Introduction

The extract from the leaves of *Ginkgo biloba* (GBE) plays a role in cellular physiological and pharmacological functions. GBE is used for treating impaired brain functions in elderly and peripheral arterial occlusive diseases (1,2). Pharmacological experiments have recently demonstrated that GBE (i) increases cerebral blood flow, decreases viscosity and antagonizes platelet activating factor receptors (3,4); (ii) increases tolerance for anoxia by preventing the decrease in ATP level (5); (iii) improves neurotransmitter disturbances (6,7); and (iv) prevents cell damage induced by free radicals (8–10).

Effects of the constituents from *Ginkgo biloba* extract (GBE) on the action potentials and the ionic currents in guinea pig ventricular cardiomyocytes were investigated using whole-cell and current-clamp techniques. The constituents, ginkgolides A, B, C and quercetin, had depressant effects at 0.1–3 μM on the action potential configuration. Ginkgolide A (1–3 μM) prolonged the action potential (action potential duration: APD) at 75% and 90% repolarizations (APD75 and APD90). However, ginkgolides B and C at low concentrations prolonged APD, but at higher concentrations (>1 μM) shortened APD. Quercetin at 3 μM prolonged the APD, but not at the lower concentrations. These constituents also inhibited the Vmax. The resting potential was unaffected. In voltage-clamp experiments, ginkgolides A and B (0.1–3 μM) markedly and concentration-dependently increased the Ca2+ current (ICa) and the delayed rectifier K+ current (IK), and decreased the inwardly rectifying K+ current (IK1). On the other hand, ginkgolide C failed to affect the ICa, but increased the IK by 14.0 ± 2.3% (n = 6, P < 0.05) at 1 μM. Quercetin inhibited ICa, and enhanced IK but decreased IK1. These responses to the constituents were almost reversible (80–90% of control) after a 10- to 20-min washout. These results indicate that even at acute administrations, these constituents produce the effective actions on the APD and the underlying ionic currents in cardiomyocytes. Each constituent does not exhibit a uniform response, although GBE acts as a net.

Keywords: action potential – ionic currents – ginkgolide A – ginkgolide B – ginkgolide C – *Ginkgo biloba* extract – guinea pig ventricular cardiomyocytes – quercetin

Original Article

Comparative Electropharmacological Actions of Some Constituents from *Ginkgo biloba* Extract in Guinea-pig Ventricular Cardiomyocytes

Hiroyasu Satoh

Department of Pharmacology, Division of Molecular and Cellular Biology, Nara Medical University, School of Medicine, Japan

GBE contains many chemical constituents. The major constituents are terpenes such as bilobalide, ginkgolide A, ginkgolide B and ginkgolide C, and flavonoids such as quercetin, kaempferol and isorhamnetin (6,11). In the cardiovascular system, GBE does not affect heart rate and contractility, but produces a concentration-dependent increase in coronary flow (12,13). In our previous experiments, however, we have found that GBE prolongs the action potential duration (APD), whereas bilobalide, a main constituent, shortens the APD in cardiomyocytes (14). The alterations of the action potential configurations clinically would lead to the pathophysiological changes in the electrocardiogram (ECG). In addition, Tamago et al. (15) have shown that ginkgolide B is a PAF-antagonist and acts on the APD, consistent with our results.

The mechanisms of the therapeutic effectiveness of GBE are expected to be extremely complex, because of the large number of its chemical constituents. The present study was conducted to investigate the electropharmacological actions of some major constituents of GBE.

For reprints and all correspondence: Hiroyasu Satoh, Department of Pharmacology, Division of Molecular and Cellular Biology, Nara Medical University, School of Medicine, Kashihara, Nara 634-8521, Japan. Tel: +81-744-29-8831; Fax: +81-744-25-7657; E-mail: hysat@naramed-u.ac.jp

The online version of this article has been published under an open access model. Users are entitled to use, reproduce, disseminate, or display the open access version of this article provided that: the original authorship is properly and fully attributed; the Journal and Oxford University Press are attributed as the original place of publication with the correct citation details given; if an article is subsequently reproduced or disseminated not in its entirety but only in part or as a derivative work this must be clearly indicated.

© 2004, the authors

*Evidence-based Complementary and Alternative Medicine, Vol. 1, Issue 3 © Oxford University Press 2004; all rights reserved*
number of its constituent substances (6,8). Unknown mecha-

nisms of these constituents for the ionic channel currents still

remain. The aim of the present experiments was to examine,

by use of a patch-clamp technique, how these constituents of

GBE, other than bilobalide, affect the action potentials and the

underlying ionic currents. In addition, the cardiac electrophar-

macological actions of each constituent were compared.

Materials and Methods

All experiments were carried out according to the guidelines

laid down by the Nara Medical University Animal Welfare com-

mittee, and also under the terms of the Declaration of Helsinki.

Cell Preparation

Cells were prepared from tissue taken from the ventricle muscle

guinea pig hearts, using methods similar to those described

previously (14,16). Under sodium pentobarbital (30 mg/kg, i.p.)
anesthesia, the chest was opened and the aorta was cannulated

in situ. The heart was dissected out and perfused with normal

Tyrode solution on the Langendorff apparatus. After a washout

of blood, the heart was perfused with Ca2+

free Tyrode solution,

and spontaneous beating ceased. Then, the perfusate was

switched to low-Ca2+ (30–60 μM) Tyrode solution containing

0.4 mg/ml collagenase (Type I, Sigma Chemical, St Louis, MO)

for about 20 min. The heart was washed out by high-K

solution (KB solution), and was dissected with scissors.

The temperature of all solutions was maintained at 36°C.

Current- and Voltage-clamp Experiments

Current-clamp and whole-cell voltage-clamp recordings were

performed using an Axopatch patch-clamp amplifier (Axon

Instruments, Burlingame, CA) and standard techniques. Patch

pipettes from borosilicate glass capillaries were fabricated

using a two-stage puller, and had a resistance of 5–7 MΩ.

The series resistance error was <3–7 mV, and no compensation

was used. The liquid junction potential between the pipette solution

and the external solution (<10 mV) was corrected for all mem-

brane potential recordings. Experiments were carried out at a

temperature of 36.5°C. The data were stored and analyzed on

an IBM-AT microcomputer, using the PCLAMP analysis pro-

gram (Axon Instruments). Current traces were filtered using a

cut-off frequency of 1 kHz for plotting. The Ica was measured

as the difference between the peak current and the zero current,

and the IK1 was the difference between the current at the end of

a 1-s test pulse and the zero current. The IK was measured at the

peak of the outward tail current. All values are given as

mean ± S.E.M. The differences of the mean values were ana-

lyzed by Student’s t-test and ANOVA for paired data, and a

P-value of <0.05 was considered significant.

Experimental Solutions

The composition of the modified Tyrode solution was:

137 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 1.0 mM MgCl2,

0.3 mM NaH2PO4, 5.0 mM glucose and 5.0 mM HEPES.

The pH was adjusted to 7.4 with NaOH. The constituents of

Ginkgo biloba extract, ginkgolides A, B and C, and quercetin

(Tokiwa Phytochemical Co., Tokyo, Japan) were dissolved

with DMSO. Bath solutions with the desired concentrations

were made and superfused. The pipette solution (intracellular)

contained: 110 mM K-aspartate, 20 mM KCl, 1 mM MgCl2,

10 mM EGTA, 5 mM Mg-ATP, 5 mM creatine phosphate and

5 mM HEPES (pH 7.2). The pipette solution for the measure-

ment of Ica alone contained: 110 mM CsOH, 20 mM CsCl,

2 mM MgCl2, 10 mM EGTA, 5 mM MgATP, 5 mM creatine

phosphate, 100 mM aspartic acid and 5 mM HEPES (pH 7.2).

Results

Gingko biloba Effects on the Action Potentials

To examine the effects of each constituent on the action

potential configuration, a current-clamp experiment was

carried out. The isolated single cell was stimulated at 1 Hz. As

shown in Fig. 1A, ginkgolide A (at >1 μM) markedly

prolonged the APD; at 3 μM, 75% and 90% repolarizations of

APD (APD 75 and APD 90) were

43.8 ± 3.6% (n = 8, 278

Electropharmacology of constituents of GBE

Figure 1. Modulation of the action potentials by the constituents of Ginkgo biloba extract in guinea pig ventricular cardiomyocytes. (A) Effects of ginkgolide A on the action potentials. (B) Action potentials in ginkgolide B. (C) Action potentials in ginkgolide C. (D) Quercetin on action potentials. Each constituent from 0.1–3 μM was cumulatively added to bath solution. Horizontal lines indicate 0 mV. Symbols used are control (C) and washout (W).
decreased by ~18–20% (n = 8, P < 0.001), respectively (Fig. 2A). Ginkgolide B at low concentrations (0.1–0.3 μM) markedly prolonged APD75 and APD90, but at high concentrations (1–3 μM) it decreased both of the APDs (although the APDs were still enhanced in comparison with the control) (Fig. 1B). The APD75 and APD90 at 0.3 μM were +63.6 ± 4.8% (n = 9, P < 0.001) and +58.3 ± 3.6% (n = 6, P < 0.001), respectively, and at 3 μM were +16.2 ± 3.9% (n = 9, P < 0.05) and +15.0 ± 3.7% (n = 9, P < 0.05), respectively (Fig. 2B).

On the other hand, ginkgolide C at low concentrations (0.3 μM) prolonged APD, whereas at high concentrations (1–3 μM) it markedly shortened APD (Fig. 1C). The APD75 and APD90 at 0.3 μM were altered by +15.5 ± 3.7% (n = 8, P < 0.05) and +9.9 ± 2.6% (n = 8, P < 0.05), respectively, and at 3 μM they were altered by −38.5 ± 2.8% (n = 8, P < 0.001) and −37.2 ± 3.5% (n = 8, P < 0.01), respectively (Fig. 2C). Quercetin at 0.1–1 μM tended to prolong the APD, and at 3 μM prolonged the APD75 by +11.9 ± 3.4% (n = 8, P < 0.05) and APD90 by +17.9 ± 3.0% (n = 8, P < 0.05) (Figs 1D and 2D).

The maximum rate of depolarization (Vmax) significantly decreased by ~18–20% (n = 8–9) at higher concentrations (1–3 μM) of these constituents. Other action potential parameters were also unaffected in the presence of all the constituents. The modulation of the action potential configurations by the constituents is summarized in Fig. 3. A washout of the drugs for 15–20 min recovered to ~70–80% of the control value.

Constituents of *Gingko biloba* and Quercetin Exert Contrasting Effects on Ionic Currents

Whole-cell patch voltage-clamp experiments were performed to examine the effects of the constituents on the underlying ionic currents. Test pulses (1 s duration) were applied to −20 to 60 mV and −40 to −120 mV from a holding potential of −30 mV. The average capacitance was 86.1 ± 2.0 pF (n = 44).

As shown in Fig. 4, ginkgolide A (1 μM) enhanced both the Ca2+ current (ICa, by 68.2 ± 2.8% (n = 7, P < 0.001), and the delayed rectifier K+ current (IK) by 66.3 ± 3.8% (n = 7, P < 0.001). Each constituent (0.1–3 μM) was cumulatively administered to the bath solution. Ginkgolide B at 0.3 μM also increased the ICa at 10 mV by 58.1 ± 2.8% (n = 6, P < 0.001) (Fig. 5). Increasing the concentrations (1–3 μM) still enhanced the ICa, but the maximal response at 0.3 μM decreased. The IK at 60 mV increased by 89.3 ± 3.8% (n = 6, P < 0.001) at 3 μM, but the inwardly rectifying K+ current (IK1) was not affected significantly. Ginkgolide C had a smaller or no effect on the IK, but markedly increased the IK1 by 59.2 ± 2.8% (n = 7, P < 0.001) at 3 μM (Fig. 6). On the other hand, the application of quercetin (0.1–3 μM) inhibited the ICa (Fig. 7A and B). The ICa at 10 mV decreased by 34.9 ± 3.2% (n = 8, P < 0.05) at 0.3 μM and by 56.8 ± 3.3% (n = 8, P < 0.05) at 3 μM. The responses were produced in a concentration-dependent manner. Simultaneously, the IK at 60 mV increased by 60.4 ± 2.7% (n = 8, P < 0.001) at 0.3 μM and by 89.7 ± 3.3% (n = 8, P < 0.001) at 3 μM. Quercetin simultaneously did not affect the IK, but markedly increased the IK1 by 12.4 ± 2.1% (n = 8, P < 0.05). The responses to the constituents for the ICa and IK currents are summarized in Fig. 8. The responses to all the constituents were recovered to ~80–90% of the control value after a 20-min washout.

**Discussion**

The present experiments showed that: (i) ginkgolide A prolonged the APD, but ginkgolides B and C shortened APD; (ii) quercetin prolonged the APD; (iii) the constituents also inhibited the Vmax and the RP was unaffected; (iv) ginkgolides A and B increased ICa and IK, and decreased IK1; (v) ginkgolide C failed to affect the ICa but increased the IK; (vi) quercetin inhibited ICa, and enhanced IK but decreased IK1; and (vii) these responses were almost reversible after a washout. These results indicate that acute administrations of these constituents can produce the effective actions on cardiomyocytes. Each constituent never exhibits a uniform response.

In recent clinical and experimental experiments, GBE has been found to be effective against ischemic brain injury (11,17).
Figure 3. Summary of the effects on action potential parameters. (A) Ginkgolide A, (B) ginkgolide B, (C) ginkgolide C, (D) quercetin. APA, action potential amplitude; MDP, maximum diastolic potential; APD 75, 75% repolarization of the action potential duration; APD 90, 90% repolarization of the action potential duration; V max, maximum rate of depolarization. Values are represented as mean ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.001, with respect to control value.

Figure 4. Modulation by ginkgolide A of the ionic currents. (A) Current traces in control. (B) Current traces in ginkgolide A (3 μM). Test pulses for 1 s were applied to −20 to 60 mV and −40 to −120 mV from a holding potential of −30 mV. Horizontal line before the current traces indicates zero current level. (C) Current−voltage relationship for Ca 2+ current. (D) Current−voltage relationship for the delayed rectifier K+ current and the inwardly rectifying K+ current. Symbols used are control (open circles), 0.3 μM (triangles) and 1 μM (squares) of ginkgolide A, and washout (filled circles).
and cerebral disorders due to aging (18). The mechanisms of the beneficial effects of GBE are considered to be improvements in: (i) hemodynamic disorders (6,7,19); (ii) PAF-associated abnormalities (3,4,20,21); (iii) cell damage induced by free radicals (8–10); and (iv) a decrease in ATP level during anoxia (5). In fact, these effects have recently been recognized clinically by several double-blind studies with GBE versus placebo (22–25).

**APD Regulation**

In experiments for the cardiovascular system, GBE does not affect heart rate and contractility, but produces a concentration-dependent increase in coronary flow (12,13). In our laboratory, however, GBE caused a negative chronotropic effect in rabbit SA nodal cells (unpublished data). Also, GBE inhibited $I_{Ca}$ and $I_K$, but bilobalide (a constituent) increased them in guinea pig ventricular cardiomyocytes (14). Simultaneously, GBE prolonged the APD, whereas bilobalide shortened it. In the present experiments, a major action of the constituents was the alteration of APD involved with the effects on the ionic channels. The APD means a period for the repolarization of the membrane, making T wave on ECG. Thus, the APD of cardiac myocytes is clinically reflected directly to the QT interval. The QT interval is a reflection of the repolarization of action potential, which is mainly responsible for the modulation of $I_K$. The APD prolongation increases the refractory period and simultaneously elevates the cellular Ca$^{2+}$ concentration (26–28). Therefore, the constituents would play an important role as ionic channel modulators of

---

**Figure 5.** Modulation by ginkgolide B of the ionic currents. (A) Current traces in control. Test pulses for 1 s were applied to −20 to 60 mV and −40 to −120 mV from a holding potential of −30 mV. (B) Current traces in ginkgolide B (0.3 μM). Test pulses for 1 s were applied to −20 to 60 mV. Holding potential was −30 mV. (C) Current traces at 3 μM. Test pulses for 1 s were applied to −20 to 60 mV and −40 to −120 mV from a holding potential of −30 mV. Horizontal line before the current traces indicates zero current level. (D) Current–voltage relationship for Ca$^{2+}$ current. (E) Current–voltage relationship for the delayed rectifier K$^+$ current and the inwardly rectifying K$^+$ current. Symbols used are control (open circles), 0.1 μM (triangles), 0.3 μM (squares) and 1 μM (filled circles) of ginkgolide B.
Figure 6. Modulation by ginkgolide C of the ionic currents in guinea pig ventricular cardiomyocytes. (A) Current traces in control. (B) Current traces in ginkgolide C (3 µM). Test pulses for 1 s were applied to −20 to 60 mV and −40 to −120 mV from a holding potential of −30 mV. Horizontal line before the current traces indicates zero current level. (C) Current–voltage relationship for the Ca²⁺ current. (D) Current–voltage relationship for the delayed rectifier K⁺ current and the inwardly rectifying K⁺ current. Symbols used are control (open circles), 0.3 µM (triangles) and 3 µM (squares) of ginkgolide C.

Figure 7. Modulation by quercetin of the ionic currents in guinea pig ventricular cardiomyocytes. (A) Current traces in control. (B) Current traces in quercetin (3 µM). Test pulses for 1 s were applied to −20 to 60 mV and −40 to −120 mV. Holding potential was −30 mV. Horizontal line before the current traces indicates zero current level. (C) Current–voltage relationship for Ca²⁺ current. (D) Current–voltage relationship for the delayed rectifier K⁺ current and the inwardly rectifying K⁺ current. Symbols used are control (open circles), 0.3 µM (triangles) and 3 µM (squares) of quercetin.
cadiomyocytes, although the effects of each constituent on the APD and the ionic currents were not exhibited in a uniform direction. Quercetin, a kind of flavonoid, had a smaller or no effect on the action potentials, but caused the APD prolongation only at high concentrations. Also, quercetin inhibited ICa and enhanced IK. Quercetin has been reported to possess many pharmacological effects (29,30); the modification of eicosanoid synthesis, prevention of platelet aggregation, and vasorelaxation due to the inhibition of PK-C.

Modulation of the Ionic Currents

Furthermore, these constituents also acted on the V_max, although they did not affect other action potential parameters. In general, the V_max is used as an activator of the Na^+ channel current (I_Na). Thus, the constituents have an inhibitory action on the I_Na, resulting in an inhibition of the conduction velocity and a suppression of excitability. In addition, the constituents simultaneously modulated the ionic channel currents such as I_Ca, I_K, I_K1, and I_Na. These effects might cause antiarrhythmic actions. In all the constituents, I_K enhancement was finally produced, indicative of a cell protection due to an APD shortening and a decline of cellular Ca^{2+} concentration.

The channel activity of single myocytes might be caused a run-down, especially Ca^{2+} channel and rapidly activating K^+ channel. The cells not causing run-down were chosen and used for the experiments. However, there may be a limitation to patch-clamp experiments. The responses to the constituents were considered to be due to the effects of constituents, because of the reversible response. Therefore, these constituents would exert many helpful and protective actions upon cardiac cells.

Clinical Uses and Summary

GBE can be administered to patients with mild to moderate symptoms of cerebral insufficiency (1,2). The half-life of GBE is 2–3 h (11). In pharmacokinetic analysis, the bioavailabilities of ginkgolides A and B are practically high, but that of ginkgolide C is very low (31). Of the GBE-containing flavonoids, quercetin has the highest percentage (8.91%). With regard to the distribution of radioactivity in the cardiovascular system, the tissues in areas such as vein, heart and aorta are relatively higher 3–7 h after oral administration (32). Quercetin possesses a vasodilating action (33,34). Also, the histamine-induced contraction of isolated guinea pig intestine was inhibited by a mixture of flavonoids (35). In our laboratory, GBE as a mixture exhibited a potent vasodilatory action (34). Therefore, these responses are most likely produced by the modulation of ion channel currents that have been demonstrated in this study.

It has been reported that a single dose of GBE does not produce potent pharmacological activities, and repeated doses are needed to produce beneficial effects over a long period of administration (36–38). In the present in vitro experiments, however, acutely single administrations produced marked actions on the ion channel currents and the action potentials. Since GBE is a mixture of its constituents, the total response to GBE would be a result of complex interactions with the constituents. Therefore, the constituents from GBE would play an important role in modulating the strong APD prolongation by interacting with each other. Although relatively higher concentrations were used in this study, because the pharmacological effects on the ion channels were so remarkable, GBE and its constituents would be helpful in the treatment of cerebral disorders due to their effects on central nervous system (CNS) neurons. Further experiments are needed to elucidate the detailed mechanisms of the cardiac actions of Ginkgo biloba.

Acknowledgements

The author wishes to thank Tokiwa Phytochemical Co. Ltd. for providing the constituents of Ginkgo biloba extract (ginkgolides A, B and C, quercetin).

References


Received July 27, 2004; accepted September 16, 2004