Br. J. Pharmac. (1984), **82**, 485–491

Vascular effects of ginsenosides *in vitro*

Xiu Chen[1], C. Norman Gillis & Richard Moalli

Departments of Anesthesiology and Pharmacology, Yale University School of Medicine, New Haven, CT 06510, U.S.A.

1 Ginsenosides (saponins extracted from *Panax ginseng*) elicit qualitatively and quantitatively different responses in isolated, contracted ring preparations of different blood vessels from rabbits, dogs and humans.

2 Ginsenosides themselves did not affect the tone of 'resting' isolated blood vessels directly, but contracted slightly the renal vein of rabbits at the maximum concentration tested. The mixture caused relaxation of the noradrenaline (NA) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced contraction of the pulmonary artery and intrapulmonary artery of rabbits, and the $PGF_{2\alpha}$-induced contraction of the canine mesenteric vein.

3 Ginsenosides potentiated, in a concentration-dependent manner, the contractile responses of renal veins of dogs and rabbits to $PGF_{2\alpha}$

4 The reason for such heterogeneous responses of different blood vessels to ginsenosides in unknown. It is suggested that either potentiation of contraction or relaxation of contracted blood vessels might be mediated by interaction with endogenous vasoactive substances. The potentiation of $PGF_{2\alpha}$-induced contraction may be related to the reduction of renal blood flow observed in anaesthetized dogs. The simultaneous contraction and relaxation effects may explain its biphasic actions on blood pressure.

Introduction

Panax ginseng has been used for more than two thousand years as a general tonic in traditional Chinese medicine. Recently it was reported that ginsenosides, saponins extracted from *Panax ginseng*, provide some protection against experimental myocardial infarction in rabbits (Chen *et al.*, 1980). In order to elucidate the mechanism of this action, the cardiovascular and haemodynamic effects of ginsenosides were studied in experiments in intact dogs. It was found that the mixture significantly reduced vertebral and femoral vascular resistance but increased renal vascular resistance mainly by decreasing renal blood flow (Chen *et al.*, 1982a,b). These findings suggested that ginsenosides may produce different responses in different blood vessels. The purpose of the present study was to examine the validity of this assumption by evaluating the response to ginsenosides of a variety of isolated blood vessels prepared from rabbits, dogs and man.

[1] Present address: Department of Pharmacology, Hunan Medical College, Changsha 410008, People's Republic of China.

Methods

Male New Zealand albino rabbits (2.2–2.8 kg) were anaesthetized by intravenous injection of allobarbitone (125 mg kg^{-1}) and urethane (500 mg kg^{-1}) followed by administration of heparin (500 units kg^{-1}). The lungs and heart were removed and placed in Krebs-bicarbonate solution and aerated continuously with 95% O_2 and 5% CO_2. Segments (2–3 cm) of the ascending aorta and right pulmonary artery (RPA) were removed. Right intrapulmonary artery segments were carefully isolated and cannulated with a fine polypropylene tubing (1 mm o.d.) and removed. After opening the abdominal cavity, the right renal artery and vein segments were isolated, cannulated and removed.

Male mongrel dogs (18–23 kg) were anaesthetized with pentobarbitone (30 mg kg^{-1}) followed by heparin (500 units kg^{-1}). Segments of right renal artery and vein and mesenteric vein were carefully isolated and removed.

Human saphenous veins employed were segments of unused tissue removed from patients (60–70 years of age) undergoing coronary bypass surgery.

Only vessels from 2.5 to 6.0 mm diameter were

used in this study. Each vessel was carefully cleaned of surrounding tissue, cut into cylindrical segments 5–6 mm in length and mounted on fine stainless steel hooks (Hooker *et al.*, 1977; Altiere *et al.*, 1983a). In each experiment four different blood vessels were suspended in 10 ml organ baths containing Krebs-bicarbonate solution at 37°C and continuously aerated with 95% O_2 plus 5% CO_2. Changes in force were measured with semi-isometric force transducers (FT03 for rabbit: pulmonary and renal arteries, load 5g; dog: renal artery, load 7–10g; renal vein 3–6g; mesenteric vein 2–5g; human saphenous vein 8–10g; Statham G7A for rabbit intrapulmonary artery load 5g; G10B for rabbit renal vein load 0.5g) and recorded on a Grass Model 7 polygraph. Preload tension was determined as described previously (Altiere *et al.*, 1983a). Ring preparations were allowed to equilibrate for 2 h under optimal applied load. The bath solution was changed by overflow every 15 min during the equilibration period.

Cumulative ginsenoside concentration-response curves were produced by the addition of solutions of ginsenosides to attain final concentrations of 10 μg to 1000 μg ml^{-1} in the bathing medium. After repeated washing and equilibration for approximately 60 min, cumulative concentration-response curves were generated with freshly prepared solutions of noradrenaline (NA) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). When preparations were maximally contracted by the agonists (NA, 5×10^{-6} M; $PGF_{2\alpha}$, 5×10^{-5} M), ginsenoside solution was added to the organ baths in a cumulative manner to observe its effect on NA or $PGF_{2\alpha}$-induced contractile responses.

Drugs

Ginsenosides were extracted from *Panax ginseng* C.A. Meyer according to the Shibata method (Shibata *et al.*, 1982). Ginsenoside, as well as L-

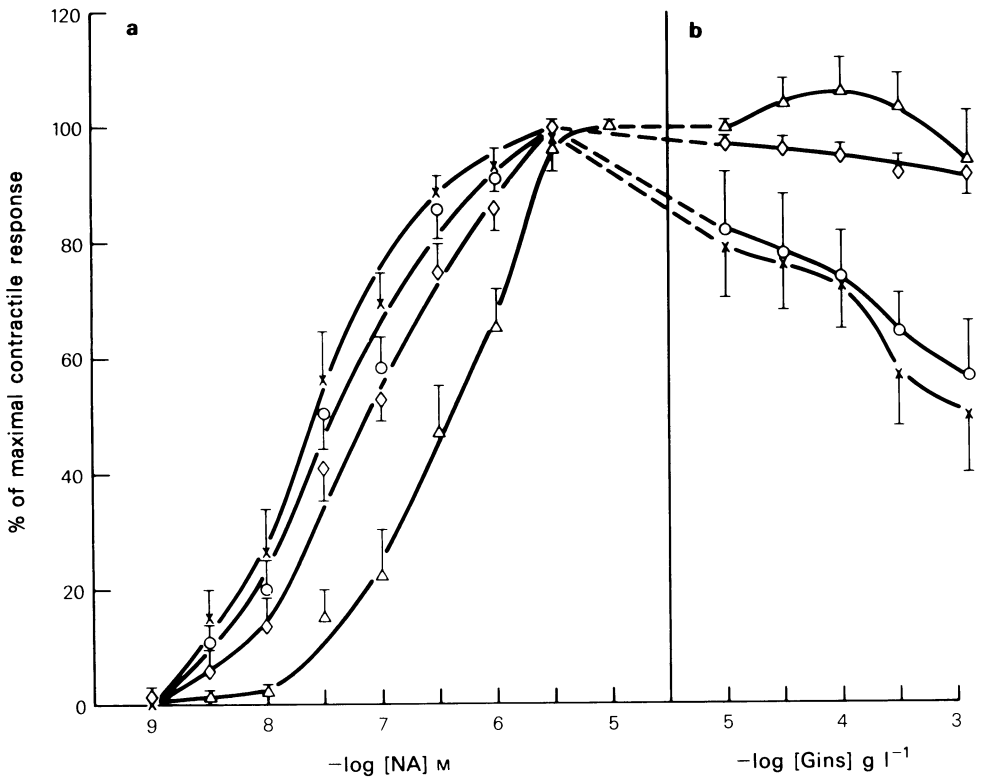

Figure 1 Effect of ginsenosides on noradrenaline (NA)-induced contractile responses of rabbit blood vessels. (a) Cumulative dose-response curves of the intrapulmonary artery (×), pulmonary artery (○), aorta (◇) and renal artery (△) to NA. (b) The effect of ginsenosides on the maximal contraction induced by noradrenaline in the different blood vessels. Data points represent mean ± s.e.mean (vertical bars) of 6 vessels (except renal artery, $n = 5$)

noradrenaline bitartrate (NA, Sigma Chemical Co.) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Upjohn, Kalamazoo, MI) were prepared in the Krebs solution immediately before use. All solutions were kept on ice and protected from light during the experiments.

Analysis of data

Concentration-response data were expressed in grams of developed force and normalized as % of the maximum response (either contraction or relaxation) of the vessel in each experiment. The mean and standard error of the mean were calculated for each vessel. Statistical significance was evaluated by the *t* test for individual or group comparisons. *P* values of 0.05 or less were considered to be significant.

Results

Rabbit blood vessels

Addition of ginsenosides (10–1,000 $\mu g\,ml^{-1}$ in the bathing medium) did not contract rabbit pulmonary, intrapulmonary or renal arteries or aorta in the absence of an agonist. The maximal concentration (1000 $\mu g\,ml^{-1}$) slightly contracted the renal vein, causing the development of a tension of 55 mg, equivalent to 17% of the maximal contraction induced by

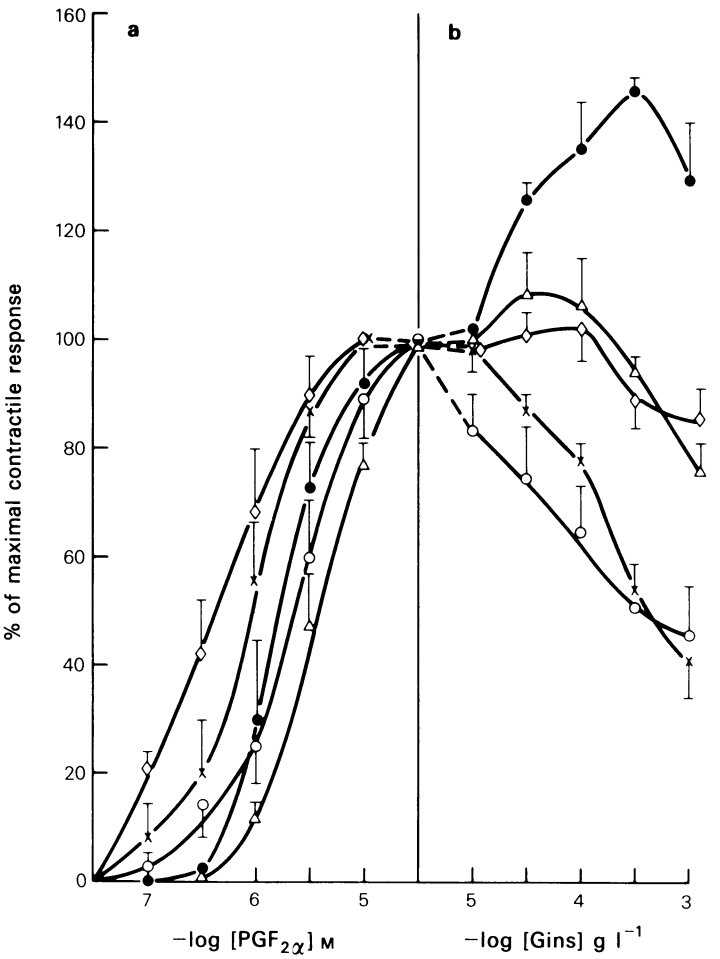

Figure 2 Effect of ginsenosides on the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced contractile responses of rabbit blood vessels. (a) Cumulative dose-response curves of pulmonary artery (○), renal vein (●), renal artery (△), intrapulmonary artery (×) and aorta (◇) to $PGF_{2\alpha}$. (b) Effects of ginsenosides on the $PGF_{2\alpha}$-contracted blood vessels. Data points represent mean ± s.e.mean (vertical bars) of 4–7 vessels.

$PGF_{2\alpha}$. However, in some blood vessels ginsenosides did affect the contraction induced by either NA or $PGF_{2\alpha}$. Figure 1a shows the cumulative concentration-response curve to NA of the intrapulmonary and pulmonary artery, and renal artery (from left to right). When the maximal contraction was obtained to 5×10^{-5} M NA and ginsenosides were added to the organ bath in a cumulative manner they significantly relaxed the pulmonary and intrapulmonary arteries in a concentration-dependent manner (from 10 $\mu g\,ml^{-1}$ to 1000 $\mu g\,ml^{-1}$). However, ginsenosides only slightly contracted the renal artery and had no apparent effect on the previously contracted aorta (Figure 1). Similar effects were observed when blood vessels were contracted by $PGF_{2\alpha}$. Thus, when a maximal contraction was induced by $PGF_{2\alpha}$ ginsenosides also relaxed the pulmonary and intrapulmonary arteries in a concentration-dependent manner. However, ginsenosides produced no significant effect on the contracted renal artery or aorta, although at very high concentrations (500–1,000 $\mu g\,ml^{-1}$) these vessels relaxed slightly (Figure 2). A unique effect shown in Figure 2 is that ginsenosides potentiated the contractile response of the renal vein to $PGF_{2\alpha}$. Thus the rabbit renal vein responds to ginsenosides in a qualitatively different manner from the other arteries studied.

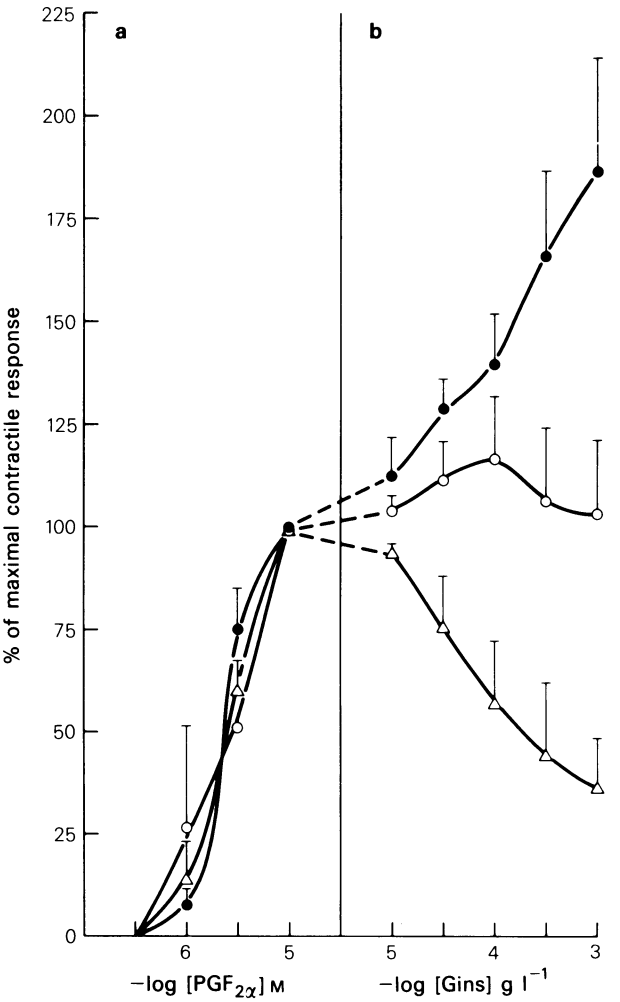

Figure 3 Effect of ginsenosides on prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced contraction of dog blood vessels. (a) The cumulative dose-response curves of renal artery (○), mesenteric vein (△) and renal vein (●) to $PGF_{2\alpha}$. (b) Effects of ginsenosides on these contracted vessels. Data points represent mean± s.e.mean (vertical bars) of 3–4 vessels.

Canine blood vessels

Ginsenosides alone did not significantly affect the canine isolated renal artery and vein and mesenteric vein. However, as shown in Figure 3, the mixture also potentiated the contractile response of the canine renal vein to $PGF_{2\alpha}$, but had no significant effect on the contracted renal artery. In contrast, it markedly relaxed $PGF_{2\alpha}$-induced tone in the canine mesenteric vein. Thus, ginsenosides also acted qualitatively differently in the canine renal artery and vein.

Human vessels

As shown in Figure 4a, ginsenosides alone in high concentrations slightly contracted the saphenous vein. This small contraction could also be elicited when the tissue was previously contracted by $PGF_{2\alpha}$, although no potentiation was seen.

Discussion

In the present study we found that ginsenosides had little direct effect on 'resting' isolated blood vessels. Only at high concentrations did ginsenosides contract the rabbit renal vein. However, the saponin mixture significantly relaxed the contracted (NA or $PGF_{2\alpha}$-induced contraction) rabbit pulmonary and intrapulmonary artery, and the contracted ($PGF_{2\alpha}$-induced contraction) canine mesenteric vein. A qualitatively different effect was observed with the rabbit or dog renal vein. In these preparations, ginsenosides potentiated the contractile response to $PGF_{2\alpha}$. This potentiation of the contractile response is recognized as being true potentiation, since ginsenosides alone produced only 17% of the maximum contractile response to $PGF_{2\alpha}$; however, if added at the peak of the $PGF_{2\alpha}$ induced contractile response ginsenosides further increased the contraction of the renal vein by an additional 46%; that is, three times the response

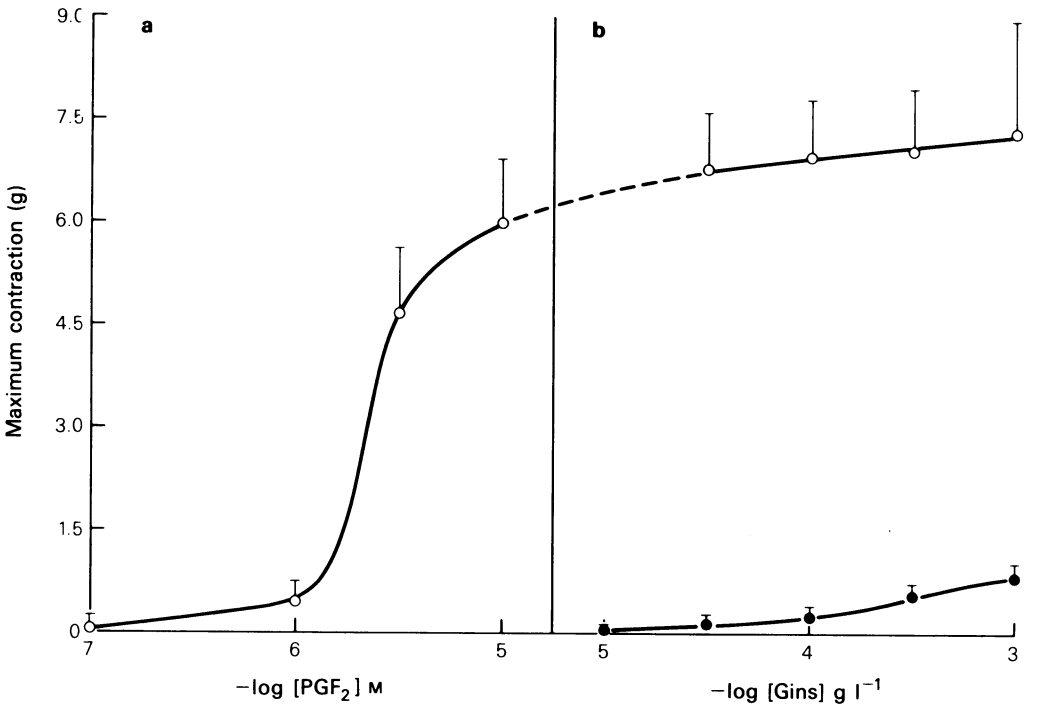

Figure 4 Effect of ginsenosides on human saphenous veins by itself and in combination with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). (a) The dose-response curve of human saphenous veins to $PGF_{2\alpha}$. (b) The effect of ginsenosides on human saphenous vein normally (●) and after the vein had contracted to $PGF_{2\alpha}$ (○). Data points represent mean ± s.e.mean (vertical bars) values.

produced by ginsenosides alone.

Considerable evidence has been published indicating that $PGF_{2\alpha}$ can be synthesized in the kidney of rats, rabbits and man (Dusing *et al.*, 1978; Oliw, 1980; Sraer *et al.*, 1982) and released in the canine renal vein (Chandler & Giri, 1981; Lonigro *et al.*, 1982). There is also evidence that prostaglandins play an important role in the regulation of renal blood flow (Terragno *et al.*, 1977). Furthermore, it has been shown that among the prostaglandins $PGF_{2\alpha}$ is the most potent vasoconstrictor of canine isolated renal vein (Chand & Altura, 1981). It is also of interest to note that the pressor activity of $PGF_{2\alpha}$ is considered primarily to result from venoconstriction (Ducharme *et al.*, 1968). Therefore it seems reasonable to speculate that the reduction in renal blood flow induced by ginsenosides observed with the intact dog (Chen *et al.*, 1982a) may result from a potentiation of the renal vasoconstriction caused by endogenous $PGF_{2\alpha}$.

In recent years the heterogeneity of responses in different blood vessels has been noted. For example, veins from different locations and of different sizes were found to have different innervation patterns and sensitivity to NA. Bevan *et al.* (1974) interpreted these differences as reflecting different functional requirements. Recently we demonstrated that extrapulmonary and intrapulmonary arteries of the rabbit have different responses (Altiere *et al.*, 1983b). Even in the same rabbit ear artery, the density of innervation in the proximal region is about twice that in the distal region, and the former also responded more quickly than the latter (Griffith *et al.*, 1982). However, the qualitative difference we observed in the effects of ginsenosides on different blood vessels has not been described previously. It is conceivable that the relaxant effect of ginsenosides in the pulmonary and intrapulmonary arteries might be mediated through release of endogenous vasodilator substances. It has been shown, for example, that the endothelial cell of the aorta contains acetylcholine, which can be released by injury (Furchgott & Zawadski, 1980). Acetylcholine, acting on muscarine receptors, stimulates formation of cyclic GMP which then relaxes arterial smooth muscle (Furchgott & Jothianandan, 1983).

Our data may also explain the complicated and seemingly conflicting haemodynamic effects of ginsenosides described in the literature. For example, *Panax ginseng* has been described as having either hypotensive (rabbits; Hsu, 1956), hypertensive (dogs; Chang, 1959); or biphasic effects on blood pressure of dogs and rats (Chen *et al.*, 1982a,b; wood *et al.*, 1964). As shown in the present study, ginsenosides can cause both vasoconstriction or vasodilatation in different vessels. Thus, the net effect on blood pressure and other haemodynamic parameters will reflect the predominant vascular site of action. In addition, ginsenosides used in our study are a complex mixture of ginseng saponins, each of which may have different effects (Kaku *et al.*, 1975), thus further complicating prediction of the cardiovascular effect of ginseng.

We gratefully acknowledge the careful technical assistance of Ms Mary Beth Clark and secretarial assistance of Ms Suzanne Abrams. We are very grateful to Dr Graeme L. Hammond, Department of Surgery, Yale University School of Medicine, for making available to us samples of human saphenous vein.

This work was supported by U.S. Public Health Service Grants HI-07410 and HL-13315.

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(*Received October 28, 1983.*)