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ABBREVIATION AH = nucleus anterior hypothalami; AMYG = nucleus amygdaloidens; ARC = nucleus arcuatus; CG = substantia grisea centralis; CP = nucleus caudatus putamen; DMH = nucleus dosomedialis hypothalami; DR = nucleus raphe dorsalis; HABL = nucleus habenulae lateralis; HABM = nucleus habenulae medialis; HI = formatio hippocampi; IC = colliculus inferior; LH = nucleus lateralis hypothalami; MM = nucleus corporis mamillaris medialis; PF = nucleus parafascicularis; PH = nucleus posterior hypothalami; POL = nucleus preopticus lateralis; POM = nucleus preopticus medialis; PVH = nucleus paraventricularis hypothalami; PVT = nucleus paraventricularis thalami; RE = nucleus reuniens; RGC = nucleus reticularis gigantocellular; RH = nucleus rhomboideus; RMG = nucleus raphe magnus; RP = nucleus raphe pontis; SC = colliculus superior; SL = nucleus septi lateralis; SM = nucleus septi medialis; SN = substantia nigra; ST = nucleus striae terminalis; TA = nucleus anterior thalami; TD = nucleus tractus diagonalis; TL = nucleus lateralis thalami; TM = nucleus medialis thalami; TPO = nucleus posterior thalami; TV = nucleus ventralis thalami; VMH = nucleus ventromedialis hypothalami.

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外周伤害性热刺激诱发清醒大鼠中枢 c-*fos* 蛋白表达

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摘要 实验采用免疫组织化学法观察外周伤害性热刺激所致的中枢 c-*fos* 蛋白的表达状况. 将清醒大鼠尾浸入50℃水浴中引起在脊髓腰、骶段背角 I、II 层，中缝背核，中央灰质腹侧部，丘脑，下丘脑和大脑皮层等脑区 c-*fos* 蛋白表达显著升高，表明伤害性热刺激在清醒大鼠上诱发的 c-*fos* 蛋白可显示痛相关神经元.

关键词 痛；原癌基因蛋白 c-*fos*；脑；脊髓；免疫组织化学法；物理刺激

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Effects of 3,6-dimethamidodibenzopyriodonium citrate on slow inward calcium current in isolated guinea pig ventricular cells

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ABSTRACT The effects of 3,6-dimethamidodibenzopyriodonium citrate (I-65) on action potentials and slow inward calcium current (I_{si}) were examined on isolated guinea pig ventricular myocardial cells. I-65 (30—100 μmol·L^{-1}) depressed the action potential duration at 20% repolarization (APD_{20}) and, under voltage-clamp conditions, reduced the amplitude of I_{si} without changing the I-V relations. I-65 also showed use-dependent effects on I_{si}. These suggest that I-65 may block I_{si} by acting on the inactivated state of Ca channels.

KEY WORDS iodonium compounds; I-65; action potentials; myocardium; calcium channel blockers; membrane potentials

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3,6-Dimethamidodibenzopyriodonium citrate (I-65) has been shown to cause a concentration-dependent depression of contraction and a plateau of action potential in guinea pig papillary muscles, suggesting that I-65 may block the calcium channels[1]. But the precise mode of action of this drug under voltage-clamp conditions has not been studied. This experiment examined the effects of I-65 on the slow inward calcium current (I_{si}) in isolated guinea pig ventricular myocardial cells.

MATERIALS AND METHODS

Experiments were performed on single cardiac ventricular cells isolated from guinea pig. The guinea

pig was killed by cervical dislocation. Heart was excised. Single myocardial cells were isolated by collagenase (type II, Worthington Biochemical Corp, USA)[2] and superfused with a solution gassed with 95% O_2 + 5% CO_2 at 36℃ containing: NaCl 118.5, $NaHCO_3$ 14.5, KCl 4.2, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11.1 and $CaCl_2$ 2.0 mmol·L^{-1}.

Cells were impaled with glass microelectrode at a resistance of 20 — 30 MΩ (containing K_2SO_4 0.5 mol·L^{-1} + KCl 0.3 mmol·L^{-1}). Action potential was obtained by 2 ms current pulse stimulation injected at 1 Hz. A switch voltage-clamp method with a single-electrode system[3] (Dagan 8500) was used. The function of the electrode was switched rapidly between current passing and voltage recording. The rate of switching was in the range of 2 — 8 kHz. The cells were clamped at a holding potential of −40 mV to inactivate the sodium current. Voltage and current were recorded on a digital audio recorder (DAT 800, Sony). Records were displayed and plotted on a digital storage oscilloscope. The magnitude of the current was measured as the difference between the peak inward current and the steady current at the end of the pulse[4,5].

RESULTS

Effects of I-65 on action potentials In the presence of I-65 (30 μmol·L^{-1}), APD_{20} decreased from 98±*s* 10 to 84±*s* 7 ms (n=6, P<0.01). An increase of the concentration of I-65 to 100 μmol·L^{-1} caused further reduction of APD_{20} to 64±13 ms (n=6, P<0.01). The depression of APD began at 2 min and reached a steady value in 6 — 9 min. The effects of I-65 on action potential were partially reversible after 20 min of washing.

Effects of I-65 on I_{si} Voltage-clamp pulses (200 ms) to different membrane potentials were applied from a holding potential −40 mV at 0.3 Hz and current-voltage (I-V) curves were constructed (Fig 1). In the presence of I-65, the amplitude of I_{si} decreased in a concentration-dependent manner without changing the I-V relations. The potential at which I_{si} was maximum (0 mV) and the apparent reversal potential remained unaltered. Fig 2 illustrated an example of this experiment.

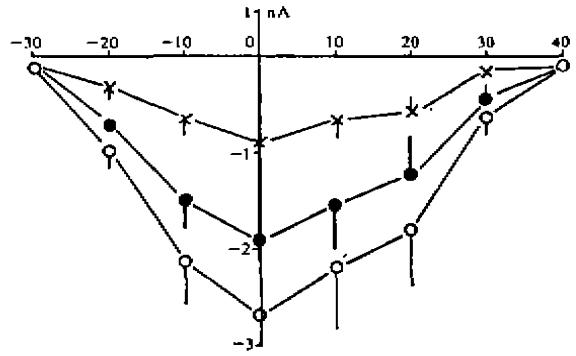

Fig 1. Effects of I-65 on I-V curves of I_{si} on isolated guinea pig ventricular myocardial cells. Control (○); After applying I-65 30 (●) and 100 (×) μmol·L^{-1}. n=9, $\bar{x}\pm s$.

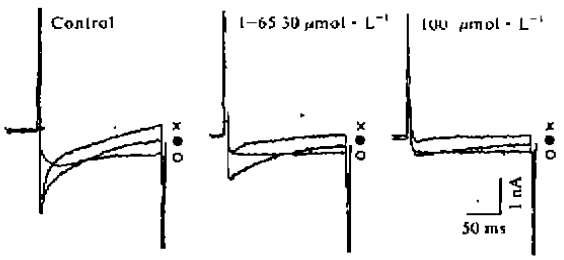

Fig 2. Effects of I-65 on I_{si} induced by 200 ms voltage clamp pulse from a holding potential of −40 mV to −30 mV (○), 0 mV (●), and +20 mV (×) at a rate of 0.3 Hz.

Use-dependent block by I-65 The depolarizing clamp applied in basic frequency of 0.3 Hz after 8 min rest (Fig 3). I-65 50 μmol·L^{-1} caused a staircase decline of I_{si}. There was no obvious change detected in the first I_{si} between the control and I-65 (Tab 1).

DISCUSSION

In guinea pig cardiac ventricular cells, I_{si} is dominant at plateau phase allowing the generation of long duration action potential. Suppression of the plateau of action potential by I-65 in this experiment was consistent with

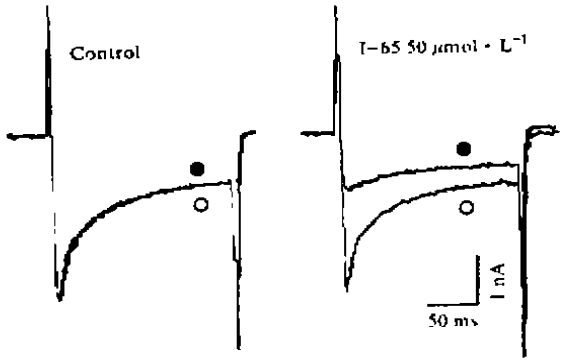

Fig 3. Use-dependency of blockade of I_{si} by I-65. I_{si} activated by the 1st (○) and 18th (●) pulses after 8 min rest.

Tab 1. Effects of I-65 on I_{si} activated respectively by the 1st and 18th pulse after 8 min rest in isolated guinea pig ventricular myocardial cells. $n=7$, $\bar{x}\pm s$. $^{*}P>0.05$ *vs* the 1st I_{si} of control, $^{\dagger\dagger\dagger}P<0.01$ *vs* the 1st I_{si} of I-65.

	I_{si}/nA	
	1st	18th
Control	2.53±0.65	2.51±0.64*
I-65 50 μmol·L^{-1}	2.47±0.68*	0.99±0.27†††

the suggestion that I-65 might block I_{si}.

The effect of I-65 on I_{si} was directly tested in voltage-clamp experiments. Under these conditions, I_{si} was thought to be carried predominantly by Ca entry through the "L" type calcium channels[7,8]. The observation that I-V curve of I_{si} was consistently suppressed by I-65 strongly supported the blockade of I-65 on I_{si}.

Some calcium channel blockers, like verapamil, have been reported to act on cardiac Ca channels in an inactivated state, increasing the time for recovery from inactivation, which was described as use-dependent enhancement of inhibition ofi I_{si}[9,10]. Because of the frequency-dependent negative inotropic effects of I-65 found in previous experiments, the question arose that I-65 might also preferably act on an inactivated state of I_{si}. The results that I-65 had no effect on I_{si} activated by the first pulse after rest, but decreased markedly the I_{si} activated by consecutive pulses accorded well with the suggestion that the inactivated state of I_{si} was its major targets.

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3,6-(二甲氨基)-二苯骈碘杂六环构檬酸盐对豚鼠单个离体心室肌细胞慢内向钙电流的影响

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摘要 I-65 30—100 μmol·L⁻¹可缩短豚鼠单个离体心室肌细胞的 APD_{20}。采用单微电极电压钳方法，发现上述浓度的 I-65可明显抑制心室肌细胞的 I_{si}，但不影响其阈电压和反转电压。I-65对 I_{si}的抑制呈使用依赖性。提示：I-65可阻断钙通道，并可能作用于钙通道的失活态。

关键词 碘化合物；I-65；动作电位；心肌；钙通道阻滞剂；膜电位

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Effect of *Panax notoginseng* saponins on increased proliferation of cultured aortic smooth muscle cells stimulated by hypercholesterolemic serum

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ABSTRACT *Panax notoginseng* saponins (PNS) was extracted from a Chinese herb medicine. After preparation of cultured aortic smooth muscle cell (SMC) from primary aortic explants, the cytotoxicity of hypercholesterolemic serum (HCS) for cultured cells was determined by trypan blue exclusion test, and [^{3}H]thymidine incorporation and cell numbers were counted at the same time. The results showed that HCS (0.5 mg cholesterol·ml^{-1}) increased the incorporation of [^{3}H]thymidine into cultured cells (3722 ± 440 *vs* 1655 ± 288 dpm/μg cell protein, $P<0.01$), stimulated the proliferation of SMC [$(6.5\pm1.5)\times10^6$ *vs* $(4.3\pm1.2)\times10^6$ cells/plate, $P<0.01$], and that high concentration HCS (final cholesterol concentration 2 mg·ml^{-1}) was cytotoxic to the cultured cells. PNS (100 and 400 μg·ml^{-1}) decreased the incorporation of [^{3}H]thymidine into SMC in culture with or without HCS (1292 ± 260 and 982 ± 314 or 4111 ± 886 and 2361 ± 751 dpm/μg cell protein), and inhibited the proliferation of the cultured cells [$(3.3\pm0.7)\times10^5$ and $(2.9\pm0.7)\times10^6$ or $(4.7\pm1.4)\times10^6$ and $(4.1\pm1.2)\times10^6$ cells/plate). We conclude that PNS can inhibit the proliferation of aortic SMC stimulated by HCS. These results also suggest that HCS may play an atherogenic role in the arterial wall and that PNS may prevent atherosclerosis and inhibit progression of the atherosclerotic lesions by interfering with the proliferation of arterial SMC.

KEY WORDS ginseng; saponins; cultured cells; thoracic aorta; vascular smooth muscle; cell count; hypercholesterolemia; thymidine

Hypercholesterolemic serum (HCS) is known to cause proliferation and necrosis in cultured smooth muscle cells (SMC)[1]. In this study, we used HCS to stimulate the proliferation of cultured SMC, and studied the effects of *Panax notoginseng* saponins (PNS) on the HCS-induced DNA synthesis and cell growth of cultured aortic SMC. As PNS can retard the progress of atherosclerosis in rabbits[2], we raise the possibility that PNS can inhibit the development and progress of atherosclerotic lesions by interfering with the proliferation of arterial SMC.

MATERIALS AND METHODS

PNS, which has seven stains in thin-layer chromatographic identification[3], was purchased from Wuzhou Third Pharmaceutical Factory. Trypsin and Medium 199 (M199) were obtained from Grand Biological Co, Grand Island NY, USA. [^{3}H]Thymidine

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