# Blocking effects of heteroclitin D and gomisin J on L-type calcium channels in ventricular cells of guinea pig[1]

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**KEY WORDS** *Kadsura heteroclita*；patch-clamp techniques；calcium channels；lipid peroxidation；myocardium

**AIM**：To study the effects of heteroclitin D（HD）and gomisin J（GJ），two lignans from *Kadsura* medicinal plants，on L-type calcium channels in ventricular cells of guinea pig. **METHODS**：The calcium currents were measured by whole-cell patch-clamp recording technique. **RESULTS**：HD 1 and 10 μmol/L decreased the L-type calcium current from（770 ± 155）to（482 ± 104）and（384 ± 85）pA，respectively. GJ 10 μmol/L inhibited calcium current from（822 ± 169）to（436 ± 143）pA. Neither HD nor GJ affected the steady-state activation curve. But they had impact on steady-state inactivation curve. HD 10 μmol/L changed the half inactivation voltage（$V_{0.5}$）from －22.7 to －40.9 mV，and slope factor（κ）from 10.2 to 20.6（$n=4$ cells from 3 guinea pigs，$P<0.05$）. GJ 10 μmol/L changed the $V_{0.5}$ from －17.7 to －33.3 mV，and κ from 15.9 to 27.8（$n=5$ cells from 3 guinea pigs，$P<0.05$）. **CONCLUSION**：HD and GJ inhibited L-type calcium channels.

## INTRODUCTION

Heteroclitin D（HD）and gomisin J（GJ）were isolated from *Kadsura* plants which were used for the treatment of blood deficiency，menstrual disorder，and stomachache in Chinese folk medicine[1,2]. Among the great number of lignan compounds extracted from *Kadsura* medicinal plants，HD and GJ have higher biological activities.

Calcium channel blockers are advocated as cardioprotective agents against ischemia. However，they were proved not to have an impact on mortality in long-term clinical studies after infarction[3]. It is well known that reperfusion of the ischemic heart can produce a large number of oxygen free radicals and accumulation of intracellular calcium ions. The free radicals attack the cell membranes，and result in lipid peroxidation and loss of membrane integrity. But existing calcium channel blockers have little effect on lipid peroxidation and calcium overload[4]. Therefore calcium channel blockers with both $Ca^{2+}$ overload inhibition and anti-lipid peroxidative activity can be a new direction for drug R&D[5,6].

Several studies have demonstrated that GJ[7－9] and HD[10] have strong anti-lipid peroxidation activities，but their calcium channel blocking activities are still being investingated. In this study，we further investigate the blocking effects of HD and GJ on L-type calcium channel in ventricular myocytes of guinea pig.

## MATERIALS AND METHODS

**Preparation of ventricular myocytes** Single ventricular myocytes were isolated from adult guinea pigs，weighing 331 g ± *s* 28 g（supplied by the Experimental Animal Center of Shanghai Medical University，Grade Ⅱ）by enzymatic disaggregation[11]. After the guinea pig was stunned，the heart was rapidly put into oxygenated calcium-free Tyrode's solution. The aorta was cannulated and the heart was perfused on Langendorff apparatus at 37 ℃. Following perfusion with calcium-free Tyrode's solution for about 5 min，the low calcium（50 μmol/L）enzymatic solution containing 0.03 % type Ⅱ collagenase and 1 % bovine serum albumin（BSA）was used. The enzymatic process was not finished until the heart became soft. The ventricles were chopped，minced，and gently agitated to obtain myocyte suspension. It was filtered through a 200-μm nylon mesh and

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the calcium concentration was gradually increased to 1.0 μmol/L.

**Whole-cell patch-clamp recording** The cells were put in a 1-mL pool on the platform of inverted microscope (Nikon, Japan). Only the cells with rod-shape and clear striations were used in experiments. The pool was perfused with test solution at rate of 1.5 mL/min. Microelectrodes were pulled in two steps with a microelectrode puller (Narrishage, Japan) and had a resistance of 3 – 4 MΩ when filled with intracellular solution. Ag-AgCl electrode was used as the reference electrode. After gigaseal formation and membrane rupture the test solution was changed to the $Na^+$-free solution in which $Na^+$ was substituted by TEA-Cl. Calcium current was recorded with Axopatch 200A amplifier (Axon Instrument, USA) and pClamp 6.0 software (Axon Instrument, USA) was used to produce protocols, acquire, and process data. Capacitive transients and series resistance were compensated and linear leakage currents were subtracted using the p/4 protocol.

**Chemicals and solution** HD and GJ were provided by the Department of Pharmacognosy, School of Pharmacy, Shanghai Medical University, and they were both spectrum pure[1,2]. Type-Ⅱ collagenase, MgATP, Tris-GTP were purchased from Sigma. TEA-Cl was a Merck product. Tetrodotoxin (TTX) was purchased from Hebei Ocean Product Institute of China. 3-(*N*-morpholino) propanesulfonic acid (MOPS), BSA, CsCl were purchased from Sino-American Biotechnology Co. Other reagents were of AR grades produced in Shanghai Chemical Reagent Plant.

The calcium-free solution contained: NaCl 100, KCl 10, $NaH_2PO_4$ 1.2, $MgSO_4$ 5.0, glucose 20, taurine 10, MOPS 10 mmol/L; pH was adjusted with KOH to 7.2. Test solution was composed of: TEA-Cl 140, $MgCl_2$ 2.0, $CaCl_2$ 1.8, HEPES 10, glucose 10, TTX 0.002 mmol/L; pH was adjusted with TEAOH to 7.3. The pipette solution was: CsCl 140, egtazic acid 10, HEPES 10, MgATP 3, Tris-GTP 0.4 mmol/L; pH was adjusted with CsOH to 7.2.

**Statistics** The data were expressed as $\bar{x} \pm s$ and analysed with paired *t*-test. The steady-state activation or inactivation curves were fitted with Boltzman equation: $I/I_{max} = 1/\{1 + EXP[(V - V_{0.5})/\kappa]\}$. $I$ is the calcium current, $I_{max}$ is the maximal amplitude of calcium current; $V$ is the voltage of conditioning pulse, $V_{0.5}$ is the half activation or inactivation voltage and $\kappa$ is the slope factor.

## RESULTS

**Effects of HD and GJ on L-type calcium current** The inward calcium current in single ventricular myocyte appeared when a step pulse from holding potential of −40 to 0 mV at 1 Hz was given. HD 1 and 10 μmol/L inhibited the current from (770 ± 155) to (482 ± 104) ($n = 6$ cells from 3 guinea pigs, $P < 0.05$) and (384 ± 85) pA ($n = 6$ cells from 3 guinea pigs, $P < 0.01$), respectively (Fig 1). GJ 10 μmol/L inhibited the current from (822 ± 169) to (436 ± 143) pA ($n = 6$ cells from 3 guinea pigs, $P < 0.01$) (Fig 1). The current could partly recover by perfusing with drug-free solution.

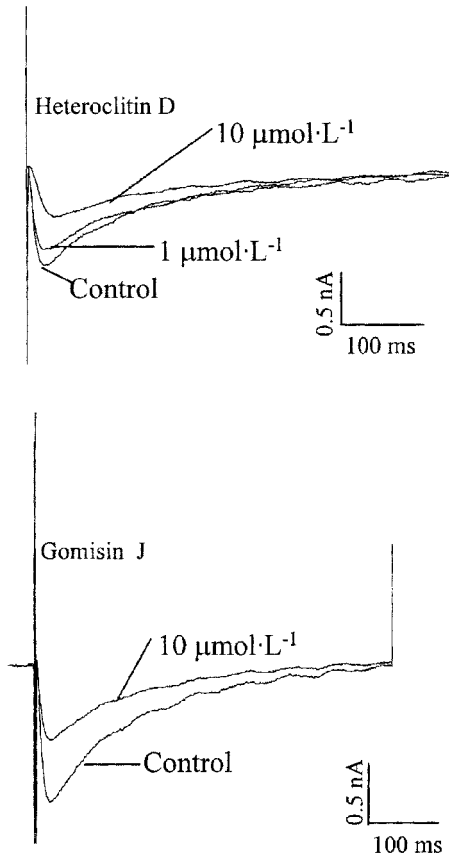


**Fig 1. Effects of heteroclitin D and gomisin J on L-type calcium current in ventricular myocytes of guinea pigs.**

**Current-voltage relationship** The membrane potential was held at −40 mV and the current-voltage relationship was obtained by depolarizing the membrane potential from −40 to +60 mV in 10 mV increment. The step pulse lasted for 500 ms. HD 1 and 10 μmol/L inhibited $I_{Ca}$ (Fig 2) without affecting the shape of the cur-

rent-voltage curves. The maximal inhibitory rates were 37.45 % and 50.05 % respectively. GJ 10 μmol/L also inhibited $I_{Ca}$ (Fig 2) and its maximal inhibitory rate was 48.00 %.

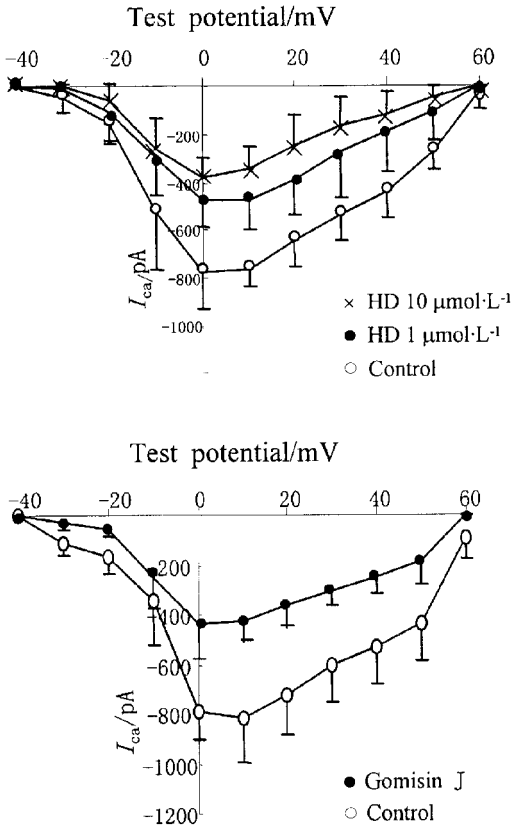


**Fig 2. Effect of heteroclitin D (HD) and gomisin J on $I-V$ relationship of L-type calcium current in myocytes. $n=6$ cells from 3 guinea pigs. $\bar{x} \pm s$.**

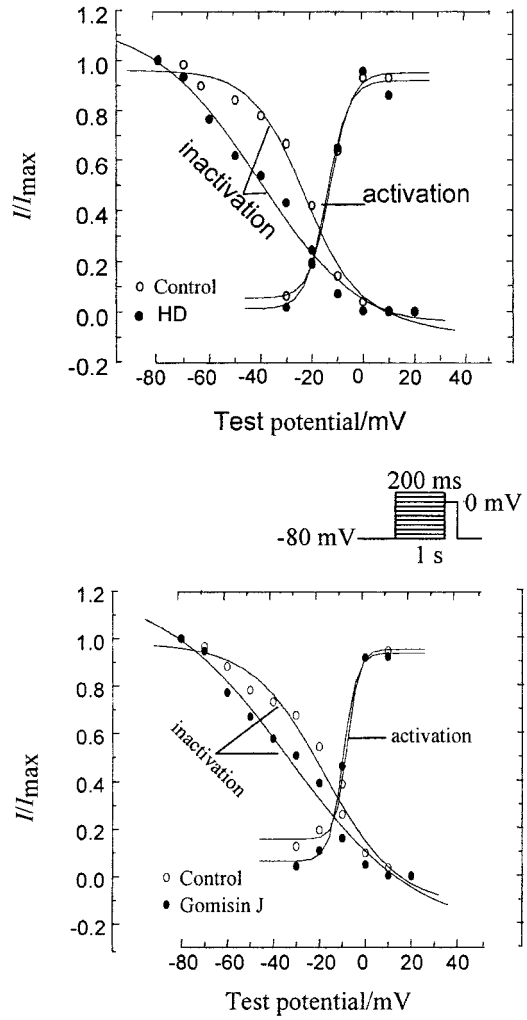


**Fig 3. Effects of HD ($n=4$ cells from 3 guinea pigs) and Gomisin J ($n=5$ cells from 3 guinea pigs) 10 μmol/L on steady-state activation and inactivation kinetics of L-type calcium current in myocytes.**

**Activation and inactivation kinetics** The protocol for inactivation curves were described as previous[12]. In brief, the holding potential was held at −80 mV and variable amplitude conditioning potentials from −80 to +20 mV in every 10 mV were followed by a test potential at 0 mV. Neither HD nor GJ affected the steady-state activation but they changed the steady-state inactivation of calcium channels. HD changed the half inactivation voltage ($V_{0.5}$) from −22.7 to −40.9 mV, slope factor ($\kappa$) from 10.2 to 20.6 ($n=4$ cells from 3 guinea pigs, $P<0.05$) (Fig 3). GJ changed the $V_{0.5}$ from −17.7 to −33.3 mV and from 15.9 to 27.8 ($n=5$ cells from 3 guinea pigs, $P<0.05$) (Fig 3).

## DISCUSSION

HD and GJ are both natural products extracted from *Kadsura* medicinal plants. Our results showed that HD 1 and 10 μmol/L blocked calcium current and GJ 10 μmol/L reduced $Ca^{2+}$ current significantly. All the results suggest that HD and GJ block L-type calcium channels which is similar to the results from isolated vessel experiments[13]. Neither HD nor GJ affected the steady-state activation, indicating that their effect on decreasing $I_{Ca}$ had no relation with activated state of calcium channels. Their effect of accelerating the inactivation of $Ca^{2+}$ channels contributed to their blocking effect, so it can be concluded that HD and GJ may have high affinity to the inactivated channels.

The "run-down" phenomenon has long been noticed in recording L-type calcium current. Generally speaking, it decreases relatively slowly within 20 − 60 min and then abruptly reduces to zero. Supplying the pipette solution with ATP[14] can double the survival time and the

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existence of GTP[15] in pipette solution also prolongs the survival time. In our experiment the low concentration drugs were added within 10 min and the high concentration drugs within 20 min. So in order to distinguish the decrease in effect caused by drugs from the "run-down" phenomenon, we compared the drug groups with controls at 10 and 20 min respectively. Only when there was significant difference, the decrease in effects of the drugs were confirmed.

The pharmacological properties of HD and GJ regarding their L-type channel blocking and anti-lipid peroxidative effect, not shared by other calcium channel blockers, may be potentially useful to treat ischemic heart disease and atherosclerosis clinically.

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## 异型南五味子丁素和戈米辛 J 对豚鼠心室肌细胞 L-型钙离子通道的阻断作用[1]

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**关键词** 异型南五味子；膜片箝技术；钙通道；脂质过氧化；心肌

**目的**：研究异型南五味子丁素(HD)和戈米辛 J (GJ)对豚鼠心肌 L-型钙离子通道的作用. **方法**：全细胞膜片箝记录. **结果**：异型南五味子丁素 1，10 μmol/L 及戈米辛 J 10 μmol/L 可抑制 L-型 $Ca^{2+}$ 电流. HD 和 GJ 对钙电流稳态激活都无影响，但它们可改变钙电流的稳态失活，提示两种药物作用于 L-型钙通道的失活态. **结论**：HD 和 GJ 对豚鼠心室肌细胞 L-型钙离子通道有阻断作用.

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