J. Pharm. Pharmacol. 1988, 40: 838-843
Received February 22, 1988

Effects of Various Ginseng Saponins on 5-Hydroxytryptamine Release and Aggregation in Human Platelets

YOSHIYUKI KIMURA, HIROMICHI OKUDA AND SHIGERU ARICHI

Second Department of Medical Biochemistry, School of Medicine, Ehime University, Shigenobu-cho, Onsen-gun, Ehime 791-02, Japan, and The Research Institute of Oriental Medicine, Kinki University, Osakasayama, Osaka 589, Japan

Abstract—The effects of various ginseng saponins isolated from red ginseng roots, on aggregation and 5-hydroxytryptamine release (5-HT) human platelets have been investigated. Among the six saponins tested, only ginsenoside Rg_1 inhibited adrenaline- and thrombin-induced platelet aggregation and 5-HT release dose-dependently, at concentrations of 5 to 500 $\mu g\ mL^{-1}$. Ginsenoside Rg_1 had no effect on adrenaline- and thrombin-induced arachidonic acid release and diacylglycerol production. But it did reduce the elevation of cytosolic free calcium concentration $(Ca^{2+})i$ shown in the second phase induced by adrenaline and thrombin, at concentrations of 10 to 500 $\mu g\ mL^{-1}$. Those data suggest that the inhibitory effects of ginsenoside Rg_1 on 5-HT release from, and aggregation of, platelets might be due to the reduction of $(Ca^{2+})i$ elevation at the second phase induced by adrenaline and thrombin. The results suggest that ginsenoside Rg_1 in red ginseng roots may be active as a drug in the treatment of artheroscleorosis and thrombosis.

The roots of red ginseng (*Panax ginseng* C. A. Meyer) have long been used for the treatment of various diseases including diabetes mellitus, thrombosis, hyperlipemia and arteriosclerosis. The chemical structures of ginseng saponins including red ginseng roots have been investigated by Shibata et al (Nagai et al 1971; Sanada et al 1974a,b; Sanada & Shoji 1978; Yahara et al 1979). The extract of red ginseng roots has been reported to prevent the decrease of blood platelet and serum fibrinogen, and to increase serum fibrin degradation products (FDP) in disseminated intravascular coagulation (DIC)-rats induced by endotoxin (Kubo et al 1982). Furthermore, ginsenoside Rg_1 (1 mM) has been found to inhibit the formation of thromboxane B_2, but it did not inhibit the formation of prostacyclin induced by ADP in a reaction mixture of platelets and aorta rings (Sekiya & Okuda 1982). When platelets are activated by various stimuli, such as, adrenaline, thrombin, and ADP, aggregation, 5-HT release, arachidonic acid release, diacylglycerol production and an elevation of $(Ca^{2+})i$ are caused (Imai et al 1982; Kajikawa et al 1983; Connolly & Limbird 1983; Sweatt et al 1985; 1986).

The present investigation describes the effects of various ginseng saponins on 5-HT release and aggregation induced by adrenaline and thrombin in washed human platelets. Furthermore, the $(Ca^{2+})i$ movements, arachidonic acid release and diacylglycerol production in the cells have been investigated to clarify the mechanisms of actions of ginseng saponins on platelet aggregation and 5-HT release in human washed platelets.

Materials and Methods

[5,6,8,9,11,12,14,15-^{3}H] Arachidonic acid (100·0 Ci $mmol^{-1}$) and [1,2-^{3}H] 5-hydroxytryptamine (5-HT) binoxalate (29·7 Ci $mmol^{-1}$) were purchased from New England Nuclear. Bovine serum albumin (BSA, fraction V) was obtained from Wako Pure Chemical Co. (Tokyo, Japan) and adrenaline from Daiichi Pharm. Co. (Tokyo, Japan). Bovine fibrinogen, human thrombin and imipramine were purchased from Sigma Chemical Co. (USA). Fura 2-acetoxymethyl ester (fura 2-AM) as a calcium indicator was obtained from Dojindo Lab. Precoated silica gel 60 TLC plastic sheets were from Merck Co. (Germany). Other chemicals were of reagent grade. Ginseng saponins (ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_2) were isolated from the red ginseng root by the procedure of Shibata et al (Nagai et al 1971; Sanada et al 1974a,b; Yahara et al 1979) (Fig. 1).

Correspondence to: Y. Kimura, Second Department of Medical Biochemistry, School of Medicine, Ehime University, Shigenobu-cho, Onsen-gun, Ehime 791-02, Japan.

Preparation of human platelets

Human platelets were obtained from male donors who had not taken aspirin for 10 days. Whole blood obtained by venipuncture was collected in tubes with 1/10 volume of sodium citrate (3·8%, w/v) as the anticoagulant. Platelet-rich plasma (PRP) was isolated from the other blood components by centrifugation for 15 min at 150 *g*. The PRP fraction was further centrifuged at 1500 *g* for 10 min at 4°C. The isolated platelets were washed twice with Tyrode-Hepes solution (mM) NaCl 137, KCl 2·7, $MgCl_2$ 1·0, NaH_2PO_4 0·4, $NaHCO_3$ 11·9, glucose 5·5, Hepes 25) containing 0·35% bovine serum albumin and 2 mM EDTA (pH 7·4) and then suspended in the same medium containing 0·35% bovine serum albumin and 1 mM $CaCl_2$.

Measurement of human platelet aggregation induced by adrenaline and thrombin

Human platelet suspensions (2×10^8 cells) were added to cuvettes in a NBS HEMA Aggregometer (Niko Bioscience, Inc.) and warmed to 37°C with stirring at 1000 rev min^{-1}. Fibrinogen, at a final concentration of 0·3 mg mL^{-1}, was also added as it was required for the adrenaline-induced aggregation (having been removed by the platelet isolation procedure). Platelets were preincubated with the indicated amounts of various ginseng saponins for 2 min at 37°C, and

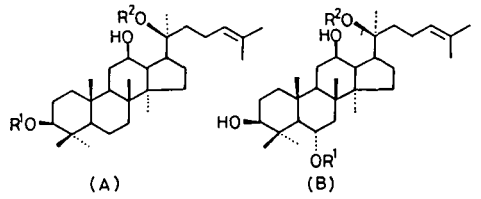

Fig. 1. The structures of various ginseng saponins.
(A) 20(S)-Protopanaxadiol: $R^1=R^2=H$: Ginsenoside Rb_1: R^1=glucose 2—1 glucose, R^2=glucose 6—1 glucose; ginsenoside Rb_2: R^1=glucose 2—1 glucose, R^2=glucose 6—1 arabinose(pry); ginsenoside Rc: R^1=glucose 2—1 glucose, R^2=glucose 6—1 arabinose(fur); ginsenoside Rd: R^1=glucose 2—1 glucose, R^2=glucose.
(B) 20(S)-Protopanaxatriol: $R^1=R^2=H$: Ginsenoside Re: R^1=glucose 2—1 rhamnose, R^2=glucose; ginsenoside Rg_1: $R^1=R^2$=glucose

then adrenaline 10 μM, or thrombin 0·1 u mL^{-1}, was added to the cuvette, and aggregation was monitored for 5 min. Platelet aggregation was measured as an increase in light transmission through the sample.

Measurement of [^{3}H]5-HT release from human platelets

To measure platelet [^{3}H]5-HT secretion, platelet-rich plasma was incubated for 30 min at 32°C with [^{3}H]5-HT at a final concentration of 7 nM. After the incubation period, the platelets were washed twice with the Tyrode-Hepes solution containing 0·35% bovine serum albumin and 2 mM EDTA (pH 7·4). In experiments in which release of [^{3}H]5-HT was to be measured, imipramine 10μM was included in the suspending medium (Tyrode-Hepes solution containing 0·35% bovine serum albumin and 1 mM $CaCl_2$) to prevent reuptake of [^{3}H]5-HT. Human platelets (2×10^8 cells) were preincubated with the indicated amounts of ginsenoside Rg_1 for 2 min at 37°C. Then, thrombin (0·1 u mL^{-1}) was added in a final volume of 0·5 mL and the mixture was incubated for 1 min and 2 min at 37°C with stirring. To measure adrenaline induced [^{3}H]5-HT release, human platelets (2×10^8 cells) were preincubated with the indicated amounts of ginsenoside Rg_1 for 2 min at 37°C and further incubated with the fibrinogen (0·3 mg mL^{-1}) for 30 s. Then, adrenaline 10 μM was added and the mixture incubated for 1 and 2 min in a final volume of 0·5 mL. The reaction mixture cooled to 4°C immediately, was mixed with formaldehyde (50 μL, final concn 1·5%) to stop the release reaction and to prevent any reuptake of [^{3}H]5-HT (Costa & Murphy 1975). After centrifugation for 10 min at 1250g and 4°C, aliquots (200 μL) of the clear platelet-free supernatants were counted in a Packard Liquid Scintillation counter. Total [^{3}H]5-HT contents in the cell suspension were determined in the presence of 0·1% Triton X-100.

Measurement of arachidonic acid release

Arachidonic acid labelling of platelets was accomplished by incubating platelet-rich plasma with 1 μCi mL^{-1} of [^{3}H]ε rachidonic acid for 30 min at 37°C. The platelets were resuspended to a final concentration of $4\cdot3\times10^8$ cells mL^{-1}. To measure adrenaline-induced [^{3}H] arachidonic acid release, human platelets (2×10^8 cells) were preincubated with the indicated amounts of ginsenoside Rg_1 for 2 min at 37°C and further incubated with the fibrinogen (0·3 mg mL) for 30 s. Then, 10 μM adrenaline was added and the mixture incubated for 2 min in a final volume of 0·5 mL with stirring at 1000 rev min^{-1}. The reaction mixture were terminated by adding 0·5 M formic acid, and centrifuging for 10 min at 4°C and 1250 g. Then a 450 μL aliquot of the supernatant was poured into 4 mL of ethylacetate and extracted. The ethyl acetate layer was removed under a nitrogen gas stream. The residue was dissolved in a small amount of ethyl acetate (40 μL), applied to precoated silica gel 60 TLC and developed

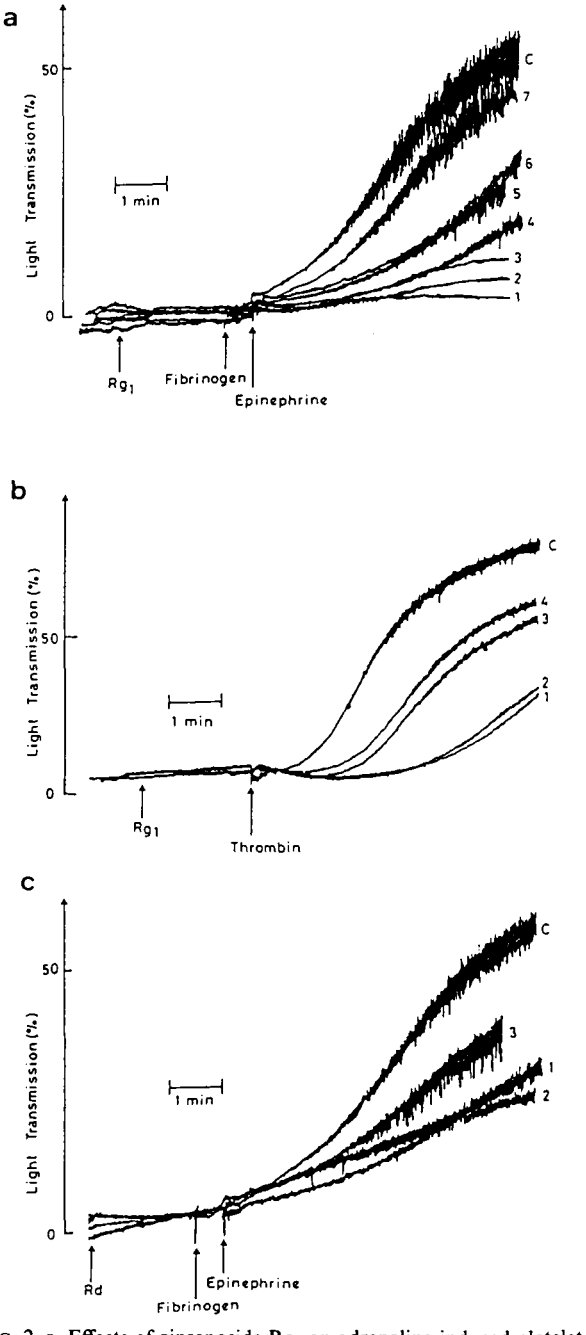

FIG. 2. a. Effects of ginsenoside Rg_1 on adrenaline-induced platelet aggregation. C, fibrinogen (0·3 mg mL^{-1}) adrenaline(10 μM); 1, ginsenoside Rg_1 500; 2, Rg_1 250; 3, Rg_1 100; 4, Rg_1 50; 5, Rg_1 25; 6, Rg_1 10; 7, Rg_1 5 μg mL^{-1}.
b. Effects of ginsenoside Rg_1 on thrombin-induced platelet aggregation. C, thrombin (0·1 u mL^{-1}) alone; 1, ginsenoside Rg_1 500; 2, Rg_1 100; 3, Rg_1 10; 4, Rg_1 5 μg mL^{-1}.
c. Effects of ginsenoside Rd on adrenaline-induced platelet aggregation. C, fibrinogen (0·3 mg mL^{-1}) plus adrenaline (10 μM); 1, ginsenoside Rd 500; 2, Rd 250; 3, Rd 100 μg mL^{-1}.

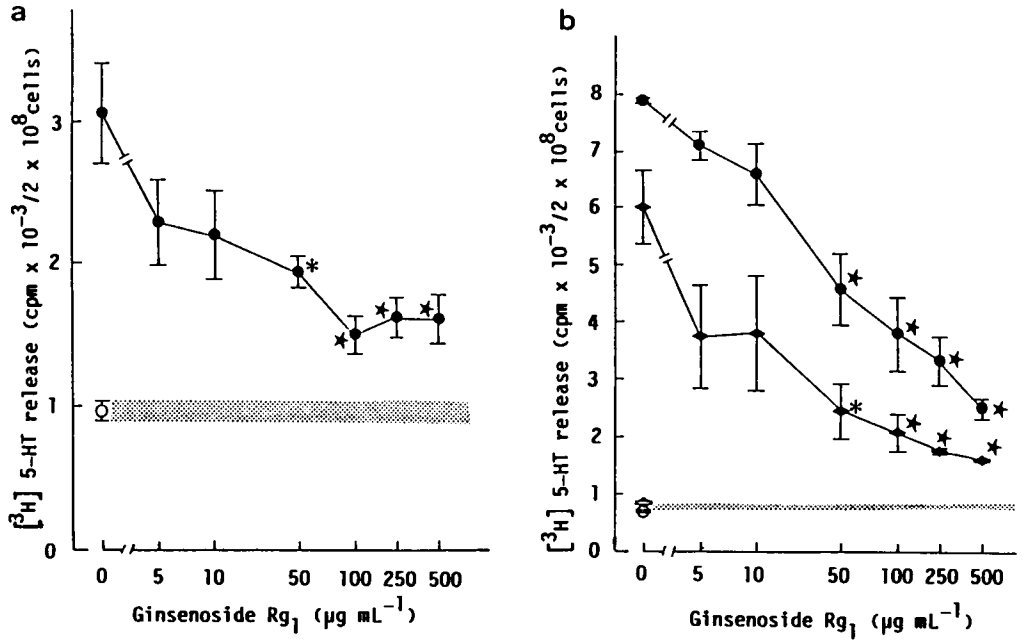

FIG. 3. a. Effects of ginsenoside Rg_1 on adrenaline-induced [^{3}H]5HT release from human platelets. The incubation time was 2 min at 37°C with stirring at 1000 rev min^{-1}. The other conditions were described in "Materials and Methods" section. ○, fibrinogen(0·3 mg mL^{-1}) alone; ●, fibrinogen plus 10 μM adrenaline. Values are means ± s.e. for 4 experiments. Significance of difference from fibrinogen plus adrenaline; $*P < 0.05$, $★P < 0.01$.

b. Effects of ginsenoside Rg_1 on thrombin-induced [^{3}H]5-HT release from human platelets. The incubation times were 1 min(◆) and 2 min(●), respectively. The other conditions were described in "Materials and Methods" section. ◇○, without thrombin; ◆●, thrombin (0·1 u mL^{-1}). Values are means ± s.e. for 4 experiments. Significance of difference from thrombin alone; $*P < 0.05$, $★ < 0.01$.

with light petroleum (bp 40–60°C)–ether–acetic acid (50:50:1, v/v) together with an authentic sample of arachidonic acid. The radioactive spot corresponding to arachidonic acid was detected by I_2 staining, and the amount of free [^{3}H]arachidonic acid was quantitated by scintillation counting. Assay of thrombin (0·1 u mL^{-1})-induced [^{3}H]arachidonic acid release was also performed using the above conditions in the absence of fibrinogen.

Measurement of diacyclglycerol production

In experiments where diacylglycerol production was measured, incubation of [^{3}H]arachidonic acid-labelled platelets was terminated by the addition of 3 volumes of chloroform–methanol (1:2, v/v). Platelet lipids were extracted by the method of Schacht (1981) and dried under vacuum. Diacylglycerol was separated from other lipids using the precoated silica gel 60 TLC by the method of Calderon et al (1979), the diacylglycerol was detected by I_2 staining, identified by comparison to diolein standard, and the amount of [^{3}H]arachidonic acid-labelled diacylglycerol was determined by scintillation counting.

Measurement of cytosolic free calcium concentration (Ca^{2+})i induced by adrenaline and thrombin in human platelets

To load fura 2 into the platelets, fura 2-AM 3μM was added to the platelet-rich plasma and incubated at 37°C for 30 min with gentle agitation in a water bath. The permeable acetoxymethyl ester of fura 2 is hydrolysed by cellular esterases on entering the cell and the fura 2 so formed is relatively impermeable and becomes trapped in the cytoplasm. After the incubation period, the reaction mixture was centrifuged at 1250 *g* to remove the plasma containing fura 2-AM solution. Then, the cells were washed twice with Tyrode-Hepes solution containing 0·35% bovine serum albumin and 2 mM EDTA (pH 7·4) and suspended in Tyrode-Hepes solution containing 0·35% bovine serum albumin and 1 mM $CaCl_2$ (pH 7·4). The assay of (Ca^{2+})i induced by adrenaline and thrombin was carried out by the same methods described for platelet aggregation. The fura 2-Ca fluorescence was measured using a fluorimeter (JACO CAF-100, Ca^{2+} Analyzer) with a fluorescence rate (short/long wavelengths) of dual excitation at 340 and 380 nm and emission at 500 nm.

Results

Effects of various ginseng saponins on adrenaline- and thrombin-induced aggregation in human washed platelets

As shown in Fig. 2a, b, of the six ginseng saponins used, ginsenoside Rg_1 was found to inhibit dose-dependently both adrenaline- and thrombin-induced platelet aggregation at concentrations of 5 to 500 μg mL^{-1}, Ginsenoside Rd also inhibited adrenaline-induced platelet aggregation at concentrations of 100 to 500 μg mL^{-1} (Fig. 2c), and inhibited thrombin-induced aggregation at a concentration of 500 μg mL^{-1}, though less strongly (data not shown). Other ginseng saponins, such as, ginsenoside Rb_1, Rb_2, Rc and Re inhibited neither adrenaline- nor thrombin-induced platelet aggregation (data not shown).

Effects of ginsenoside Rg_1 on adrenaline- and thrombin-induced [^{3}H]5-HT release from human platelets

Among the six ginseng saponins, ginsenoside Rg_1 strongly inhibited both adrenaline- and thrombin-induced platelet

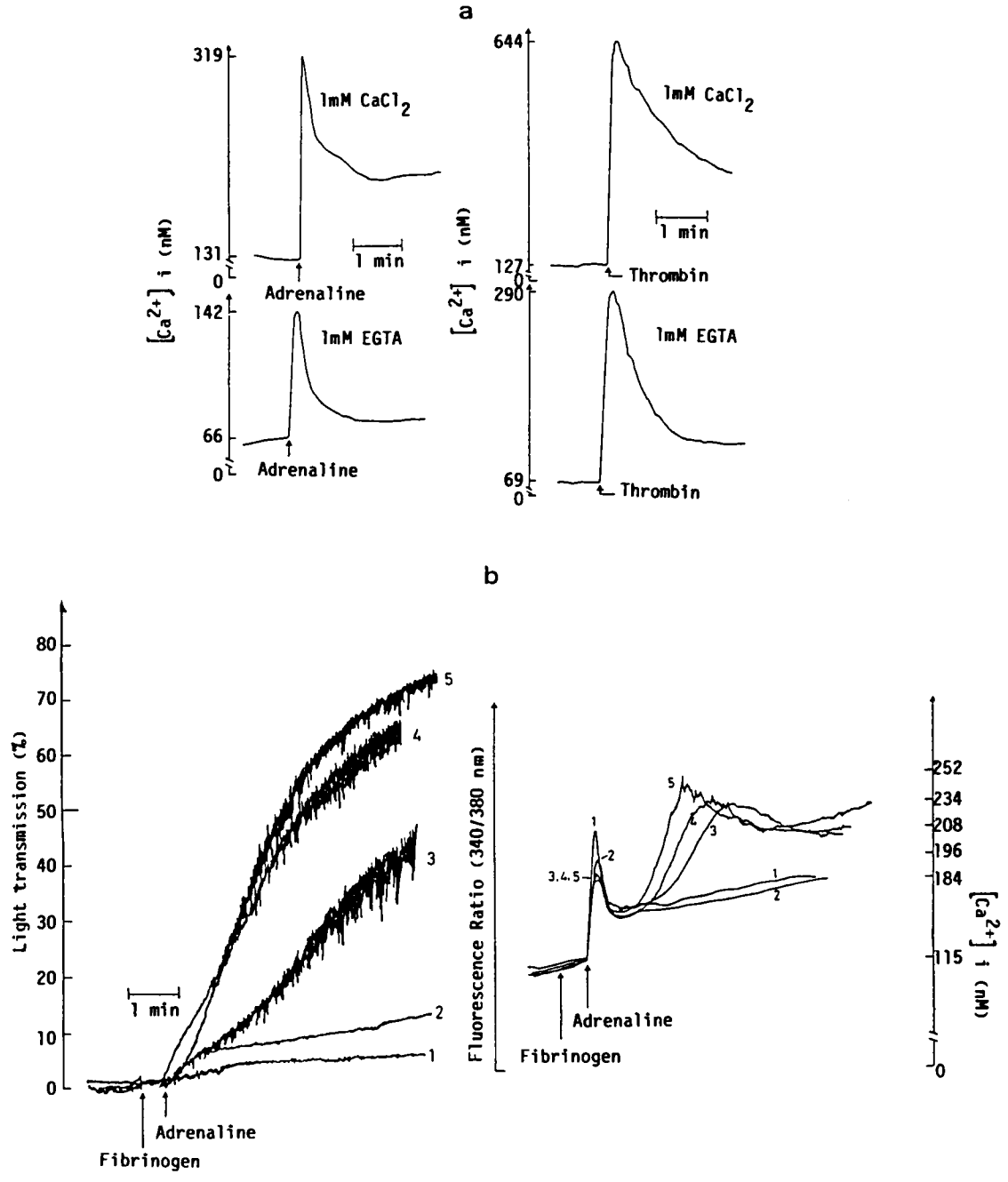

FIG. 4. a. Effects of adrenaline and thrombin on cytosolic free calcium concentration $(Ca^{2+})i$ in human platelets. Human platelets were incubated wtih Tyrode-Hepes solution (pH 7·4) in the absence of 0·35% bovine serum albumin.

b. Effects of fibrinogen on adrenaline-induced aggregation and $(Ca^{2+})i$ in human platelets. Washed human platelets suspended in Tyrode-Hepes containing 1 mM $CaCl_2$ and 0·35% bovine serum albumin were incubated with the various concentrations of fibrinogen and 10 μM adrenaline. The other conditions were described in "Materials and Methods" section. 1, fibrinogen 0; 2, fibrinogen 50; 3, fibrinogen 200; 4, fibrinogen 400; 5, fibrinogen 600 μg mL^{-1}.

aggregation. Therefore, we investigated the effects of ginsenoside Rg$_1$ on [^{3}H]5-HT release induced adrenaline and thrombin. As shown in Fig. 3, ginsenoside Rg$_1$ inhibited the [^{3}H]5-HT release induced by adrenaline and thrombin.

Effects of ginsenoside Rg$_1$ on arachidonic acid release and diacylglycerol production in [^{3}H]arachidonic acid-labelled platelets

Adrenaline and thrombin caused the arachidonic acid release and diacylglycerol production in [^{3}H] arachidonic acid-labelled platelets. The basal values of arachidonic acid release and diacylglycerol production were 2510±120 counts min^{-1} and 83·7±4·67 dmin^{-1} per 10^8 cells, respectively. And the values of arachidonic acid release and diacylglycerol production induced by adrenaline (10μM) and fibrinogen (0·3 mg mL^{-1}) were increased to 4970±185 counts min^{-1} and 125·6±9·81 dmin^{-1} per 10^8 cells, respectively. Following thrombin (0·1 u mL^{-1}), the values of arachidonic acid release and diacylglycerol production were increased 5120±450 counts min^{-1} and 126·8±4·30 dmin^{-1}

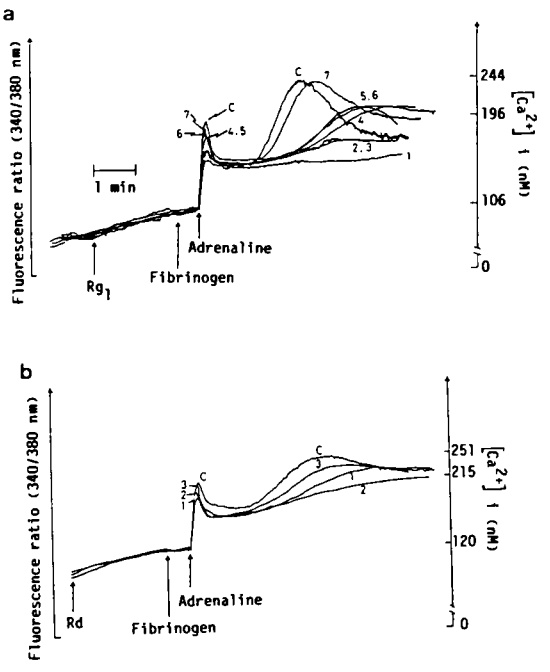

FIG. 5. (a) Effects of ginsenoside Rg_1 (b) ginsenoside Rd on adrenaline-induced $(Ca^{2+})i$ in human platelets. a. C, fibrinogen (0·3 mg mL^{-1}) plus 10 μM adrenaline 1, ginsenoside Rg_1 500; 2, Rg_1 250; 3, Rg_1 100; 4, Rg_1 50; 5, Rg_1 25; 6, Rg_1 10; 7, Rg_1 5 μg mL^{-1}.
b. Effects of ginsenoside Rd on adrenaline-induced $(Ca^{2+})i$ in human platelets. b. 1, ginsenoside Rd 500; 2, Rd 250; 3, Rd 100 μg mL^{-1}.

per 10^8 cells, respectively. Ginsenoside Rg_1 had no effect on arachidonic acid release and diacylglycerol production induced by adrenaline and thrombin in [^{3}H]arachidonic acid-labelled platelets (data not shown).

Effects of ginsenosides Rd and Rg_1 on cytosolic free calcium concentration $(Ca^{2+})i$ induced by adrenaline or thrombin

As shown in Fig. 4a, adrenaline and thrombin caused a rapid rise of $(Ca^{2+})i$ in platelets suspended in Tyrode-Hepes solution (pH 7·4) containing 1 mM $CaCl_2$. The adrenaline caused two phases of $(Ca^{2+})i$ elevation in the presence of 0·35% bovine serum albumin and fibrinogen (0·3 mg mL^{-1}). Adrenaline-induced platelet aggregation, which is dependent on fibrinogen content, was closely associated with the second phase of $(Ca^{2+})i$ elevation (Fig. 4b). The ginsenoside Rg_1 dose-dependently inhibited both platelet aggregation (Fig. 2a) and the second phase of $(Ca^{2+})i$ elevation induced by adrenaline (Fig. 5a). Ginsenoside Rd also induced the second phase of $(Ca^{2+})i$ elevation induced by adrenaline (Fig. 5b). Thrombin also caused both phases of $(Ca^{2+})i$ elevation in the presence of 0·35% bovine serum albumin without fibrinogen (Fig. 6). In contrast to adrenaline, ginsenoside Rg_1 dose-dependently inhibited both phases of $(Ca^{2+})i$ elevation induced by thrombin (Fig. 6).

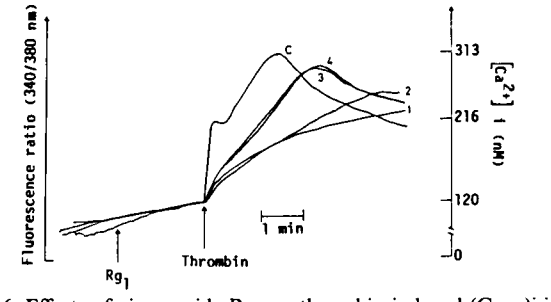

FIG. 6. Effects of ginsenoside Rg_1 on thrombin-induced $(Ca_{2+})i$ in human platelets. C, thrombin (0·1 u mL^{-1}) alone; 1, ginsenoside Rg_1 500; 2, Rg_1 100; 3, Rg_1 10; 4, Rg_1 5 μg mL^{-1}.

Discussion

The present investigation has demonstrated that various ginseng saponins (especially, ginsenoside Rg_1) isolated from red ginseng roots affect aggregation, 5-HT secretion, arachidonic acid release, diacylglycerol production and the movements of cytosolic free calcium concentration induced by adrenaline and thrombin. Adrenaline, 10μM, caused platelet aggregation and 5-HT release in the presence of fibrinogen (0·3 mg mL^{-1}) (Figs 2a, 3), but not in its absence (data not shown). On the other hand, thrombin (0·1 u mL^{-1}) caused platelet aggregation and 5-HT release in the absence of fibrinogen. Connolly & Limbird (1983) reported that adrenaline (10 μM)-, ADP (5 μM)- and thrombin- (0·004 u mL^{-1}) induced aggregation, and 5-HT release were inhibited by a cyclo-oxygenase inhibitor, indomethacin, and a Na^+-free medium. In contrast, they reported that platelet aggregation induced by a high concentration of thrombin (0·1 u mL^{-1}), and 5-HT release were not inhibited by indomethacin (Connolly & Limbird 1983). We also found that indomethacin did not inhibit platelet aggregation and that 5-HT release was induced by a high concentration of thrombin (0·1 u mL^{-1}) (data not shown). Therefore, Connolly & Limbird suggested that platelet aggregation and 5-HT release induced by adrenaline, ADP, and a low concentration of thrombin, were mediated by cyclo-oxygenase products. Platelet aggregation is induced by a wide variety of stimuli. One of the mechanisms is mediated by thromboxane A_2 synthesized from membrane-liberated arachidonic acid via the cyclo-oxygenase pathway (Hamberg & Samuelsson 1975).

Among the six ginseng saponins we used, ginsenoside Rg_1 strongly inhibited platelet aggregation and 5-HT release induced by adrenaline (in the presence of fibrinogen) and thrombin (in the absence of fibrinogen), while it had no effect on arachidonic acid release from the platelet membrance and diacylglycerol production induced by adrenaline and thrombin. These findings suggest that the inhibition of platelet aggregation and 5-HT release by ginsenoside Rg_1 cannot be explained by inhibition of arachidonate metabolite formation. Furthermore, Yamamoto et al (1987) reported that ginsenoside Rg_1 inhibited arachidonic acid- and a thromboxane A_2 agonist STA_2-induced platelet aggregation, but it had no effect on the formation of thromboxane A_2 from exogenous [1-^{14}C]arachidonic acid. Therefore, they suggested that the inhibitory actions of ginsenoside Rg_1 on platelet aggregation may be caused via the thromboxane A_2 receptor rather than the inhibition of thromboxane A_2 formation in human platelets.

To clarify the inhibitory mechanisms of ginsenoside Rg_1 on platelet aggregation and 5-HT release induced by adrenaline and thrombin, we then studied the effects of ginsenoside Rg_1 on $(Ca^{2+})i$ movements induced by adrenaline and thrombin. When the fura 2-loaded platelets, suspended in Tyrode-Hepes containing 0·35% bovine serum albumin and

1 mM $CaCl_2$, were stimulated by adrenaline (10 μM) plus fibrinogen (0·3 mg mL^{-1}) or 0·1 u mL^{-1} of thrombin alone, both phases of $(Ca^{2+})i$ elevation were shown. It seems likely that the first phase might be due to calcium mobilization from calcium pools, while the second phase might be due to Ca^{2+} influx from the extracellular medium, since that phase was reduced by addition of EGTA while the first phase was not. It is generally thought that, in many cells, $(Ca^{2+})i$ elevation is the trigger for secretory excytosis. Although calcium is regarded as the final common activator for the secretion, shape-change and aggregation of blood platelets (Detwiler et al 1978), some investigators consider that platelet aggregation and secretion are independent of $(Ca^{2+})i$ elevation (Rink et al 1982, 1983). Therefore, the contribution of $(Ca^{2+})i$ to platelet aggregation and secretion remains to be elucidated. However, the present study demonstrates that platelet aggregation induced by adrenaline or thrombin is associated with $(Ca^{2+})i$ elevation of the second phase. This finding suggests that aggregation induced by these agents might be due to $(Ca^{2+})i$ elevation through Ca^{2+} influx from extracellular medium. Furthermore, it has been reported that extracellular Ca^{2+} are required for platelet aggregation and 5-HT secretion in response to ADP or adrenaline (Bennett & Vilaire 1979; Gogstad et al 1982; Brass & Shatti 1984; Sweatt et al 1985). The present investigation also demonstrates that adrenaline-induced aggregation and 5-HT release are not found in the absence of fibrinogen which suggests a critical role of fibrinogen in the platelet aggregation induced by adrenaline.

In the present experiments, both ginsenosides Rg_1 and Rd inhibited the second phase of $(Ca^{2+})i$ elevation induced by adrenaline, while ginsenoside Rg_1 also inhibited both phases of $(Ca^{2+})i$ elevation induced by thrombin.

From these results, it seems likely that the inhibitory actions of ginsenoside Rg_1 on platelet aggregation and 5-HT release might be due to inhibition of the $(Ca^{2+})i$ elevation induced by adrenaline and thrombin. Recently, it has been reported that ginsenoside Rg_1 inhibits the platelet aggregation induced by adrenaline, ADP, thrombin and collagen in the human platelet-rich plasma (PRP) system, and that the enhancement of platelet aggregation induced by adrenaline, ADP and thrombin in PRP of diabetic is reduced by ginsenoside Rg_1 taken orally Nakanishi et al (1983). Furthermore, Odani et al (1983) reported that the amount of ginsenoside Rg_1 absorbed after oral administration seemed to be in the range of 1·9—20·0% of the dose. This, suggests that ginsenoside Rg_1 in red ginseng roots may be active as a drug in the treatment of atheroscleorosis and thrombosis.

References

Bennet, J. S., Vilaire, G. (1979) Exposure of platelet fibrinogen receptors by ADP and epinephrine. J. Clin. Invest. 64: 1393–1401

Brass, L. F., Shatti, S. J. (1984) Identification and function of the high affinity binding sites for Ca^{2+} on the surface of platelets. Ibid. 73: 626–632

Calderon, P., Furnelle, J., Christoque, J. (1979) In vitro lipid metabolism in the rat pancreas I. Basal lipid metabolism. Biochim. Biophys. Acta 574: 379–390

Connolly, T. M., Limbird, L. E. (1983) Removal of extraplatelet Na^+ eliminates indomethacin-sensitive secretion from human platelets stimulated by epinephrine, ADP and thrombin. Proc. Natl. Acad. Sci. USA 80: 5320–5324

Costa, J. L., Murphy, D. L. (1975) Platelet 5-HT uptake and release stopped rapidly by formaldehyde. Science 255: 407–408

Detwiler, T. C., Charo, I. F. & Feinman, R. D. (1978) Evidence that calcium regulates platelet function. Thromb. Haemostas. 40: 207–211

Gogstad, G. O., Brosstad, F., Krutnes, M-B., Hagen, I., Solum, N. O. (1982) Fibrinogen-binding properties of the human platelet glycoprotein IIb–IIIa complex: A study using crossed-radioimmunoelectrophoresis. Blood 60: 663–671

Hamberg, M., Samuelsson, B. (1975) Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc. Natl. Acad. Sci. USA 72: 2994–2998

Imai, A., Ishizuka, Y., Kawai, K., Nozawa, Y. (1982) Evidence for coupling of phosphatidic acid formation and calcium influx in thrombin-activated human platelets. Biochim. Biophys. Res. Commun. 108: 752–759

Kajikawa, N., Kaibuchi, K., Matsubara, T., Kikkawa, U., Takai, Y., Nishizuka, Y. (1983) A possible role of protein kinase C in signal-induced lysosomal enzyme release. Ibid. 116: 743–750

Kubo, M., Matsuda, H., Tani, T., Arichi, S. (1982) Effects of Panax ginseng on the experimental disseminated intravascular coagulation in rat. Proc. Symp. WAKAN-YAKU 15, 36–45 (In Japanese)

Nagai, Y., Tanaka, O., Shibata, S. (1971) Chemical studies on the oriental plant drugs—XXIV Structure of ginsenoside-Rg_1, A neutral saponin of ginseng root. Tetrahedron 27: 881–892

Nakanishi, K., Kaneko, H., Murakami, A. Kuwashima, K. (1983) The inhibitory effect of Panax ginseng on platelet. Proc. Symp. WAKAN-YAKU 16: 110–113 (In Japanese)

Odani, T., Tanizawa, H., Takino, Y. (1983) Studies on the absorption, distribution, excretion and metabolism of ginseng saponins. II. The absorption, distribution and excretion of ginsenoside Rg_1 in the rat. Chem. Pharm. Bull. 31: 292–298

Rink, T. J., Smith, S. W., Tsien, R. Y. (1982) Cytoplasmic free Ca^{2+} in human platelets: Ca^{2+} thresholds and Ca-independent activation for shape-change and secretion. FEBS Letters, 148: 21–26

Rink, T. J., Sanchez, A., Hallam, T. J. (1983) Diacylglycerol and phorbol ester stimulate secretion without raising cytoplasmic free calcium in human platelets. Nature 305: 317–319

Sanada, S., Kondo, N., Shoji, J., Tanaka, O., Shibata, S. (1974a) Studies on the saponins of ginseng. I. Structures of ginsenoside-Ro, -Rb_1-Rb_2, -Rc and -Rd. Chem. Pharm. Bull. 22: 421–428

Sanada, S., Kondo, N., Shoji, J., Tanaka, O., Shibata, S. (1974b) Studies on the saponins of ginseng. II. Structures of ginsenoside-Re, -Rf and -Rg_2. Ibid. 22: 2407–2412

Sanada, S., Shoji, J. (1978) Studies on the saponins of ginseng. III. Structures of ginsenoside-Rb_3 and 20-glucoginsenoside-Rf. 26: 1694–1697

Schacht, J. (1981) Extractino and purification of polyphosphoinositides. Methods Enzymol. 72: 626–631

Sekiya, K., Okuda, H. (1982) Effects of medical plants components on arachidonic acid metabolism in platelets. Proc. Symp. WAKAN-YAKU 15: 58–61 (In Japanese)

Sweatt, J. D., Johnson, S. L., Cragoe, E. J., Limbird, L. E. (1985) Inhibitors of Na^+/H^+ exchange block stimulus-provoked arachidonic acid release in human platelets. J. Biol. Chem. 260: 12910–12919

Sweatt, J. D., Blair, I. A., Cragoe, E. J. & Limbird, L. E. (1986) Inhibitors of Na^+/H^+ exchange block epinephrine- and ADP-induced stimulation of human platelet phospholipase C by blockade of arachidonic acid release at a prior step. J. Biol. Chem. 261: 8660–8666

Yahara, S., Kaji, K., Tanaka, O. (1979) Further study on dammarane-type saponins of root, leaves, flower-buds, and fruits of Panax ginseng C. A. Meyer. Chem. Pharm. Bull. 27: 88–92

Yamamoto, K., Hirai, I., Terano, T., Tamura, T., Yoshida, H. (1987) Inhibitory effects of red ginseng saponins on platelets. J. Med. Pharm. Soc. WAKAN-YAKU 4: 342–343 (In Japanese)