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Effects of ginkgo biloba extract on cation currents in rat ventricular myocytes

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Abstract

Ginkgo biloba extract (GBE), a valuable natural product for cerebral and cardiovascular diseases, is mainly composed of two classes of constituents: terpene lactones (e.g., ginkgolide A and B, bilobalide) and flavone glycosides (e.g., quercetin and kaempferol). Its electrophysiological action in heart is yet unclear. In the present study, using whole-cell patch clamp technique, we investigated electrophysiological effects of GBE on cation channel currents in ventricular myocytes isolated from rat hearts. We found that GBE 0.01–0.1% inhibited significantly the sodium current (I_{Na}), L-type calcium current (I_{Ca}) and transient outward potassium current (IK_{to}) in a concentration-dependent manner. Surprisingly, its main ingredients, ginkgolide A (GB A), ginkgolide B (GB B) and bilobalide (GB BA) at 0.1 mM did not exhibit any significant effect on these cation channel currents. These results suggested that GBE is a potent non-selective cation channel modulator in cardiaomyocytes. Other constituents (rather than GB A, GB B and GB BA) might be responsible for the observed inhibitory effects of GBE on cation channels.

Keywords: Ginkgo biloba extract; Sodium channel current; Potassium channel current; Calcium channel current; Ventricular myocytes

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Introduction

Medicinal use of ginkgo biloba can be traced back almost 5,000 years in Chinese herbal medicine. Its extract, named Ginkgo Biloba Extract (GBE), is now the most widely prescribed herbal medicine in Germany and France, for treatment of cerebral insufficiency and peripheral vascular disease (Le Bars et al., 1997). Its therapeutic effects are mainly attributed to nitric oxide mediated vasorelaxation, antagonism of platelet activating factor (PAF) receptor and antioxidant effects (Maclennan et al., 2002). It is not clear whether GBE or its constituents exert its effect by modulating ionic channels, such as calcium channels, calcium activated potassium channels, since both of them are important regulators of the tone of smooth muscle (Nelson et al., 1995; Moosmang et al., 2003). However, the electrophysiological profiles of GBE in living cells are poorly understood. Until recently, Chatterjee and his colleague (Chatterjee et al., 2003) showed that GBE and its constituents inhibited receptor-gated chloride channels in hippocampal neuronal cells. Cermak et al. (Cermak et al., 2002) found that quercetin (one of the GBE constituents) activated basolateral potassium channels in colon epithelium. More recently, Satoh group reported GBE prolonged action potential duration, inhibited the calcium channel currents, delay rectifier potassium channel currents and inward rectifier potassium current in guinea pig ventricular myocytes (Nishida and Satoh, 2003; Satoh, 2003; Satoh and Nishida, 2004). However, other study has shown no effects in cardiovascular system by GBE (Kalus et al., 2003).

To better understand the electrophysiological profiles of GBE in heart, the present study was designed to determine whether ginkgo biloba extract and ginkgolide A, B and bilobalide could influence the cation channels, using whole cell patch clamp techniques.

Materials and methods

Materials

TTX, CsCl, collagenase type II, ginkgolide A, ginkgolide B and bilobalide were purchased from Sigma Co. (St. Louis, MO, USA). All other chemicals are of high purity and commercially available. Ginkgo biloba extract (GBE) was provided by Sichuan Guangsong pharmaceutics Co. (Sichuan, China). The extract, a 50:1 concentrated extract, is manufactured according to the standard specifications, consisting of 24% ginkgo flavone glycosides and 6% terpene lactones. Sprague-Dawley (SD) rats were provided by Kunming Medical College Laboratory Animal Center.

Isolation of cardiac myocytes

Single ventricular myocytes were isolated using the method described by Mitra (Mitra and Morad, 1985). Young adult SD male rats (body weight 250–300 g) were housed and allowed free access to water and food. Animal care and studies were performed in compliance with institutional animal care and use committee guidelines. Animals were anesthetized by 60 $mg \cdot kg^{-1}$ pentobarbital sodium IP and were sacrificed by a sudden blow on the neck. The heart was rapidly explanted and transferred to cold Ca^{2+}-free solution (described below). The pericardium and part of the vasa were removed, the aorta was cannulated, and the cannula was attached to a Langendorf apparatus for retrograde intra-arterial perfusion of the heart with the aforementioned oxygenated (5% CO_2 and 95% O_2) Ca^{2+}-free solution (37 °C) for approximately 5