Effects of Ginkgolide B on action potential and calcium, potassium current in guinea pig ventricular myocytes[1]

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KEY WORDS *Ginkgo biloba*; patch-clamp techniques; myocardium; action potentials; potassium channels; calcium channels

ABSTRACT

AIM: To investigate the effect of Ginkgolide B (GB) on action potential (AP) , delayed rectifier potassium current (I_K), and L-type calcium current ($I_{Ca\text{-}L}$) in guinea pig ventricular myocytes. **METHODS:** Single ventricular myocytes were isolated by an enzymatic dissociation method. AP, I_K, $I_{Ca\text{-}L}$ were recorded by whole-cell patch-clamp technique in either current or voltage clamp mode. **RESULTS:** GB shortened APD in a concentration-dependent manner. GB 0.1, 1, and 10 μmol/L shortened APD_{50} by 7.9 % (n=5, P>0.05), 18.4 % (n=5, P<0.01), and 28.9 % (n=6, P<0.01), respectively; APD_{90} by 12.4 % (n=5, P>0.05), 17.6 % (n=5, P<0.01), 26.4 % (n=5, P<0.01), respectively. GB increased I_K in a concentration-dependent manner. GB 0.1, 1, and 10 μmol/L increased I_K by 20.1 % (n=6, P<0.05), 43.1 % (n=6, P<0.01), 55.6 % (n=6 , P<0.05); increased I_K tail by 10.7 % (n=6, P<0.05), 25.1 % (n=6, P<0.05), and 37.7 % (n=6, P<0.05), respectively at testing potential of +50 mV and shift the *I-V* curve of I_k upward. But GB had no significant effect on $I_{Ca\text{-}L}$ at above concentrations. **CONCLUSION:** GB significantly shortened APD in a concentration-dependent manner which mainly due to increase of I_K.

INTRODUCTION

Ginkgo biloba extract (*Gb*E) is extracted from the leaves of *Ginkgo biloba*. *Gb*E is a multicomponent drug with a polyvalent action. In Germany and France, such extracts were used effectively to treat cerebral dysfunction and peripheral circulatory disturbances[1]. The results of clinical trails support new indications for *Gb*E in the treatment of cardiovascular disease, particularly in the prevention of ischemic heart syndromes[2]. The primary active constituents of *Gb*E include ginkgoflavone glycosides (24 %) and unique diterpenes (6 %) known as ginkgolide. Many pharmacological actions of *GbE* are attributed to its ginkgolides, a diverse group of terpenoids. Ginkgolides present in the extract can be divided into types A, B, C, M, J. Ginkgolide B ($C_{20}H_{24}O_{10}$, GB) is one active component[3,4]. Previous investigations suggested that GB is a specific antagonist of platelet activating factor (PAF) receptor, and could prevent PAF-induced ischemia-like cellular damage[5]. A growing body of data suggested that GB possessed anti-ischemia and protective effect on heart, and also against ischemia-reperfusion-induced cardiac arrhythmia[6-8]. But, the electro-physiological effect of GB on cardiac myocardium was uncertain. This study sought to determine the effects of the GB on AP, I_K, and $I_{Ca\text{-}L}$ in guinea pig

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ventricular myocytes by using whole-cell patch-clamp techniques.

MATERIALS AND METHODS

Preparation of ventricular myocytes Isolation of single myocytes were prepared from ventricles of adult guinea pigs (male, n=10, 215 g±19 g) by enzymatic dissociation according to Farmer *et al* with small modifications[9]. The heart was excised quickly and placed into oxygenated calcium-free Tyrode's solution at 4 ºC. The aorta was cannulated and the heart was retrograde perfused on Langendorff apparatus at 37 ºC and at a constant rate of 9 mL/min with the following solutions: 5 min with Ca^{2+}-free Tyrode's solution, and then 4 min with the same solution to which had been added $CaCl_2$ 40 μmol/L, 0.03 % type II collagenase and 1 % bovine serum albumin (BSA). All solutions were gassed with 100 % O_2, pH was 7.2. After perfusion, the ventricles were chopped, minced and gently agitated with a pipette in low Ca^{2+} Tyrode's solution (80 μmol/L) containing 1 % BSA at 37 ºC for 10 min. Cells were filtered through a 200-μm nylon mesh and calcium concentration in Tyrode's solution was gradually increased to 1 mmol/L.

Chemicals and solutions GB, collagenase type-II, Na_2ATP, K_2ATP, *tris*-GTP, CsCl, CsOH, TEA-Cl, BSA, HEPES, egtazic acid, taurine, aspartic acid ,and 3-(*N*-morpholino)-propanesulfonic acid (MOPS) were purchased from Sigma. Other reagents were products of Shanghai Chemical Reagent Plant.

The calcium-free solution contained (in mmol/L): NaCl 100, KCl 10, NaH_2PO_4 1.2, $MgSO_4$ 5.0, glucose 20, taurine 10, MOPS 10; pH was adjusted to 7.2 with KOH. For AP recording, external solution (in mmol/L): NaCl 137, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, HEPES 10, glucose 10; pH was adjusted to 7.4 with NaOH. Pipette solution (in mmol/L): potassium aspartic acid 120, KCl 20, $MgCl_2$ 2.0, HEPES 10, egtazic acid 10, Na_2ATP 10; pH was adjusted to 7.2 with KOH. For potassium current recording, external solution (in mmol/L): NaCl 137, KCl 5.4, $MgCl_2$ 1.0, HEPES 10, glucose 10; pH was adjusted to 7.4 with NaOH. Pipette solution (in mmol/L): potassium aspartic acid 120, KCl 20, $MgCl_2$ 0.5, HEPES 10, egtazic acid 10, K_2ATP 10; pH was adjusted to 7.2 with KOH. For L-type calcium current recording, external solution (in mmol/L) : NaCl 137, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, HEPES 10, glucose 10, TTX 0.01, TEA-Cl 20; pH was adjusted to 7.4 with NaOH. Pipette solution (in mmol/L) : CsCl 140, egtazic acid 10, HEPES10, MgATP 3.0, Tris-GTP 0.4; pH was adjusted to 7.2 with CsOH 0.1 mol/L.

Whole cell patch-clamp techniques A drop of cell suspension was added to the Tyrode's solution in recording chamber (volume 1 mL) mounted on an inverted microscope (Olympus IX 70, Japan). After the cells attached the bottom, the bath was perfused at a flow rate at 2 mL/min with different external solution continuously gassed with O_2. Membrane currents and AP were recorded using whole-cell patch-clamp technique in either voltage or current clamp mode with a patch-clamp amplifier[10] (Axopatch 200B, Axon Instrument, USA). Micropipette was pulled with a vertical puller PP-830 (Narishige, Tokyo, Japan), which had a tip resistance of 2-4 MΩ, when filled with pipette solution. Ag-AgCl electrode was used as the reference electrode. After the whole cell configuration was achieved, AP was recorded in current clamp mode and membrane currents were recorded in voltage clamp mode under control condition, first till the record was stable, then the external solution containing different concentration of GB was changed and the experiments were repeated again. The experiment protocol and data acquisitions were performed with Pclamp 6.0.3 running on a personal computer. Single GB concentration was applied on each guinea pig ventricular myocyte.

Statistics Data were analyzed by paired Student's t-test. P<0.05 was considered as statistically significant difference. All data presented as mean±SD.

RESULTS

Effect of GB on AP AP in guinea pig ventricular myocytes was evoked by a step current pulse of 500 pA , 5-ms duration at the frequency of 1 Hz. GB shortened the APD in a concentration-dependent manner (Fig 1). GB 0.1, 1, and 10 μmol/L decreased APD_{50} from 364±25 to 336±23 ms (n=5, P>0.05), 348±35 to 285±33 ms (n=5, P<0.01), and(446±33 to 317±33 ms (n=5, P<0.01) , respectively; APD_{90} from 430±24 to 376±33 ms (n=5, P>0.05), 438±74 to 361±48 ms (n=5, P<0.01), 527±18 to 389±41 ms (n=5, P<0.01), respectively. RP and other parameters of AP had no significant change (Tab 1).

Effect of GB on I_K and $I_{K\,tail}$ I_K in guinea pig ventricular myocyts were recorded by a depolarizing pulse from holding potential -40 mV to +50 mV with step of 10 mV at the frequency of 0.2 Hz. The step pulse

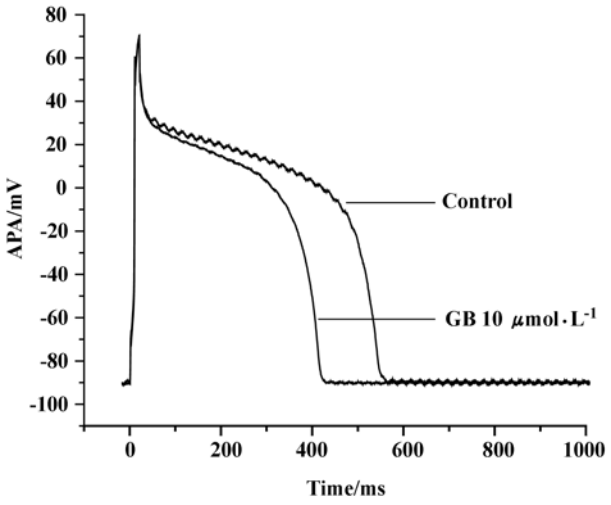

Fig 1. Effect of GB on action potential in guinea pig ventricular myocyte. APA: action potential amplitude.

Tab 1. Effect of GB on resting potential (RP), action potential amplitude(APA), APD in guinea pig ventricular myocytes. *n*=5. Mean±SD. $^{c}P<0.01$ *vs* control.

Group	RP/mV	APA/mV	APD_{50}/ms	APD_{90}/ms
Control	88±4	138±8	364±25	430±24
GB 0.1 μmol/L	89±3	133±3	336±23	376±33
Control	89±1	141±11	348±35	438±74
GB 1 μmol/L	90±2	134±6	285±33^{c}	361±48^{c}
Control	89±1	140±8	446±33	527±18
GB 10 μmol/L	89±2	138±4	317±33^{c}	389±41^{c}

duration was 5 s. Holding potential of -40 mV were used to inactivate I_{Na} and T-type Ca^{2+} current. External solution contained nifedipine 1 μmol/L and glybenclamide 10 μmol/L to block $I_{Ca\text{-}L}$ and $I_{K\text{-}ATP}$. The results showed that GB increased the density of I_K in a concentration-dependent manner (Fig 2). The current density-voltage relationship of I_K was shown in Fig 3. GB 0.1, 1, and 10 μmol/L increased I_K from (8.1±0.9) to (9.7±0.9)

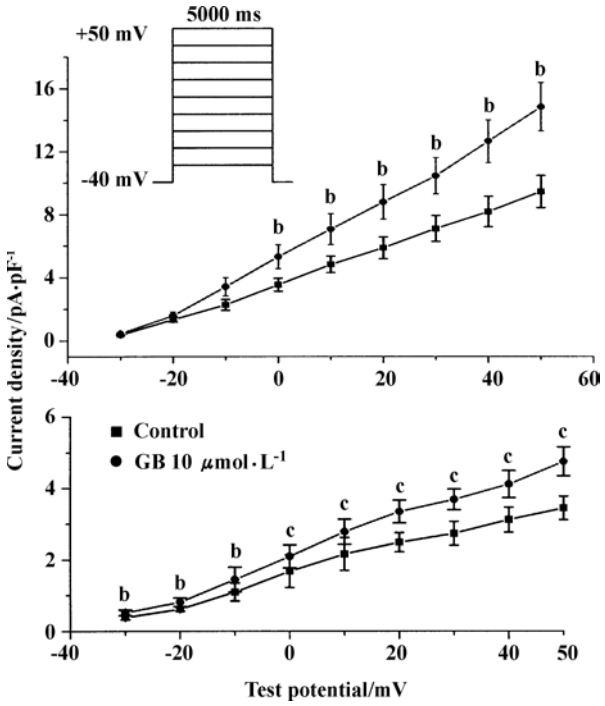

Fig 3. *I-V* relations for I_k and $I_{k\text{-}tail}$ before and after perfusion of GB. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control. Mean±SD. *n*=6.

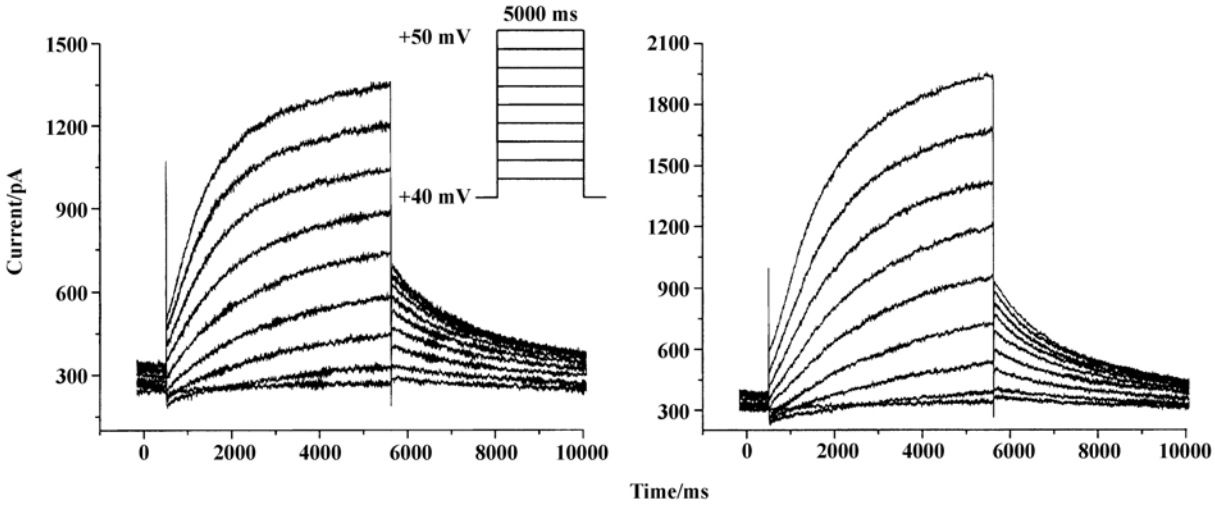

Fig 2. Effect of GB on delayed rectifier potassium current in guinea pig ventricular myocytes. A: control; B: GB 10 μmol/L.

(n=6, P<0.05), (7.5±1.1) to (10.7±1.4) (n=6, P<0.01), (9.5±1.0) to (14.8±1.5) pA/pF (n=6, P<0.05), respectively at the testing potential of +50 mV. GB also increased $I_{K\text{-tail}}$ in a concentration-dependent manner, GB 0.1, 1, and 10 μmol/L increased $I_{K\text{-tail}}$ from (3.1±0.3) to (3.4±0.3) pA/pF (n=6, P<0.01), (3.3±0.3) to (4.1±0.5) pA/pF (n=6, P<0.01), (3.5±0.8) to (4.8±1.0) pA/pF (n=6, P<0.01), respectively at the testing potential of +50 mV.

Effect of GB of $I_{Ca\text{-}L}$ $I_{Ca\text{-}L}$ was recorded when the cell depolarized from the holding potential -40 mV to 0 mV for 500 ms at the frequency of 0.5 Hz. External solution contained TEA-Cl 20 mmol/L and TTX 10 μmol/L to block I_k and I_{Na}. GB had no significant effect on $I_{Ca\text{-}L}$ at 0.1, 1, and 10 μmol/L concentration (Fig 4).

DISCUSSION

In this study, the effect of GB, a potent inhibitor of platelet activating factor, on action potential and delayed rectifier potassium current were investigated. Previous data demonstrated that GB caused the shortening of APD. The shortening of APD can be explained by both an enhancing effect on potassium currents and a reduction on calcium current. Previous study showed 4-aminopyridine could partially prevent the shorting of APD induced by PAF antagonist BN52021, glybenclamide could nearly completely prevent the strong shortening of APD induced by BN52021[11]. Those data suggest that the shortening of APD maybe related to transient outward potassium current and ATP-dependent potassium current. In the present study it was shown that GB shortened APD in a concentration-dependent manner. By using nifedipine and glybenclamide to block $I_{Ca\text{-}L}$ and $I_{K\text{-}ATP}$, it was shown that GB increased the current density of I_k and shifted the I-V curve of I_k upward also in a dose-dependent manner. On the other side, it had no effect on $I_{Ca\text{-}L}$. GB shortened APD mainly by increasing of I_k. Our results suggested that GB might be a delayed rectifier potassium channel modulator.

The delayed rectifier outward potassium current is the major outward current responsible for repolarization of cardiac action potential. In guinea pig ventricular myocytes, I_k is relatively large current compared with that measured in ventricular cell isolated from cat, rabbit or rat[12-14]. I_k recorded from guinea pig ventricular cell is characterized by a very slow onset activation after a step depolarization several seconds are required for near complete activation. Sanguinetti and Jurkiewicz[15] had identified two components of I_k in guinea pig ventricular myocytes: a rapidly activating component termed I_{kr} and a slowly activating component termed I_{ks}. In present study the I_k included I_{kr} and I_{ks} both.

A growing body of data suggested that GB possess anti-ischemia and protective effect on heart, and also against cardiac arrhythmia by ischemia-reperfusion induced. In our lab, we have observed that GB could inhibit the increase the left ventricular end diastolic pressure during ischemia-reperfusion and improved cardiac pump function after ischemia-reperfusion. Using confocal microscope, we found that GB could decrease intracellular calcium concentration and against the calcium overload reaction induced by myocardial ischemia[16]. It was reported that potassium channel opener could prevent calcium overlord in cardiac myocytes[17]. Be-

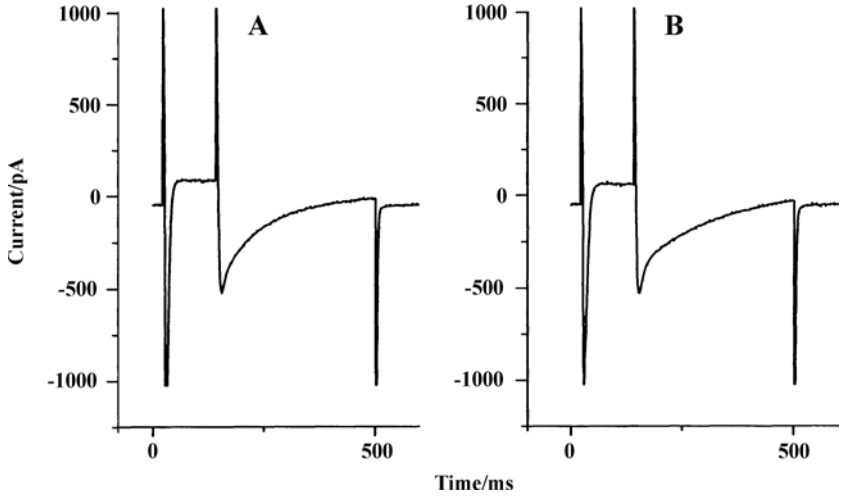

Fig 4. Effect of GB on L-type calcium current in guinea pig ventricular myocytes. A: control; B: GB 10 μmol/L.

cause potassium channel opener could decrease the intracellular free calcium release[18]. Recently potassium channel opener as nicoradil was already used clinically to improve myocardial ischemia[19]. The relationship between the potassium channel opening effect and the calcium overlord preventing effect is not clean yet. It is possible that the potassium channel opening effect is one of the mechanisms to prevent ischemia-induced calcium overlord, so that GB could against the ischemia-induced cardiac arrhythmia.

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