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Vasorelaxing Effect by Protopanaxatriol and Protopanaxadiol of *Panax ginseng* in the Pig Coronary Artery

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Abstract□Saponin of *Panax ginseng* (C.A. Meyer) is composed of protopanaxatriol (PT) and protopanaxadiol (PD). We investigated the effects of PT and PD on the contractility and ^{45}Ca uptake in the pig coronary artery. Isometric tension in the helical strips and ^{45}Ca uptake in the ring strips were measured in the presence or absence of PT and PD. PT and PD did not affect the high K^+ (40 mM)-induced contraction but relaxed the ACh-induced contraction in a dose-dependent manner (1~10 mg/dl). The vasorelaxing effect of PT on the ACh-induced contraction was more potent than that of PD. Those relaxations were partially suppressed by the rubbing of endothelium removal. ACh-induced contraction in the Ca^{2+}-free Tyrode's solution was suppressed by the pretreatment of PT or PD. Following the depletion of ACh-sensitive intracellular Ca^{2+} pool, ACh-induced contraction was suppressed by the pretreatment of PT or PD. With the pretreatment of PT or PD, ^{45}Ca uptake by high K^+ (40 mM) was not changed but that by ACh was suppressed in the pig coronary artery. From the above results, we suggested that the vasorelaxing effect of PT and PD of *Panax ginseng* was due to iinhibition of intracellular Ca^{2+} release, inhibition of Ca^{2+} uptake via receptor-operated Ca^{2+} channels and in part a release of vasorelaxing factor from endothelium in pig coronary artery.

Key words□*Panax ginseng*, protopanaxatriol, protopanaxadiol, pig coronary artery, receptor-operated Ca channels.

Introduction

Panax ginseng (C.A. Meyer) has been used for more than two thousand years as a general tonic in traditional medicine in Korea for a variety of disorders. In spite of a number of studies on the biological effects of ginseng in the past, the pharmacological action of this herb is still mysterious.

There were reports about effects of *Panax ginseng* extract or saponins of *Panax ginseng* on the several vascular smooth muscles. Hah[1]) reported that noradrenaline-induced contraction was inhibited by ginseng alcohol extract in isolated guinea pig aortic strips. Also Lee[2]) reported that panaxatriol and panaxadiol of *Panax ginseng* inhibited noradrenaline-induced contraction in rabbit aorta in a dose-dependent manner and this relaxing effect of panaxatriol is more potent than that of panaxadiol. It was found that ginsenosides of *Panax ginseng* significantly reduced veterbral and femoral vascular resistance but increased renal vascular resistance.[3,4]) These findings suggest that ginsenosides may produce different responses in different blood vessels. It was also reported that ginsenosides and saponins extracted from *Panax ginseng* had cadioprotective function in the experimental model of myocardiac infraction in rabbits.[5,6]) However, the effects of saponins of *Panax ginseng* on the contractility in the coronary artery were not still unknown.

It is generally accepted that an increase of intracellular Ca concentration ([Ca]i) induces the contraction while decrease of [Ca]i induces the relaxation in vascular smoooth muscle. Extracellular Ca can enter into the cell through potential-dependent Ca channel or receptor-operated Ca channel in the vascular smooth muscle.[7])

Lee[2] reported that saponins of *Panax ginseng* inhibited Ca influx in the microsomal fraction of myocardium. However, there is few report about effects of saponins of *Panax ginseng* on the Ca movements in the vascular smooth muscles.

In the present study, we examined the vasorelaxing effects and mechanisms of PT and PD of *Panax ginseng* to clarify the effect of PT or PD on contractilty and ^{45}Ca uptake in the pig coronary artery.

Materials and Methods

1. Materials

Pig hearts were obtained from local slaughter house and transported to the laboratory in ice cold (4℃) oxygenated Tris-buffered Tyrode's solution. Branches of left anterior descending coronary artery (about 1 mm of outer diameter) were carefully dissected and cleaned of fat and periarterial connective tissue. Coronary artery was carefully cut into helical strips (2 mm wide and 10 mm long).

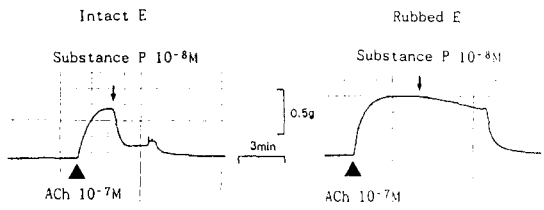

Fig. 1. Confirmation of endothelial removal by substance P on the ACh-induced contraction in the pig coronary artery. ACh (10^{-6} M)-induced contraction in the strips with endothelum (Intact E) was relaxed by substance P (10^{-8} M) but was not relaxed in the strips without endothelium (Rubbed E).

2. Isometric tension recording

The helical strips of coronary artery were mounted vertically between glass hooks in a thermostatically controlled organ baths containing 50 ml of the Tris-buffered Tyrode's solution contained (mM): NaCl 158, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, Tris 5, glucose 6 (pH 7.4 at 37℃). The temperature of organ bath solution was maintained at 37℃ and bubbled with a 100% O_2. The hook anchoring the upper end of the strip was connected to the lever of force transducer (F-60, Narco-Bio system) and the strips were suspended under a tension of 0.5 g. Each preparation was allowed to equilibrate for at least one hour. Isometeric tensions were recored on physiograph (MK-IV, Naro-Bio system).[8] High K^+ Tyrode's solution was prepared by replacing NaCl in the Tyrode's solution with an equivalent concentration of KCl and Ca^{2+}-free Tyrode's solution was prepared with addition of EGTA (0.1 mM).

To avoid the possible influence of the EDRF, endothelium was removed by gently rubbing the intimal surface with a cotton ball. And loss of endothelial function was tested by the absence of substance P-induced relaxing effect on ACh-induced contraction (Fig. 1). For depletion of Ca^{2+} from ACh-sensitive intracellular Ca^{2+} pool, ACh-induced contraction was repeated in the Ca^{2+}-free Tyrode's solution with washing until the contraction disappeared (Fig. 2).

Cumulative concentration-response curves were produced by the stepwise addition of PT or PD

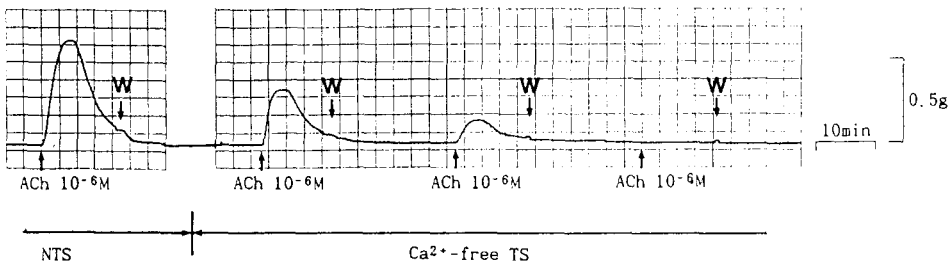

Fig. 2. Depletion of Ca^{2+} from ACh-sensitive intracellular Ca^{2+} pool. ACh (10^{-6} M)-induced contractions were progressively decreased by repeated administration of ACh in the Ca^{2+}-free Tyrode's solution (Ca^{2+}-free TS). NTS : Normal Tyrode's solution, W : Washout.

to the bath solution.

3. Measurement of ^{45}Ca uptake

The ^{45}Ca uptake was measured as described by Shibata *et al.*[9] Arterial rings were placed in test tubes containing HEPES-buffered Tyrode's solution with ^{45}Ca (1 μCi/m*l*) in the presence of PT or PD and incubated for 10 minutes in ACh-induced ^{45}Ca uptake or for 30 minutes in high K^+-induced ^{45}Ca uptake. After the incubation, the tissues were moved into test tubes containing 10 m*l* of ice-cold La^{3+}-substituted solution ($LaCl_3$ 73.8 mM, glucose 5 mM, Tris-HCl 10 mM adjusted to pH 6.8) and left them alone for 30 minutes. The tissues were than picked out, lightly blotted with filter paper, dried for 12 hours at 80℃ and weighed. Dried tissue was dissolved in nitric acid (0.2 m*l*) at 60℃ for 2 hours. After scintillation cocktail (Luma-gel, Lumac) was added, the radioactivity was counted by a liquid scintillation counter (Tri Carb 300C).

4. Data analysis and drugs

The results were expressed as means and S.E. Student's t-test was used for statistical analysis. P values of less than 0.05 were considered to be statistically significant.

Drugs used were acetylcholine, substance P, HEPES, Trizma base, Lanthanum and EGTA (Sigma), Luma-Gel (Lumac). ^{45}Ca (specific activity: 18.7 mCi/mg) was purchased fro New England Nuclear. Protopanaxatriol and Protopanaxadiol of *Panax ginseng* (C.M. Meyer) were donated by Korea Ginseng and Tobacco Research Institute.

Results

The effect of PT or PD on the contraction induced by high K^+ (40 mM) in the pig coronary arterial strips was examined (Fig. 3). When the contraction induced by 40 mM K^+ reached maximum, PT or PD were added to the bath solution in a cumulative manner. As shown in Fig. 3, they did not significantly affected the contraction at the range of 10 to 20 mg/dl of PT or PD.

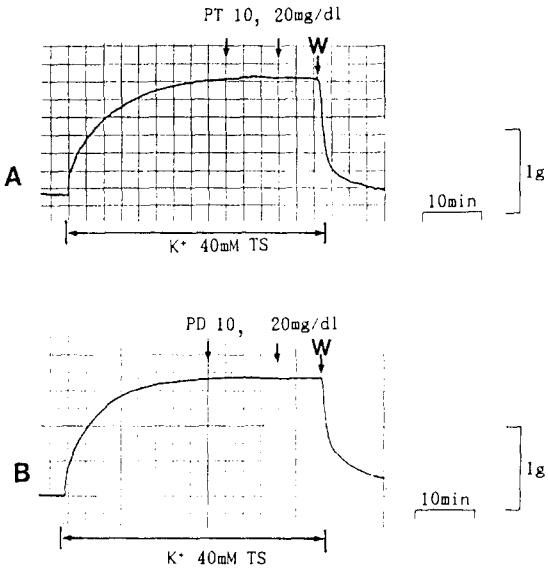

Fig. 3. Effects of protopanaxatriol (PT) (A) or protopanaxadiol (PD) (B) on the 40 mM K^+-induced contractions in the strips of pig coronary arteries. 40 mM K^+-induced contractions were not affected by PT or PD (10, 20 mg/dl). W : Washout.

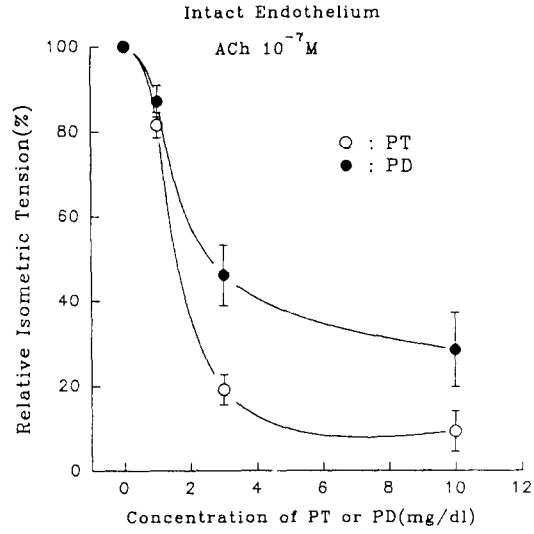

Fig. 4. Effects of PT or PD on the ACh (10^{-7} M)-induced contraction in the strips of pig coronary arteries. ACh (10^{-7} M)-induced contractions of arterial strips with intact endothelium were relaxed by PT or PD in a dose-dependent manner at the range of 1~10 mg/dl. Ordinate was expressed as percentage of ACh-induced contraction. Each point shows mean (n=6) and vertical bar is SEM.

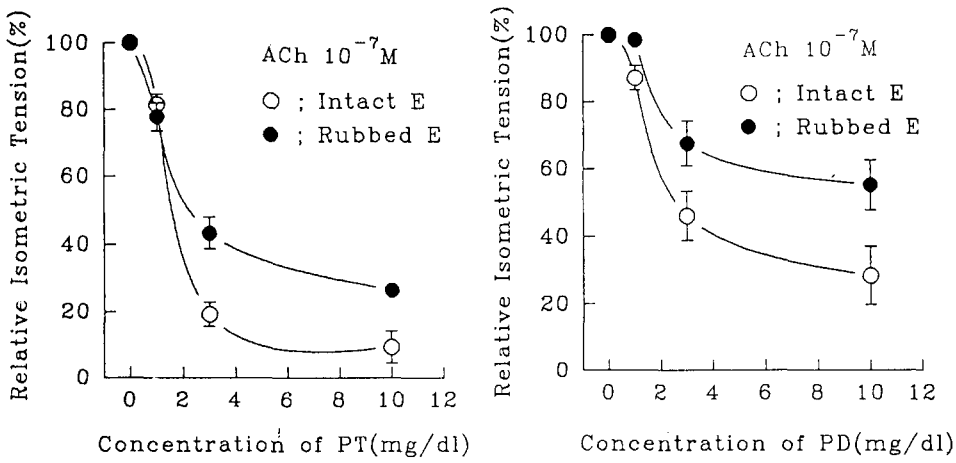

Fig. 5. Effects of endothelium on PT- or PD-induced relaxation in the strips of pig coronary arteries with endothelium (Intact E) or without endothelium (Rubbed E). PT- or PD-induced relaxation on the ACh (10^{-7} M)-induced contraction was partially decreased by the removal of endothelium. Ordinate was expressed as percentage of ACh-induced contraction. Each point shows mean (n=6) and vertical bar is SEM.

The effect of PT or PD on the contraction induced by ACh in the pig coronary arterial strips was examined (Fig. 4). 10^{-7} M ACh caused a phasic and tonic contraction in pig coronary artery, and the tension of tonic contraction cound be maintained for 20 minutes. When the contraction induced by 10^{-7} M ACh reached maximum, PT or PD were added to the bath solution in a cumulative manner. As shown in Fig. 4, both PT and PD significantly relaxed the ACh-induced contraction in a concentration-dependent manner (from 1 mg/dl to 10 mg/dl). The relaxing effect of PT was much stronger than that of PD.

We examined the effect of endothelium on the PT- or PD-induced relaxation in the ACh-induced contraction. As shown in Fig. 5A, B, the magnitude of relaxation by PT or PD was partially decreased by the rubbing of endothelium. However, the relaxation by PT or PD on the ACh-induced contraction was persisted even after the rubbing of endothelium.

In order to examine the effect of PT or PD on the intracellular Ca^{2+} release, we compared the magnitude of ACh-induced contractions in the Ca^{2+}-free Tyrode's solution in the presence or absence of PT or PD. As shown in Fig. 6, PT or PD (3 mg/dl) suppressed ACh-induced contractions in the

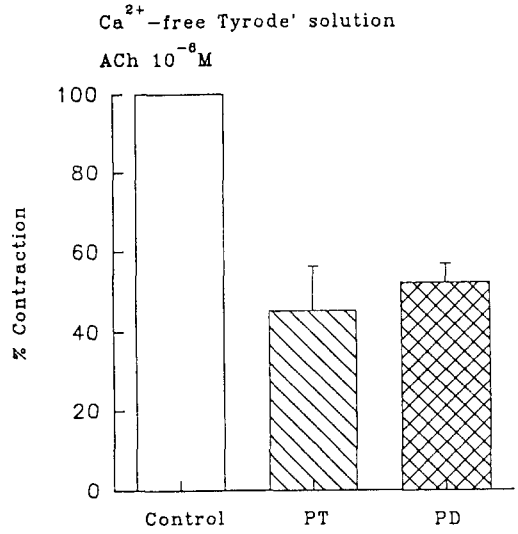

Fig. 6. Effects of PT (3 mg/dl)- or PD (3 mg/dl)-induced inhibition on the ACh (10^{-6} M)-induced contraction in the Ca^{2+}-free Tyrode's solution in the strips of pig coronary arteries. Ordinate was expressed as percentage of ACh-induced contraction. Each point shows mean (n=6) and vertical bar is SEM.

Ca^{2+}-free Tyrode's solution and magnitude of suppression was slightly greater in PT than that of PD.

In order to examine the effect of PT or PD on the Ca^{2+} uptake in the pig coronary artery, we de-

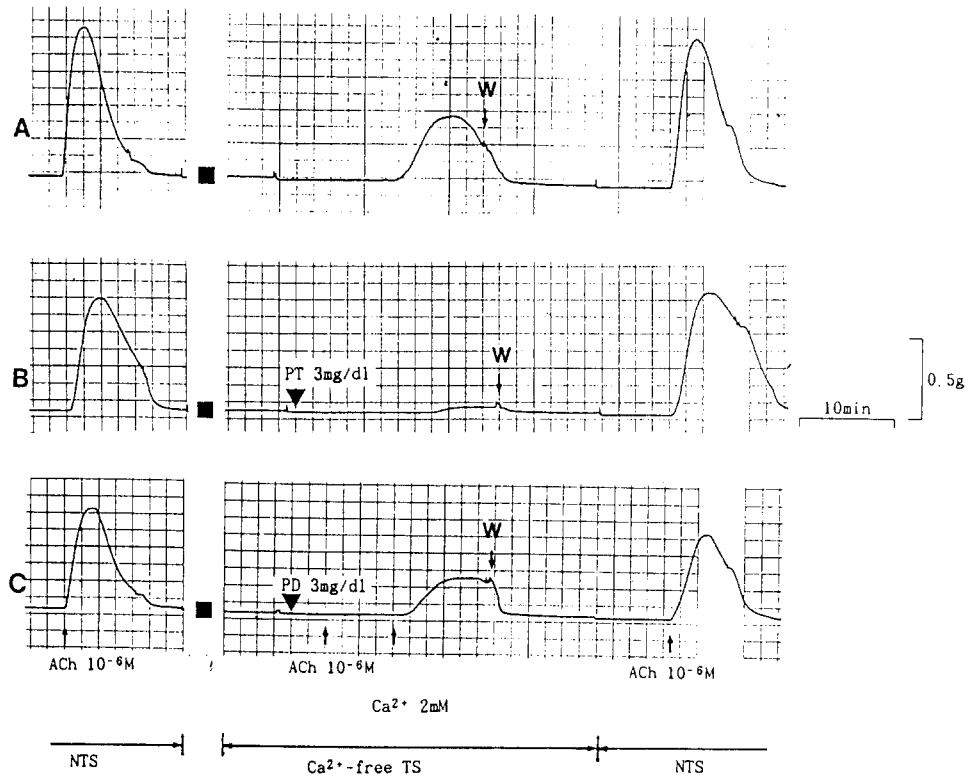

Fig. 7. Effects of pretreatment of PT or PD on the ACh (10^{-6} M)-induced contraction in the Ca^{2+}-free Tyrode's solution (Ca^{2+}-free TS) after depletion of ACh-sensitive intracellular Ca^{2+}-pool in the strips of pig coronary arteries. ■ : Depletion of ACh-sensitive intracellular Ca^{2+} pool. NTS : Normal Tyrode's solution, W : Washout.

A : no pretreatment of PT or PD (Control)
B : pretreatment of PT (3 mg/dl)
C : pretreatment of PD (3 mg/dl)

pleted the ACh-sensitive intracellular Ca^{2+} from Ca^{2+} pool of strips as a way of Fig. 2, and pretreated the arterial strips with ACh(A), PT and ACh(B), or PD and ACh(C) amd administrated Ca^{2+} for contraction (Fig. 7). As shown in Fig. 7, the contraction was significantly suppressed by the pretreatment of PT and PD (3 mg/dl), and the magnitude of suppression was more potent in PT than PD.

The effect of PT or PD on the Ca^{2+} uptake in ring strips was examined (Fig. 8, 9). We measured ^{45}Ca uptake induced by 40 mM K^+ and ACh in the presence or absence of PT or PD. ^{45}Ca uptake induced by K^+ for 30 minutes was 236.2± 16.7 μmole/kg dry weight and ^{45}Ca uptake induced by 40 mM K^+ in the presence of PT or PD was 232.5± 12.2 and 249.2± 10.1 μmole/kg dry weight, respectively (Fig. 8). PT or PD (10 mg/dl) did not significantly suppressed the uptake of ^{45}Ca induced by high K^+. However, ^{45}Ca uptake induced by ACh (10^{-6} M) for 10 minutes was 127.5± 7.3 μmole/kg dry weight and ^{45}Ca uptake induced by ACh (10^{-6} M) in the presence of PT or PD were 86.0± 5.0 and 99.4± 7.5 μmole/kg dry weight, respectively. PT or PD (10 mg/dl) significantly suppressed the ^{45}Ca uptake induced by ACh (10^{-6} M) ($p<0.001$; ACh & ACh+PT, $p<0.03$; ACh & ACh+PD). But the magnitude of inhibition by PT and PD (10 mg/dl) on the ACh-induced ^{45}Ca uptake did not significantly differ in the ring cut strips of pig coronary artery.

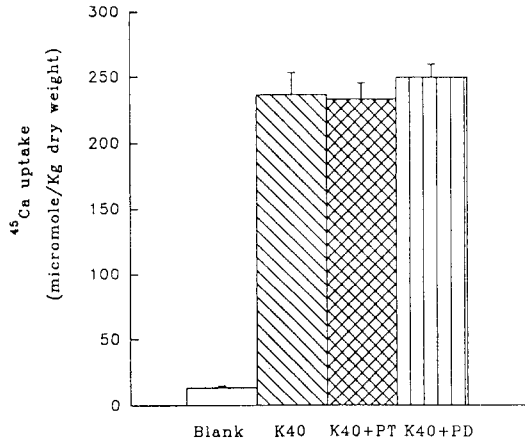

Fig. 8. Effects of PT or PD on the 40 mM K^+-induced ^{45}Ca uptake in the ring strips of pig coronary arteries. 40 mM K^+-induced ^{45}Ca uptake (1 μCi/ml) incubated for 30 minutes was not significantly affected by PT or PD. Ordinate was expressed as μmole/kg dry weight. Each bar shows mean of 6 samples and vertical line is SEM.
Blank ; ^{45}Ca+40 mM K^++La (5×10^{-3} M)
K40 : ^{45}Ca+40 mM K^+
K40+PT : ^{45}Ca+40 mM K^++PT (10 mg/dl)
K40+PD : ^{45}Ca+40 mM K^++PD (10 mg/dl).

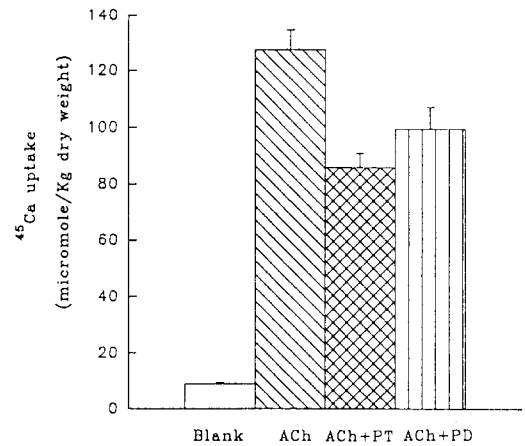

Fig. 9. Effects of PT or PD on the ACh-induced ^{45}Ca uptake in the ring strips of pig coronary arteries. ACh (10^{-6} M)-induced ^{45}Ca uptake (1 μCi/ml) incubated for 10 minutes was significantly decreased by the pretreatment of PT or PD. Ordinate was expressed as μmole/kg dry weight. Each bar shows mean of 6 samples and vertical line is SEM.
Blank : ^{45}Ca+ACh (10^{-6} M)+La (5×10^{-3} M)
ACh : ^{45}Ca+ACh (10^{-6} M)
ACh+PT : ^{45}Ca+ACh (10^{-6} M)+PT (10 mg/dl)
ACh+PD : ^{45}Ca+ACh (10^{-6} M)+PD (10 mg/dl)

Discussion

Saponins extracted from *Panax ginseng* is composed of protopanaxatriol (PT), protopanaxadiol (PD) and oleanolic acid. Each ginseng saponin is composed of several ginsenosides. It is known that PT and PD of *Panax ginseng* induced vascular relaxation in the rabbit aorta.[2] However, it was still unknown that effect and its mechanism of PT and PD of *Panax ginseng* on the contractility of coronary artery.

The present study demonstrates that PT and PD induced the relaxation on the ACh-induced contraction (Fig. 4) and these relaxation was partially reduced by the rubbing of endothelium (Fig. 5). These findings suggest that both smooth muscle cells and endothelial cells have been affected by PT or PD.

Calcium can be mobilized for contraction from extracellular fluid through potential-operated Ca^{2+} channels (POC) which are activated by high K^+.[10] PT or PD did not inhibit the high K^+-induced contraction and the uptake of ^{45}Ca induced by high K^+. This finding suggests that PT or PD may not block the Ca^{2+} uptake through potential-operated Ca^{2+} channels in the pig coronary artery.

It is known that ACh had no effect on the membrane potential and membrane resistance in the pig coronary artery.[11] The initiation and maintenance of tension in the response to ACh results primarily from mobilization of limited intracellular Ca^{2+} and Ca^{2+} entry through receptor-operated Ca^{2+} channels[7] acting on muscarinic receptors. In the present study, PT or PD relaxed ACh-induced contraction and the magnitude of relaxation by PT on the ACh-induced contraction was much greater than that by PD. Also PT or PD inhibited ACh-induced contraction following intracellular Ca^{2+} depletion from ACh-sensitive Ca^{2+} pool and inhibited the ^{45}Ca uptake induced by ACh. This finding suggests that PT or PD may block Ca^{2+} uptake through receptor-operated Ca^{2+} channels and this inhibitory action of PT on the contraction induced by ACh was more

potent than that of PD.

It is generally accepted that several agonists induced intracellular Ca^{2+} release in the absence of extracellular Ca^{2+}.[12] The pretreatment of PT or PD inhibited ACh-induced contraction in the Ca-free Tyrode's solution. Therefore, these results suggested that PT or PD inhibited the release of intracellular Ca^{2+} from the ACh-sensitive Ca^{2+} stores in the pig coronary artery.

In conclusion, we suggested that the vasorelaxing effect of PT and PD of *Panax ginseng* was due to inhibition of Ca^{2+} uptake via receptor-operated Ca^{2+} channels, inhibition of Ca^{2+} release from the ACh-sensitive intracellular Ca^{2+} stores and in part a release of vasorelaxing factor from endothelium in the pig coronary artery.

요 약

돼지관상동맥에서 고려인삼의 protopanaxatriol (PT)과 protopanaxadiol(PD)의 혈관이완작용을 알아보기 위하여 수축물질에 대한 수축반응과 ^{45}Ca 유입에 미치는 PT와 PD의 영향을 비교한 결과 다음과 같은 결론을 얻었다. PT와 PD는 고농도 K^+에 의한 수축에 영향을 주지 못하였으나 acetylcholine(ACh)에 의한 수축은 농도의존적(1~10 mg/dl)으로 억제하였다. ACh에 의한 수축에 PT의 이완정도는 PD에 의한 이완정도보다 컸으며, 이러한 이완반응은 내피세포제거에 의하여 부분적으로 억제되었다. Ca-free용액에서 ACh에 의한 수축은 PT 또는 PD 전처치에 의하여 억제되었으며, ACh에 민감한 세포내 Ca저장고를 고갈시킨 후 ACh에 의한 수축도 PT 또는 PD 전처치에 의하여 억제되었다. 고농도 K^+에 의한 ^{45}Ca유입은 PT 또는 PD 전처치에 의하여 억제되지 않았으나 ACh에 의한 ^{45}Ca유입은 PT 또는 PD 전처치에 의하여 억제되었다. 이상의 결과로 보아, 돼지관상동맥에서 고려인삼의 PT와 PD의 이완작용은 세포내 Ca유리의 억제와 수용체작동성 Ca통로를 통한 Ca유입의 억제 및 내피세포에서 유리되는 이완물질의 유리에 기인하는 것으로 사료된다.

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