	84 ± 20^a
APD_{50}/ms	100.8 ± 2.9	89.2 ± 1.9^b	102 ± 17	104 ± 15^a	106 ± 22^a
APD_{90}/ms	120 ± 5	101 ± 7^b	123 ± 8	125 ± 5^a	128 ± 8^a
APA/mV	81 ± 8	75 ± 8^b	87 ± 8	84 ± 5^a	80 ± 8^a
V_{max}/V·s^{-1}	11.6 ± 2.0	7 ± 4^b	12.0 ± 2.4	12 ± 3^a	10 ± 5^a

calmodulin.

The very poor recovery of the Fel effect suggested that Fel had no effect on the slow Ca^{2+} channel at the outer surface of the membrane, but it was more likely that Fel exerted its effect by interacting with the Ca^{2+} channel at the inner surface of the cell membrane.

The frequency-effect relationship of Fel and Nif was contrary to that of Ver. In addition, the effect of Nif was reversible, but Fel was not, suggesting that although Fel and Nif were both belong to dihydropyridine derivative, there were some differences in the site and mechanism of the effect of both drugs. The above results showed that Fel had an inhibitory effect on the cardiac Ca^{2+} channels, mainly acted on the resting state of the channel. The potency of Fel was about the same as that of Nif and about 10 times more potent than that of Ver.

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241-244

非洛地平对豚鼠右心室乳头状肌的电生理作用

郑 平，史念慈，贡沁燕，杨藻宸 （上海医科大学基础医学院药理教研室，上海 200032，中国）

关键词 非洛地平；乳头状肌；动作电位；膜片箝技术；硝苯啶；维拉帕米

R965.1 R964

A **目的**：证明非洛地平(Fel)对心肌钙通道是否有阻断作用，与硝苯啶和维拉帕米作一些比较。**方法**：利用细胞内微电极和单蔗糖电压钳方法研究Fel对豚鼠右心室乳头状肌动作电位，慢反应动作电位和慢钙电流的作用。**结果**：Fel呈浓度依赖性抑制动作电位的持续时间，慢反应动作电位的0相除极速度，幅度及持续时间和慢钙电流。Fel的作用不易冲洗掉，刺激频率增加，Fel作用减弱。Fel对心肌钙通道静息态有作用。用三氟拉嗪处理标本后，Fel作用消失。**结果**：Fel对心肌钙通道具有阻断作用。这种作用主要是对静息态钙通道的作用