

Comparison of Vasodilator Effects of Platycodin D and D₃ in Rats

Dong-Yoon Lim¹, Byeong-Cheol Kim¹, and Eun-Bang Lee²

¹Department of Pharmacology, College of Medicine, Chosun University, Gwangju 501–759, Korea; ²Natural Products Research Institute, Seoul National University, Seoul 110–460, Korea

The aim of the present study was to examine the effects of platycodin D and D₃, which are active components derived from the roots of *Platycodon grandiflorum* A. DC., on the contractile force of the isolated rat aorta and blood pressure of the anesthetized rat, and also to elucidate its mechanism of action. Both phenylephrine (an adrenergic α_1 -receptor agonist) and high potassium (a membrane-depolarizing agent) caused great contractile responses in the isolated aortic strips. Platycodin D at high concentration (24 $\mu\text{g/ml}$) inhibited contractile responses induced by phenylephrine (10^{-5} M) and high potassium (5.6×10^{-2} M), while low concentrations of platycodin D (4–8 $\mu\text{g/ml}$) did not affect those responses. However, platycodin D₃ (8–32 $\mu\text{g/ml}$) did not alter the contractile responses evoked by phenylephrine and high K⁺. Interestingly, the infusion of platycodin D₃ (1.0 mg/kg/30 min) significantly reduced the pressor responses induced by intravenous norepinephrine. However, platycodin D₃ (1.0 mg/kg/30 min) did not affect them. Taken together, these results show that intravenously administered platycodin D depresses norepinephrine-induced pressor responses in the anesthetized rat, at least partly through the blockade of adrenergic α_1 -receptors. Platycodin D also caused vascular relaxation in the isolated aortic strips of the rat via the blockade of adrenergic α_1 -receptors, in addition to an unknown direct mechanism. However, platycodin D₃ did not affect both norepinephrine-induced pressor responses and the isolated rat aortic contractile responses evoked by phenylephrine and high potassium. Based on these results, there seems to be much difference in the mode of action between platycodin D and platycodin D₃.

Key Words: *Platycodon grandiflorum* A. DC, Platycodin D and D₃, Vascular relaxation, Blockade of adrenergic α_1 -receptors.

INTRODUCTION

Platycodi radix, the roots of *Platycodon grandiflorum* A. DC, has been used traditionally as an expectorant and a remedy for bronchitis, tonsillitis, laryngitis and suppurative dermatitis in China, Korea and Japan. In China and Korea, the fresh roots of *P. grandiflorum* have been consumed as pickles for preventing obesity. Although it has been thought that pickles of *Platycodi radix* have anti-obesity activity, only hearsay evidence exists. It has been reported that *Platycodi radix* prevented hypercholesterolemia and hyperlipidemia (Kim et al, 1995). Obesity was induced by feeding female mice with high fat diet containing 40% beef tallow for 10 weeks (Han et al, 1999b), and chitosan (Han et al, 1999a) and oolong tea (Han et al, 1999c) were found to exert anti-obesity and/or anti-hyperlipidemic actions that were mediated through delaying the intestinal absorption of dietary fat by inhibiting

pancreatic lipase activity. Han and his co-workers (2000) have shown that the anti-obesity effect of the aqueous extract of *Platycodi radix* in mice, which were fed a high fat diet, was in part due to the inhibition of intestinal absorption of dietary fat by saponins of *Platycodi radix*. Platycodin D, isolated from the root of *Platycodon grandiflorum* A. DC. (Campanulaceae) at 10 and 30 μM suppressed prostaglandin E₂ production in rat peritoneal macrophages stimulated by the protein kinase C activator 12-O-tetradecanoylphorbol 13-acetate, while Platycodin D₃ and oleanolic acid showed no effect at these concentrations (Kim et al, 2001). Platycodin D and D₃ are shown to increase mucin release from rat and hamster tracheal surface epithelial cell culture and also from intact rat trachea upon nebulization (Shin et al, 2002). Crude platycodin, given intra-arterially, reduced dose-dependently coronary and hindquarter vascular resistances in anesthetized dogs, and this vasodilating effect of crude platycodin appeared to be direct and non-specific on the peripheral blood vessels as in the case of glyceryl trinitrate or papaverine. (Kato et al, 1973). Intravenously administered crude platycodin also increased coronary and hindquarter blood flows, concomitantly with a transient systemic hypotension. In spite of these published observation, however, there is no clear evidence so far whether platycodin D and produce vasodilating effect in isolated rat

Corresponding to: Dong-Yoon Lim, Department of Pharmacology, College of Medicine, Chosun University, Gwangju 501-759, Republic of Korea. (Tel) 82-62-230-6335, (Fax) 82-62-227-4693, (E-mail) dylim@chosun.ac.kr

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aortic strips. Therefore, the present study was undertaken to examine the effects of platycodin D and D₃ on the contractile force of the isolated rat aorta and blood pressure of the anesthetized rat and also to elucidate its mechanism of their action.

METHODS

Isolation of platycodin D and platycodin D₃

The root of *P. grandiflorum* was collected in the plant garden of Natural Products Research Institute, Seoul National University, Korea. A voucher specimen (No. NP 9621) was deposited in the herbarium of Natural Products Research Institute. The methanol extract (294 g) was fractionated successively with n-hexane, chloroform, ethyl acetate, and n-butanol. The n-butanol fraction (9.3 g) was loaded on a silica gel (150 g) column and eluted with chloroform-methanol-water (15 : 10 : 2) to afford 15 fractions. The fraction 8 (2.3 g) contained mixtures of platycodin D and platycodin D₃. This fraction was further chromatographed using a silica gel (80 g) column, which was eluted with ethyl acetate-methanol gradient (90 : 10, 200 ml; 80 : 20, 400 ml; 70 : 30, 200 ml) and four fractions (8-1 to 8-4) were collected. The fraction 8-4 was rich mixtures of platycodin D and platycodin D₃. This fraction was further fractionated by a silica gel column (40 g) eluted with chloroform-methanol-water-n-propanol (5 : 6 : 4 : 1.2) (400 ml), yielding pure platycodin D (0.06 g) and platycodin D₃ (0.18 g). Identification of the compounds was carried out by co-TLC with authentic samples and by NMR data (Ishii et al, 1978).

Experimental procedure

Mature Sprague-Dawley male rats, weighing 150 to 350 g, were used in the experiment. The animals were housed individually in separate cages, and food (Cheil Animal Chow) and tap water were allowed *ad libitum* for at least one week to acclimatize to experimental circumstances. On the day of experiment, the rat was anesthetized intraperitoneally with thiopental sodium (40 mg/kg) and tied in supine position on a fixing panel.

Isolation of aortic strips: The thorax was opened by a midline incision, and the heart and surrounding area were exposed by the placement of three hook retractors. The heart and portion of the lung were not removed, but pushed over to the right side and covered by saline-soaked gauze pads in order to obtain enough working space for isolation of aortic vessel. The aorta was isolated from the proximal part of the heart to the vicinity of liver and immediately immersed in cold Krebs solution. The blood within the aorta was rapidly removed. The aorta was cut into the ring of 4~5 mm length.

Preparation of arterial cannulation: Animal was tied in supine position on a fixing panel to insert a T-formed cannula into the tachea for securing free air passage. The rectal temperature was maintained at 37~38°C by a thermostatically controlling blanket and heating lamp throughout the whole experiment.

Recording of mechanical activity

The ring segment of aorta was mounted in a muscle bath

by sliding the ring over two parallel stainless-steel hooks (0.15 mm in diameter). The lower hook was fixed on bottom of the bath, and the upper was connected to isometric transducer (Grass FT. 03). The signal from the transducer was displayed on a polygraph (Grass Instruments Model 79). The volume of bath was 25 ml, and the bath solution was saturated with 95% O₂ and 5% CO₂ at 37°C (Fig. 1). The composition (mM) of Krebs solution was: NaCl, 118.4; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.18; NaHCO₃, 25; KH₂PO₄, 1.2; glucose, 11.7. The pH of the solution was maintained at 7.4~7.5. During equilibration period of 2 hours, the resting tension was adjusted to 0.5 g. After the equilibration period, the ring was challenged twice with 35 mM KCl and, if it responded with contraction, the proper experiment started. Vasoconstrictors were administered into the bath in order to obtain dose-response curves. In the subsequent experiments, some vasoconstrictors were administered under the presence of platycodin D or D₃. The data were expressed as % of the control tension.

Measurement of blood pressure

In order to observe the change of arterial pressure, one of the common carotid arteries or of the femoral arteries was catheterized with polyethylene tubing [outside diameter (o.d.): 0.5 mm]. The tubing was connected to a pressure transducer (Gould Co., USA) and pulse of mean arterial blood pressure was continuously recorded on a biological polygraph (Grass Co., USA). The chart speed was adjusted to 2 cm per minute. The artery tubing was filled with heparin solution (400 I.U.) to prevent blood coagulation during the experiment. Another cannulation with polyethylene tubing (o.d.: 0.3 mm) was made into a femoral vein for the administration of drugs and supplemental anesthetic agents to maintain light surgical anesthesia. Each rat was left undisturbed for at least 30 minutes after completion of the operative procedures to permit cardiovascular parameters to be stabilized, and drugs under investigation were administered at 60 minutes intervals.

Statistical analysis

The statistical significance between groups was determined by the Student's *t*- and ANOVA-tests. A P-value of less than 0.05 was considered to represent statistically significant changes unless specifically noted in the text. Values given in the text refer to means and the standard errors of the mean (S.E.M.). The statistical analysis of the experimental results was made using the computer program described by Tallarida & Murray (1987).

Drugs and their sources

The following drugs were used: Platycodin D and Platycodin D₃ were gift from Professor Eun-Bang Lee, Natural Products Research Institute, Seoul National University, Seoul, KOREA, phenylephrine hydrochloride, potassium chloride, and norepinephrine bitartrate were purchased from Sigma Chemical Co., USA, and thiopental sodium and heparin sodium were from Daehan Choongwae Pharm. Co., Korea. Other chemicals were of reagent grade. Drugs were dissolved in distilled water (stock) and added to the normal Krebs or 0.9% saline solution as required. Concentrations of all drugs used are expressed in molar and gram.

RESULTS

Effects of platycodin D on contractile responses induced by phenylephrine and high K⁺ in the rat aortic strips

The resting (basal) tension from the isolated rat aortic strips reached a steady state after perfusion with oxygenated Krebs-bicarbonate solution for 90 min before the experimental protocol was initiated. The resting tension was adjusted to 0.5 g. The effect of platycodin D on phenylephrine- as well as high potassium chloride-mediated contractile responses in the rat aorta were examined. In the present study, platycodin D itself did not produce any effect on the resting tension in the aortic strips isolated from the rat (data not shown). However, as shown in Fig. 1 and 2, pretreatment with platycodin D at a higher dose was found to inhibit both the phenylephrine- and high potassium-induced the contractile responses, but it did not at lower doses of platycodin D.

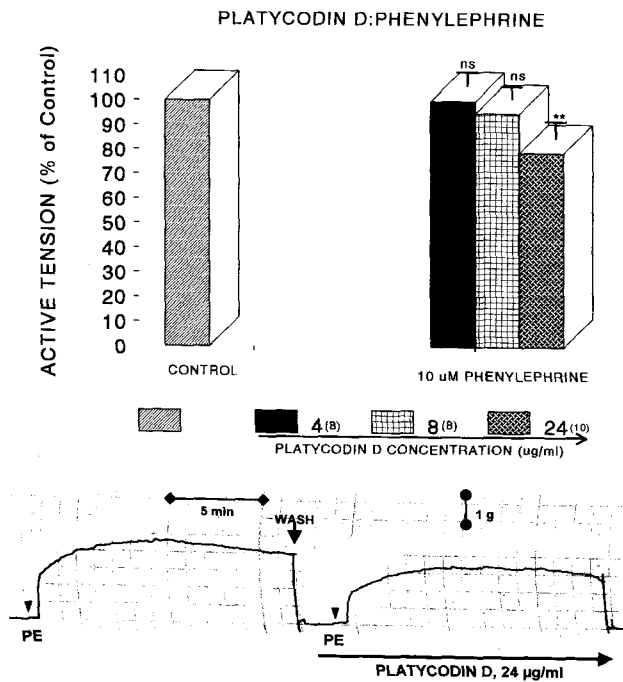


Fig. 1. Upper: Influence of platycodin D on phenylephrine (PE)-induced contractile response in the isolated rat aortic strips. The contractile response was induced by adding 10 μM PE after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol. "CONTROL" denotes active tension induced by PE before adding platycodin D (100%). Numerals in the parenthesis indicate number of experimental rat aortic strips. Vertical bar represents the standard error of the mean (S.E.M). Ordinate: the active tension (% of control). Abscissa: concentration of PE (μM). Statistical difference was obtained by comparing the control with the platycodin D-pretreated groups (4, 8 and 24 μg/ml). **: P<0.01. ns: Statistically not significant. Lower: The typical tracing showing the effect of platycodin D on phenylephrine (PE)-induced contractile responses in the rat aortic strips. Left: PE-induced contractile response. Right: PE-induced contractile response in the presence of 24 μg/ml of platycodin D. At arrow mark, the indicated dose (10⁻⁵ M) of phenylephrine was added to the bath. The chart speed was 5 mm/min.

When 10⁻⁵ M phenylephrine concentration was administered into the aortic strip in organ bath, its active tension increased to 2.4±0.2 g from the resting tension level. However, under the pre-loading with 4, 8 and 24 μg/ml platycodin D concentrations, 10⁻⁵ M phenylephrine-induced tensions were dose-dependently inhibited by ~79% of the control contractile response (2.3±0.2 g) (Fig. 1). Moreover, the lower doses of platycodin D (4 and 8 μg/ml) did not affect phenylephrine-induced contractile responses, as shown in Fig. 1.

High K exerts two distinct effects on cells: (1) depolarization of cell membrane and (2) depolarization-induced influx of calcium via voltage-dependent calcium channels (Wada et al, 1985). When added through the bath, high of 5.6×10⁻² M potassium concentration, which is a membrane-depolarizing agent, increased aortic contraction. As shown in Fig. 2, high potassium (5.6×10⁻² M)-induced contractile response after pre-loading with 4 and 8 μg/ml of platycodin D were 100±6% (ns, n=8) and 95±5% (ns, n=8) of their corresponding control response (2.4±0.2 g), respectively. However, in the presence of platycodin D at higher concentration of 24 μg/ml, high potassium (5.6×10⁻² M)-induced contractile response was 80% of its corresponding control response (Fig. 2).

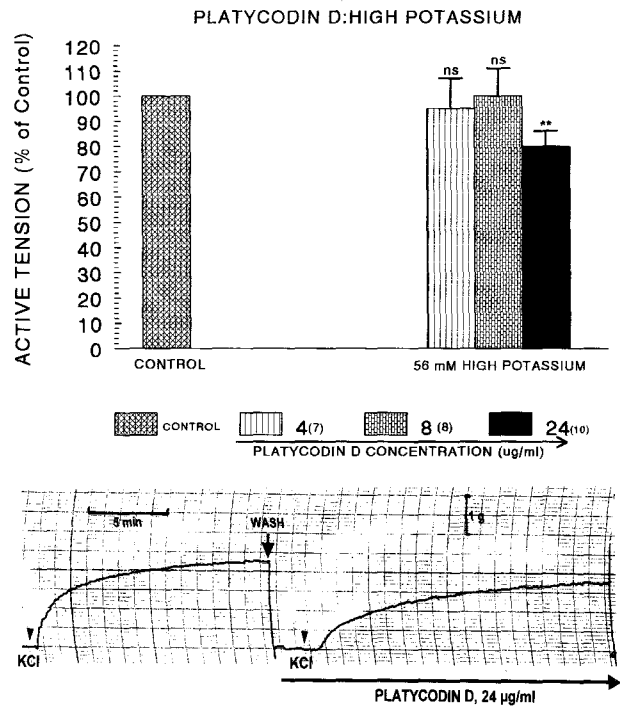


Fig. 2. Upper: Influence of platycodin D on high potassium-induced contractile responses in the isolated rat aorta. High potassium (56 mM) was added into the bath before and after pretreatment with 4, 8 and 24 μg/ml of Platycodin D, respectively. Other legends are the same as in Fig. 2. **: P<0.01. ns: Statistically not significant. Lower: The typical tracing showing the effect of platycodin D on high potassium (KCl)-induced contractile responses in the rat aortic strips. Left: KCl-induced contractile response. Right: KCl-induced contractile response in the presence of 1.2 mg/ml of GTE. At arrow mark, the indicated dose of KCl (56 mM) was added to the bath. The chart speed was 5 mm/min.

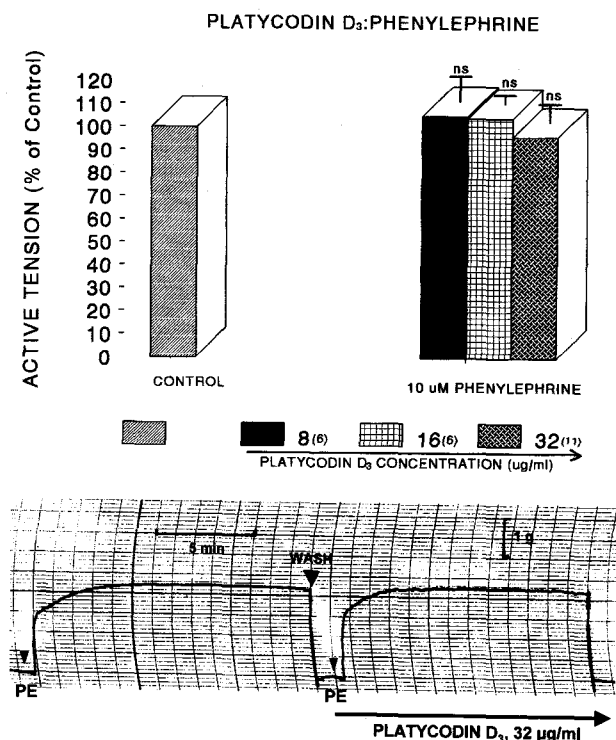


Fig. 3. Upper: Influence of platycodin D₃ on phenylephrine (PE)-induced contractile responses in the isolated rat aortic strips. The contractile response was induced by adding 10 μM PE before (CONTROL) and after adding platycodin D₃ at concentrations of 8, 16 and 32 μg/ml. Other legends are the same as in Fig. 2. ns: Statistically not significant. Lower: The typical tracing showing the effect of platycodin D₃ on phenylephrine (PE)-induced contractile responses in the rat aortic strips. Left: PE-induced contractile response. Right: PE-induced contractile response in the presence of 32 μg/ml of platycodin D₃. At arrow mark, the indicated dose (10⁻⁵ M) of phenylephrine was added into the bath. The chart speed was 5 mm/min.

Effects of platycodin D₃ on contractile responses induced by phenylephrine and high K in the rat aortic strips

Intra-arterially given crude platycodin reduced coronary and hindquarter vascular resistances dose-dependently in anesthetized dogs and this vasodilator effect of crude platycodin appeared to be direct and non-specific on the peripheral blood vessels, similar to the case of glyceryl trinitrate or papaverine. (Kato et al, 1973). Therefore, it was of interest to compare the effects of platycodin D₃, which is another component of platycodin saponins, on the contractile responses induced by high potassium and phenylephrine.

In the presence of 8, 16 and 32 μg/ml of platycodin D₃, the aortic contractile responses evoked by phenylephrine (10⁻⁵ M) were 105 ± 11% (ns, n=6), 104 ± 4% (ns, n=6) and 96 ± 8% (ns, n=11) of the control response, respectively in comparison with the corresponding control response (2.5 ± 0.2 g) (Fig. 3).

High potassium-induced contractile response before platycodin D₃ treatment was 2.4 ± 0.3 g, while after pretreatment with 8, 16 and 32 μg/ml of platycodin D₃, the re-

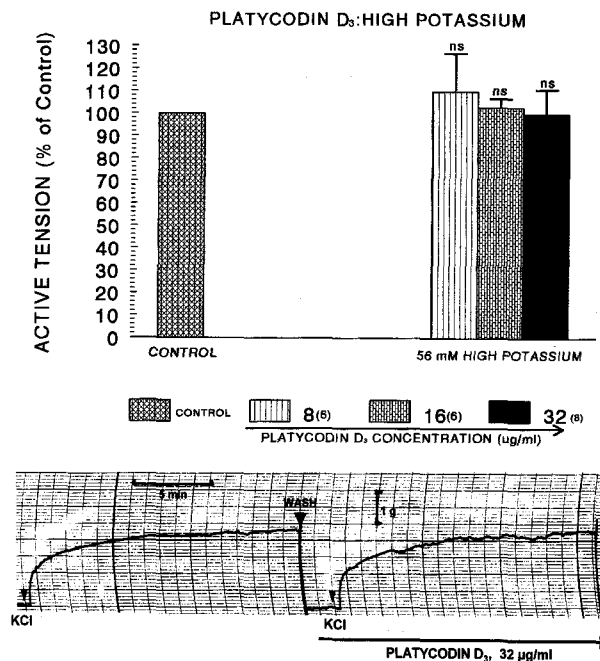


Fig. 4. Upper: Influence of platycodin D₃ on high potassium (KCL)-induced contractile responses in the isolated rat aortic strips. The contractile response was induced by 56 mM of KCL before (CONTROL) and after adding platycodin D₃ at concentrations of 8, 16 and 32 μg/ml. Other legends are the same as in Fig. 2. ns: Statistically not significant. Lower: The typical tracing showing the effect of platycodin D₃ on high potassium (KCL)-induced contractile responses in the rat aortic strips. Left: KCL-induced contractile response. Right: KCL-induced contractile response in the presence of 32 μg/ml of Platycodin D₃. At arrow mark, the indicated dose of KCL (56 mM) was added into the bath. The chart speed was 5 mm/min.

sponses were 110 ± 17% (ns, n=6), 103 ± 4% (ns, n=6) and 100 ± 11% (ns, n=8) of the corresponding control responses (Fig. 4).

Effects of platycodin D and platycodin D₃ on norepinephrine-induced hypertensive responses

Since platycodin D strongly inhibited phenylephrine-induced contractile responses of the isolated aortic smooth muscle as shown in Fig. 1, it is highly likely that platycodin D caused hypotension through the blockade of peripheral adrenergic α-receptors. Therefore, it is of interest to examine the effect of intravenously administered platycodin D on norepinephrine-evoked pressor responses. As shown in Fig. 5, in 12 rats, norepinephrine at doses of 0, 3, 1.0 and 3.0 μg/kg caused dose-dependent pressor responses of 13 ± 0.8 mmHg, 18 ± 1 mmHg and 29 ± 2 mmHg from the original baseline (122 ± 13 mmHg), respectively. However, after infusion of platycodin D with a rate of 1 mg/kg/30 min, they were significantly depressed to 9 ± 1 mmHg (P < 0.01), 13 ± 1 mmHg (P < 0.01) and 22 ± 2 mmHg (P < 0.01) at the same above doses, respectively (Fig. 5). Fig. 6 shows that norepinephrine-evoked pressor responses are greatly attenuated after pretreatment with intravenous platycodin D.

PLATYCODIN D:NOREPINEPHRINE

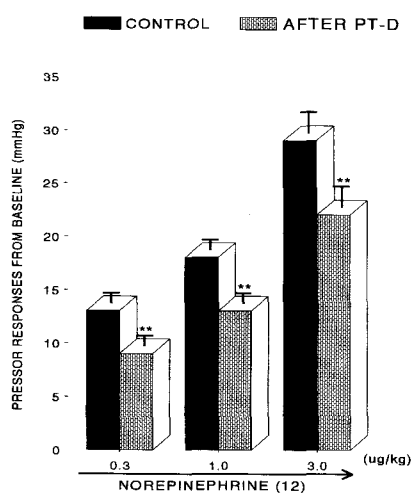


Fig. 5. Influence of intravenously administered platycodin D (PT-D) on norepinephrine-evoked pressor responses. Ordinate: Changes of blood pressure from baseline level in mmHg. Abscissa: Intravenous doses of norepinephrine in $\mu\text{g}/\text{kg}$. Vertical bar on the top of each column indicates standard error of mean. There was statistically significant difference in changes of norepinephrine-evoked pressor responses between before and after pretreatment with PT-D. PT-D was infused into a femoral vein at a rate of 1 mg/kg/30 min after obtaining the corresponding control responses of intravenous norepinephrine. Numeral in the parenthesis denotes number of animals used in the experiment. The original base-line of arterial blood pressure was 122 ± 13 mmHg. **: $P < 0.01$.

In order to compare the effect of platycodin D and D₃, the effect of platycodin D₃ on norepinephrine-evoked pressor responses was studied. In 12 rats, norepinephrine at doses of 0.3, 1.0 and 3.0 $\mu\text{g}/\text{kg}$ before the pretreatment with platycodin D₃ caused dose-dependent pressor responses of 14 ± 2 mmHg, 20 ± 2 mmHg and 32 ± 4 mmHg from the original baseline, respectively. However, after pretreatment with platycodin D₃ at a rate of 1 mg/kg/30 min, norepinephrine elicited the pressor responses of 12 ± 2 mmHg (ns), 19 ± 3 mmHg (ns) and 30 ± 3 mmHg (ns) at the same above doses, respectively (Fig. 7). Fig. 8 shows that norepinephrine-evoked pressor responses were not affected after pretreatment with intravenous platycodin D₃.

DISCUSSION

The present experimental results show that intravenously administered platycodin D caused a dose-dependent inhibition of norepinephrine-induced pressor action in the anesthetized rat, at least partly through the blockade of adrenergic α_1 -receptors. It seems that platycodin D also caused vascular relaxation in the isolated aortic strips of the rat via the blockade of adrenergic α_1 -receptors, in addition to an unknown direct mechanism. However, platycodin D₃ did not affect the contractile responses of the isolated rat aortic strips as well as norepinephrine-induced pressor action.

In support of this idea, Kato & his coworkers (1973) earlier showed that intra-arterial crude platycodin at

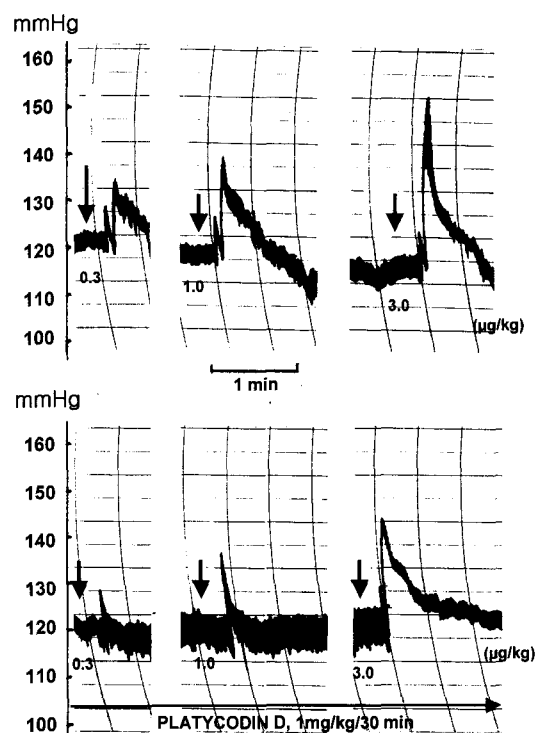


Fig. 6. The representative tracing of platycodin D's effect on intravenous norepinephrine (NE)-induced pressor response in the anesthetized rat. At arrow marks, the indicated doses (0.3, 1.0 and 3.0 $\mu\text{g}/\text{kg}$) of NE were administered into a femoral vein. Upper: NE-induced pressor responses in a non-treated rat. Lower: NE-induced pressor responses in a platycodin D-pretreated rat. Platycodin D was infused into a femoral vein at a rate of 1.0 mg/kg/30 min. ABP: Arterial blood pressure in mmHg. The chart speed was 20 mm/min.

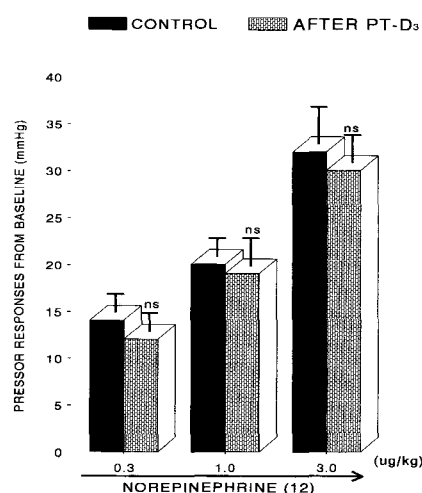
PLATYCODIN D₃:NOREPINEPHRINE

Fig. 7. Influence of intravenously administered platycodin D₃ (PT-D₃) on norepinephrine-evoked pressor responses. PT-D₃ was infused into a femoral vein at a rate of 1 mg/kg/30 min after obtaining the corresponding control responses of intravenous norepinephrine. Other legends are the same as in Fig. 10. ns: Statistically not significant.

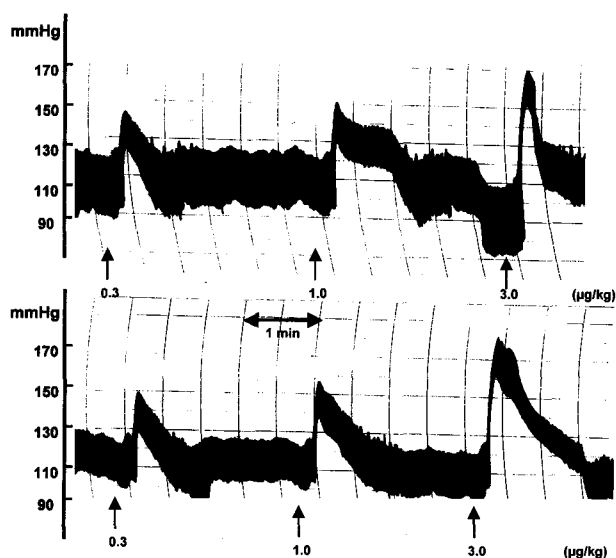


Fig. 8. The representative tracing of platycodin D₃'s effect on intravenously administered norepinephrine (NE)-induced pressor response in the anesthetized rat. At arrow marks, the indicated doses (0.3, 1.0 and 3.0 $\mu\text{g/kg}$) of NE were administered into a femoral vein. Upper: NE-induced hypertensive responses in a non-treated rat. Lower: NE-induced pressor responses in a platycodin D₃-pretreated rat. Platycodin D₃ was infused into a femoral vein at a rate of 1 mg/kg/30 min. ABP: arterial blood pressure in mmHg. The chart speed was 20 mm/min.

doses of 200 to 800 μg in dose-dependent manner reduced coronary and hindquarter vascular resistances in anesthetized dogs and the potency was comparable to that of papaverine. Crude platycodin (4 mg/kg, i.v.) also caused an increase in coronary and hindquarter blood flows, concomitantly with a transient systemic hypotension. The vasodilating effect of crude platycodin appears to be direct and non-specific on the peripheral blood vessels as in the case of glyceryl trinitrate or papaverine. Based on these findings, the present study demonstrates that platycodin D can cause the depressor effect.

Among drugs that interfere with peripheral sympathetic function, adrenergic α -receptor blocking agents alone in general cause reversal of the epinephrine pressor response (Constantine et al, 1973). When epinephrine is administered to untreated animals, its α -agonist properties predominate, resulting in a rise in mean arterial pressure. On the other hand, in the presence of adrenergic α -receptor blockade, the peripheral β_2 -agonist properties of epinephrine predominate and a fall in arterial pressure or reversal of the pressor response is observed. In contrast, the pressor responses to norepinephrine are impaired by adrenergic α -receptor blockade, but are not reversed (Freis et al, 1951), since this agent processes little β_2 -agonist activity (Ablad et al, 1975). Since phenylephrine-evoked contractile response is greatly depressed by platycodin D, platycodin D appears to have vascular dilatatory activity through the adrenergic α -receptor blockade. The present finding that platycodin D significantly suppressed the norepinephrine-induced pressor responses, together with the above observations, demonstrate that platycodin D possesses the antagonistic activity of adrenergic α_1 -receptors. Also, Crude platycodin in a relatively high concentration

(1×10^{-3} to 3×10^{-3} g/ml) has been shown to depress contractile force in isolated guinea pig atria, although the effect was mild (Takagi & Lee, 1972). However, in the present study, the pretreatment with platycodin D₃ failed to affect the hypertensive responses evoked by intravenous norepinephrine. Platycodin D₃ is another component of saponins found in *Plantaginis grandiflorum*. This finding suggests that platycodin D-induced depressor effect is not associated with platycodin D₃. Moreover, the result in the present study that platycodin D₃, a component of various saponins derived from *Plantaginis grandiflorum*, did not affect phenylephrine- as well as high potassium-induced contractile response further supports the fact that platycodin D's vasorelaxation is not associated with the effects of platycodin D₃. In support of this idea, it has recently been found that platycodin D₃ and oleanolic acid at 10 and 30 μM did not suppress prostaglandin E₂ production in rat peritoneal macrophages which were stimulated by the protein kinase C activator, 12-O-tetradecanoylphorbol 13-acetate (Kim et al, 2001).

Generally, it is well known that potassium chloride (KCl) opens voltage-dependent calcium channels by depolarizing the cell membrane of vascular smooth muscle, resulting in increased influx of extracellular Ca^{2+} (Bolton, 1979; Schwartz & Taira, 1983; Dube et al, 1985; 1988). Kim & his colleagues (1989) have shown that the contractile responses of vascular smooth muscle induced by CaCl_2 and KCl may result most likely from increased influx of extracellular Ca^{2+} through the voltage-dependent calcium channels. These results, together with the present findings, that platycodin D inhibited the contraction of rat aortic smooth muscle evoked by not only phenylephrine (an α_1 -adrenergic receptor agonist) but also by KCl (a membrane depolarizer), indicate that platycodin D's vascular relaxation is mediated by the blockade of α_1 -adrenergic receptors, in addition to an unknown mechanism of direct action.

In previous studies, three cellular mechanisms have been proposed to explain relaxant response of vascular smooth muscle: (i) blockade of extracellular Ca^{2+} entry into cells (Fleckenstein, 1977; Schwartz & Triggle, 1984), (ii) increase in binding or sequestration of intracellular Ca^{2+} (Watkins & Davidson, 1980; Imai & Kitagawa, 1981), and (iii) inhibition of the release of intracellular stored Ca^{2+} (Ito et al, 1980a, b; Imai & Kitagawa, 1981). In contrast, the contractions of vascular smooth muscles induced by neurohumoral agents have been suggested to comprise two components: Phasic contraction induced by the Ca^{2+} released from inside the cell and tonic tension related to the Ca^{2+} influx (Bevan, 1982; Dube et al, 1988), both leading to increased intracellular calcium. In light of these findings, it can not be ruled out that platycodin D can dilate the contractile responses of vascular smooth muscle evoked by phenylephrine through the blockade of extracellular Ca^{2+} entry into the muscle cells. Thus, these effects of platycodin D remain to be further investigated.

In conclusion, these experimental results demonstrate that intravenously administered platycodin D depresses norepinephrine-induced pressor responses dose-dependently in the anesthetized rat, at least partly through the blockade of adrenergic α -receptors. Platycodin D also causes vascular relaxation in the isolated aortic strips of the rat via the blockade of adrenergic α_1 -receptors, in addition to an unknown direct mechanism. However, platycodin D₃ does not affect both norepinephrine-induced pressor re-

sponses and the isolated rat aortic contractile responses evoked by phenylephrine and high potassium. Based on these results, there seems to be much difference in the mode of action between platycodin D and platycodin D₃.

REFERENCES

- Ablad B, Borg KO, Carlsson E, Johnson G, Malmfors L, Regardh CG. A survey of the pharmacological properties of metoprolol in animals and man. *Acta Pharmacol Toxicol (Copenh)* 36(5): 7–23, 1975
- Bevan JA. Selective action of diltiazem on cerebral vascular smooth muscle in the rabbit: antagonism of extrinsic but not intrinsic maintained tone. *Am J Cardiol* 46: 519–524, 1982
- Bolton TM. Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol Rev* 3: 606–718, 1979
- Constantine JW, Mcshane WK, Scriabine A, Hess HJ. Analysis of the hypotensive action of prazosin. In *Hypertension: Mechanisms and Management*, ed. by Onesti G, Kim KE, Moyer JH, New York, Grune & Stratton Inc, p 429, 1973
- Dube GP, Baik YH, Schwartz A. Effects of novel calcium channel agonist dihydropyridine analogue, Bay K 9644, on pig coronary artery: Biphasic mechanical response and paradoxical potentiation of contraction by diltiazem and nimodipine. *J Cardiovasc Pharmacol* 7: 377–389, 1985
- Dube GP, Baik YH, Van Breemen C, Schwartz A. Effects of isosorbide dinitrate and diltiazem on Ca²⁺ flux and contraction in artery. *European J Pharmacol* 145: 39–47, 1988
- Fleckenstein A. Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *Ann Rev Pharmacol Toxicol* 17: 149–166, 1977
- Freis EE, Mackey JD, Oliver WF. The effect of "sympatholytic" drugs on the cardiovascular responses to epinephrine and norepinephrine in man. *Cir Res* 3: 254, 1951
- Han LK, Kimura Y, Okuda H. Reduction in fat storage during chitin-chitosan treatment in mice fed a high-fat diet. *Int. J Obes* 23: 174–179, 1999a
- Han LK, Li J, Sumiyoshi M, Tsujita T, Kimura Y, Zheng YN, Okuda H. Reduction in fat storage during 4G-b-D-galactosylsucrose (lactosucrose) treatment in mice fed a high-fat diet. *J Traditional Med* 16: 66–71, 1999b
- Han LK, Takaku T, Li J, Kimura Y, Okuda H. Anti-obesity action of oolong tea. *Int J Obes* 23: 98–105, 1999c
- Han LK, Xu BJ, Kimura Y, Zheng YN, Okuda H. Platycodi Radix affects lipid metabolism in mice with high fat diet-induced obesity. *J Nutr* 130: 2760–2764, 2000
- Imai S, Kitagawa. A comparison of the differential effects of nitroglycerin, nifedipine, and papaverine on contractures induced in vascular and intestinal smooth muscle by potassium and lanthanum. *Jap J Pharmacol* 31: 193–199, 1981
- Ishii H, Tori K, Tozjo I, Yoshimura Y. Structures of polygalacin D₁ and D₂, platycodin D and D₃ and their monoacetates, saponins isolated from *Platycodon grandiflorum* A. D.C., determined by ¹³C nuclear magnetic resonance spectroscopy. *Chem Pharm Bull* 26: 674–677, 1978
- Ito Y, Kitamura K, Kuriyama H. Actions of nitroglycerin on the membrane and mechanical properties of smooth muscles of the coronary artery of the pig. *Br J Pharmacol* 70: 197–204, 1980a
- Ito Y, Kitamura K, Kuriyama H. Nitroglycerin and catecholamine actions on smooth muscle cells of canine coronary artery. *J Physiol (London)* 309: 171–183, 1980b
- Kato H, Suzuki S, Nakao K, LEE EB, Takagi K. Vasodilating effect of crude platycodin in anesthetized dogs. *Japan J Pharmacol* 23: 709–716, 1973
- Kim KS, Ezaki O, Ikemoto S, Itakura H. Effects of *Platycodon grandiflorum* feeding on serum and liver lipid concentrations in rats with diet induced hyperlipidemia. *J Nutr Sci Vitaminol* 41: 485–491, 1995
- Kim YP, Lee EB, Kim SY, Li D, Ban HS, Lim SS, Shin KH, Ohuchi K. Inhibition of prostaglandin E₂ production by platycodin D isolated from the root of *Platycodon grandiflorum*. *Planta Med* 67(4): 362–364, 2001
- Kim JM, Park KO, Baik YH. Effects of antiepileptic drugs on contractile responses of vascular smooth muscles. *Chonnam J Med Sci* 2(1): 50–59, 1989
- Schwartz A, Taira N. Calcium channel-blocking drugs: A novel intervention for the treatment of cardiac disease. *Circ Res (American Heart Association Monograph)* 52: 1–183, 1983
- Schwartz A, Triggle DJ. Cellular action of calcium blocking drugs. *Ann Rev Med* 35: 325–339, 1984
- Shin CY, Lee WJ, Lee EB, Choi EY, Ko KH. Platycodin D and D₃ increase airway mucin release in vivo and in vitro in rats and hamsters. *Planta Med* 68(3): 221–225, 2002
- Takagi K, Lee EB. Pharmacological studies on *Platycodon grandiflorum* A. DC. 3. Activities of crude platycodin on respiratory and circulatory systems and its other pharmacological activities. *Yakugaku Zasshi* 92(8): 969–973, 1972
- Tallarida RJ, Murray RB. *Manual of pharmacologic calculation with computer programs*. 2nd Ed. New York, Springer-Verlag, p 132, 1987
- Wada A, Takara H, Izumi F, Kobayashi H, Yanagihara N. Influx of ²²Na through acetylcholine receptor-associated Na channels: relationship between ²²Na influx, ⁴⁵Ca influx and secretion of catecholamines in cultured bovine adrenal medullary cells. *Neuroscience* 15: 283–292, 1985
- Watkins RW, Davidson IWF. Comparative effects of nitroprusside and nitroglycerin: Actions on phasic and tonic components of arterial smooth muscle contraction. *European J Pharmacol* 62: 191–200, 1980