2009년 2월
박사학위논문

Influence of Polyphenols Isolated from *Rubus coreanum* on Contractile Responses of Aortic Strips Isolated from Spontaneously Hypertensive Rats

조선대학교 대학원

의학과

민선영

Influence of Polyphenols Isolated from *Rubus coreanum* on Contractile Responses of Aortic Strips Isolated from Spontaneously Hypertensive Rats

복분자에서 분리한 폴리페놀이 자연발증 고혈압쥐의 적출대동맥편의 수축반응에 미치는 영향

2009년 2월 25일

조선대학교 대학원

의학과

민선영

Influence of Polyphenols Isolated from *Rubus coreanum* on Contractile Responses of Aortic Strips Isolated from Spontaneously Hypertensive Rats

지도교수 임 동 윤

이 논문을 의학박사 학위 신청논문으로 제출함.

2008 년 10 월

조선대학교 대학원

의학과

민선영

민 선 영의 박사학위논문을 인준함

위원장	전남대학교	교수	인
위 원	서울대학교	교수	인
위 원	조선대학교	교수	인
위 원	조선대학교	교수	인
위 원	조선대학교	교수	인

2008 년   12 월

조선대학교 대학원

CONTENTS

CONTENTS OF FIGURES

<국문초록>

복분자에서 분리한 폴리페놀이 자연발증 고혈압쥐의
적출대동맥편의 수축반응에 미치는 영향

민 선 영

(지도교수 : 임 동 윤)

조선대학교 대학원 의학과

　최근 기 및 임 (2007)은 복분자로 양조한 복분자주에서 분리한 폴리페놀 화합물 (PCRC)이 자연발증 고혈압쥐 (SHRs)의 적출 관류 부신수질에서 콜린성 (니코틴 및 무스카린) 수용체 흥분작용 및 직접적인 막탈분극에 의한 카테콜아민 (CA) 분비작용에 대하여 억제작용을 나타낸다고 보고하였다. 이러한 PCRC의 억제작용은 SHRs의 부신수질에서 내피 NO Synthase의 활성화에 의한 NO 생성증가로 인하여 부신크롬친화세포 내로 나트륨 및 칼슘유입과 세포 내 칼슘저장고로부터 칼슘유리의 억제작용에 기인된다고 하였다. 따라서, 본 연구의 목적은 PCRC가 SHRs에서 적출한 대동맥편의 수축반응 및 혈압상승반응에 대한 PCRC의 작용을 검색하고, 나아가 작용기전을 규명하는데 있으며, 본 연구를 수행하여 다음과 같은 연구 결과를 얻었다.

　PCRC (200~800 ㎍/ml)는 SHRs의 내피존재 적출 대동맥편에서

phenylephrine (α₁-아드레날린 수용체 작동제, 10 μM)에 의한 수축반응을 농도-의존적으로 억제하였다. 또한 PCRC (400 μg/ml)는 bath medium내로 투여시 고칼륨 (막탈분극제, 25 및 56 mM) 과 phenylephrine (3 및 10 μM)에 의한 수축반응을 뚜렷이 억제하였다. PCRC (400 μg/ml) 와 L-NAME (NO Synthase 억제제, 300 μM) 동시 존재 하에서 고칼륨 (56 mM) 과 phenylephrine (10 μM)에 의한 수축반응이 PCRC 단독처치 시 나타나는 억제효과에 비교하여 상응하는 대조치의 수준까지 회복되었다. 그러나 PCRC (400 μg/ml) 와 Indomethacin (선택성 Cyclooxygenase 억제제, 10 μM) 동시 존재 하에서 이들의 수축반응은 현저하게 억제되었다. 또한 CHAPS처치로 내피를 제거한 대동맥편에서 PCRC (400 μg/ml)는 고칼륨 (56 mM) 과 phenylephrine (10 μM)에 의한 수축반응에 아무런 영향을 미치지 못하였다. 흥미롭게도, 마취한 SHRs에서 PCRC (1~10mg/kg)를 30분간 주입한 후 norepinephrine에 의한 승압반응을 용량의존적으로 억제하였다.

이상의 연구결과를 종합하여보면, PCRC는 SHRs에서 분리 적출한 내피 존재 대동맥편에서 혈관이완작용을 나타내며, 이러한 PCRC의 혈관 이완작용은 적어도 SHRs의 혈관내피에서 NO Synthase의 활성화에 의한 NO 생성증가에 기인하며, Cyclooxygenase 활성화와는 관련성이 없는 것으로 사료된다. 이와 같은 PCRC의 작용으로 보아 PCRC가 고혈압 및 협심증을 비롯한 심혈관계 질환의 예방 및 치료에 유익할 것으로 생각된다.

I. INTRODUCTION

Rubus coreanum MIQUEL（覆盆子）has been presently used in treating the disease of the aged, spermatorrhea and impotence in oriental medicine. It is also the principal products of Gochang county, Chonbuk province, Korea, where is famous for wine brewed from *Rubus coreanum* MIQUEL (Bokboonja liquor, 복분자주). So far *Rubus coreanum* has been found to possess several polyphenolic compounds, such as (-)-epicatechin, (+)-catechin, proanthocyanidin, etc. Ethanol extract of *Rubus coreanum* showed the antioxidative activity with inhibitory effects on linoleic acid oxidation and LDL oxidation (Lee and Do, 2000).

Cho (2005) found that total phenol content of extract from *Rubus coreanum* M. was contained highly in hot-water extract than other extracts. These extracts elicited antioxidant protection as well as inhibitory activities on xanthine oxidase, pancreatin, α-amylase, and angiotensin converting enzyme (Cho, 2005).

Recently, it has been demonstrated that polyphenol compounds (PCRC), isolated from Bokboonja liquor, inhibits the CA secretory responses evoked by stimulation of cholinergic (both muscarinic and nicotinic) receptors as well as by direct membrane-depolarization from the isolated perfused adrenal gland of the normotensive rats (Kee and Lim, 2007) and spontaneously hypertensive rats (Lim and Hong, 2007). It seems that this inhibitory effect of PCRC is exerted by inhibiting both the Ca^{2+} influx into the rat adrenal medullary chromaffin cells and the uptake of Ca^{2+} into the cytoplasmic calcium store partly through the increased

NO production due to the activation of nitric oxide synthase (Kee and Lim, 2007; Lim and Hong, 2007*).*

Generally, the presence of polyphenolic compounds is widespread among plants and plant products (Formica and Regelson 1995; Zenebe and Pecháňová 2002). Several epidemiological studies have shown that consumption of foods rich in polyphenolic compounds is associated with lower incidence of cardiovascular disease. It was hypothesized that the cardioprotective effect of polyphenols results from their ability to protect low-density lipoprotein from oxidation, to prevent platelet aggregation and leukocyte adhesion, and to promote relaxation of vascular smooth muscle (Keli et al., 1996; Hertog et al., 1997). Polyphenols also act on other targets involved in the metabolism of mammalian cells, including nitric oxide (NO), which by itself regulates hemostasis (Palmer et al., 1987), thrombus development (Radomski et al., 1987) and vascular tone (Moncada et al., 1991; Zenebe et al., 2003). The beneficial properties of NO may therefore explain, at least in part, the beneficial effects of plant polyphenols. Several authors have reported that extracts from grapes and wine induce endothelium-dependent relaxation via enhanced generation and/or increased biological activity of NO leading to the elevation of cGMP levels (Fitzpatrick et al., 1993; Flesch et al., 1998). The critical step for the activation of NO synthase in endothelial cells is the increase in Ca^{2+} concentration leading to the production of NO and the subsequent endothelium-dependent vasorelaxation (Andriambeloson et al., 1999). The biological activity of NO can be effectively

increased by the scavengers of oxygen-free radicals (Bouloumié et al., 1997).

Thus, there are so far many reports about pharmacological effects of polyphenolic compound isolated from red grape wine on cardiovascular system. However, there have been a few reports on the effects of polyphenol compounds from Bokboonja liquor (PCRC) on cardiovascular system. Therefore, the purpose of the present study was to examine whether PCRC affects the contractile responses of the aortic strips isolated from spontaneously hypertensive rats (SHRs), blood pressure, and to clarify its mechanism of action.

Ⅱ. MATERIALS AND METHODS

Experimental Procedure

Mature male spontaneously hypertensive rats (purchased from DAMOOL SCIENCE, International Customer Service, Seoul, Korea), weighing 200 to 300 grams, 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 a week to adapt to experimental circumstances. On the day of experiment, a rat was anesthetized with thiopental sodium (50 mg/kg) intraperitoneally, and tied in supine position on fixing panel.

A) Isolation of Thoracic Aortic Strips: The thorax was opened by a midline incision, and the heart and surrounding area were exposed by placing 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 gauge pads in order to obtain enough working space for isolating thoracic 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.

B) Preparation for measurement of Arterial pressure: The animal was tied in supine position on 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 course of the 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% O_2 and 5% CO_2 at 37^0C. The composition (mM) of Krebs was: NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11.7. The final 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 with 35 mM KCl two times, and if it responded with contraction, the proper experiment was started. Vasoconstrictors were administered into the bath in order to obtain dose-response curves. In the subsequent experiments, under the presence of PCRC, some vasoconstrictors were administered, respectively. 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): 0.5mm]. The tubing was connected to a pressure transducer (Gould Co., U.S.A.) and pulse of mean arterial blood pressure was recorded on a biological polygraph (Grass Co., U.S.A.) continuously. The chart speed was adjusted to 2 cm per minute. The artery tubing was filled with heparin solution (400 I.U.) to prevent the blood coagulation during the experiment. Another cannulation with polyethylene tubing (o.d.: 0.3mm) was made into a femoral vein for the administration of drugs and supplemental anesthetic agents as needed 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 intervals of 60 minutes.

Removal of Endothelium

A solution containing 0.4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (CHAPS) was perfused for 30 s to remove the endothelium (Moore et al., 1990), followed by washout with the drug-free solution. The effect of CHAPS was confirmed by the absence of a flow increase due to 10^{-6} M acetylcholine and the presence of a response to 10^{-6} M sodium nitroprusside before the experiments were started. The vasoconstrictor-induced response of non-treated

(control) and CHAPS-treated preparations was compared in parallel.

Isolation of polyphenolic compounds

Polyphenolic compounds were prepared as described by Caderni et al (2000), using adsorption chromatography from a 1-year old wine brewed from *Rubus coreanum* Miquel (覆盆子) at the Research Institute of Bokboonja, Gochang County, Cheollabukdo Province, Korea or a 2-year-old cabernet sauvignon red wine made from Cabernet Sauvignon grapes by standard red wine making procedures at the Arzens Cooperative winery (Arzens, Aude, France), as follows (Fig. 1): alcohol was eliminated by distillation, and the remaining solution was deposited on a Diaion HP-20 column (Mitsubish Chemical Industries, Japan). After rinsing with water to remove sugars and organic acids, the phenolic pool of chemicals present in wine was eluted with 100% ethanol in water, concentrated by vacuum, evaporation and atomized, lyophilized by freezing dryer (Coldvac -80, Hanil R & D, Korea). About 2.9 g PCRC was obtained from 1 L Bokboonja wine, and 2.1 g PCRW from 1 L red grape wine. This indicates that the content of PCRC is higher in Bokboonja wine than red wine. The working solution of this PCRC was prepared by dissolving in 0.9% NaCl solution on the day of each experiment and filtered before administration.

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 by computer program described by Tallarida and Murray (1987).

Drugs and Their Sources

The following drugs were used: polyphenols isolated from *Rubus coreanum* M. (PCRC), phenylephrine hydrochloride, potassium chloride, Indomethacin, N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), and norepinephrine bitartrate (Sigma Chemical Co., U. S. A.), thiopental sodium and heparin sodium (Daehan Choongwae Pharm. Co., Korea). Drugs were dissolved in distilled water (stock) and added to the normal Krebs or saline solution as required. Concentrations of all drugs used are expressed in terms of molar base and gram.

III. RESULTS

Effects of polyphenols isolated from Rubus coreanum (PCRC) on contractile responses induced by phenylephrine and high K^+ in the thoracic aortic strips of SHRs

The resting (basal) tension from the isolated aortic strips of SHRs with intact endothelium reaches a steady state after the perfusion with oxygenated Krebs-bicarbonate solution for 90 min before the experimental protocol is initiated. The resting tension was adjusted to 0.5 g. The effects of PCRC on phenylephrine- as well as high potassium-induced contractile responses in the aorta of SHRs with intact endothelium were examined. In the present study, PCRC itself did not produce any effect on the resting tension in the aortic strips with intact endothelium isolated from the SHRs (data not shown). To establish dose-response curve of the inhibitory effects of PCRC on phenylephrine (10^{-6} M)-induced contractile responses, in the presence of PCRC at 200, 400 and 800 µg/ml, 5 min before addition of phenylephrine, the contractile responses of phenylephrine (10^{-6} M) were dose-dependently reduced to 80±10% (P< 0.01, n=8), 55±9% (P< 0.01, n=10) and 36±7% (P< 0.01, n=8) of the corresponding control response, respectively. In all subsequent experiments, a single dose of PCRC (400 µg/ml) was used.

When 10^{-6} M and 10^{-5} M of phenylephrine concentrations were administered into the aortic bath, their active tensions amounted to 1.7±0.2 g and 2.9 ±0.3 g from the resting tension level, respectively. However, in the presence of PCRC (400 µg/ml), their active tensions were reduced to 50±10% (P< 0.01, n=15) and

55±11% (P< 0.01, n=15) of the control contractile responses, respectively (Fig. 3 and 4).

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 potassium at the concentrations of 2.5 x 10^{-2} M and 5.6 x 10^{-2} M, which is a membrane-depolarizing agent, caused an increase in aortic contraction. As shown in Fig. 5 and 6, high potassium (2.5 x 10^{-2} M and 5.6 x 10^{-2} M)-induced contractile responses after pre-loading with 400 µg/ml of PCRC 5 min before high potassium were 43±12% (P< 0.01, n=10) and 58±8% (P< 0.01, n=10) of their corresponding control responses, respectively.

Influence of PCRC plus L-NAME on PCRC-induced inhibition to the contractile responses evoked by phenylephrine (PE) and high potassium (KCl) in the isolated aortic strips of SHRs

In previous study, it has been demonstrated that PCRC inhibits the CA secretion evoked by cholinergic stimulation and direct membrane-depolarization from the perfused rat adrenal medulla, which was blocked in the presence of L-NAME, a NO synthase inhibitor (Lim and Hong, 2007). These results suggest that PCRC can inhibit the CA release at least partly through the activation of nNOS in the adrenal medulla of SHRs. Therefore, in the presence of L-NAME, it was likely interesting to compare the effects of PCRC on the contractile responses induced by high potassium and phenylephrine.

In the simultaneous presence of PCRC (400 µg/ml) and L-NAME (300 µM), the aortic contractile response evoked by phenylephrine (10^{-5} M) was 111±11% (P<

0.01, n=9) of the control in comparison with the inhibitory response of PCRC-treatment alone (55±11%) from the resting tension level as shown in Fig. 7 and 8.

High potassium (5.6 x 10^{-2} M)-induced contractile response in the simultaneous presence of PCRC (400 µg/ml) and L-NAME (300 µM) was recovered to 92±8% (P< 0.01, n=7) of the corresponding control compared with the inhibitory response of PCRC-treatment alone (58±8%) from the resting tension level (Fig. 7 and 9).

Influence of PCRC plus indomethacin on PCRC-induced inhibition to the contractile responses evoked by phenylephrine (PE) and high potassium (KCl) in the isolated aortic strips of SHRs

Previously, procyanidins, oligomers of 3-flavanols (a subclass of flavonoids), induced concentration-dependent and endothelium-dependent relaxation in isolated human internal mammary artery, with a maximal vasorelaxant effect at 50 µM (Aldini et al., 2003). This effect was significantly reduced (by almost 50%) following preincubation of arterial rings with indomethacin, a cyclooxygenase inhibitor, indicating the involvement of a prostanoid (Aldini et al., 2003). Therefore, in the presence of indomethacin, it was likely interesting to compare the effects of PCRC on the contractile responses induced by phenylephrine and high potassium.

In the simultaneous presence of PCRC (400 µg/ml) and indomethacin (10 µM), the aortic contractile response evoked by phenylephrine (10^{-5} M) was not affected by relaxant effect of 33±11% (ns, n=6) of the control in comparison with the inhibitory response of PCRC-treatment alone (55±11%) from the resting tension

level as shown in Fig. 10 and 11.

High potassium (5.6×10^{-2} M)-induced contractile response in the simultaneous presence of PCRC (400 μg/ml) and indomethacin (10 μM) was also not affected by the inhibitory effect of 54±9% (ns, n=7) of the corresponding control compared with the inhibitory response of PCRC-treatment alone (64±9%) from the resting tension level (Fig. 10 and 12).

Influence of PCRC plus CHAPS on contractile responses induced by phenylephrine (PE) and high potassium (KCl) in the isolated aortic strips of SHRs

As shown in Fig. 7-9, PCRC-induced vasorelaxation was markedly blocked in the presence of L-NAME, a NO synthase inhibitor. Therefore, it is likely interesting to examine the effects of CHAPS, a detergent which suppresses endothelial function (Moore et al., 1990), on PCRC-induced inhibitory responses to the contractile active tension evoked by high potassium and phenylephrine.

In the presence of PCRC (400 μg/ml) after pretreatment with 0.4% CHAPS, the aortic contractile response evoked by phenylephrine (10^{-5} M) elicited 103±4% (ns, n=10) of the control in comparison with the corresponding control response (100%) from the resting tension level as shown in Fig. 13 and 14 (Upper panel).

High potassium (5.6×10^{-2} M)-induced contractile response in the simultaneous presence of PCRC (400 μg/ml) after pretreatment with CHAPS elicited 100±17% (ns, n=9) of the control in comparison with the corresponding control response (100%) from the resting tension level (Fig. 13 and 14-Lower panel).

Influence of intravenous PCRC on norepinephrine (NE)-evoked pressor

responses in the anesthetized SHRs

Since PCRC greatly inhibited phenylephrine-induced contractile response of the aortic strip of the SHR, as shown in Fig. 2-4, it suggests that PCRC might cause hypotension through the blockade of peripheral adrenergic α-receptors. It is also of interest to examine the effect of PCRC on norepinephrine-evoked pressor responses. When cardiovascular parameters were stabilized for 30 min before the experimental protocols were initiated, the administration of physiological saline solution in a volume of 0.2 ml into a femoral vein did not cause any changes in arterial blood pressure. Then, it was tried to test the effect of PCRC on norepenephrine-induced hypertensive responses in the anesthetized SHRs.

   In 10 SHRs, as shown in Fig. 15, norepinephrine at doses of 0,3, 1.0 and 3.0 µg/kg caused dose-dependent pressor responses of 14±1 mmHg, 22±2 mmHg and 32±2 mmHg from the original baseline (183±11 mmHg), respectively. After infusion of PCRC with a rate of 1 mg/kg/30min, hypertensive responses of norepinephrine were not altered compared to the corresponding controls (Fig.15 and 16). However, after increasing the dose of PCRC to 3 mg/kg/30min, norepinephrine-evoked hypertensive responses at doses of 0,3, 1.0 and 3.0 µg/kg were significantly inhibited to 67±7% (P< 0.01), 70±4% (P< 0.01) and 78±6% (P< 0.01) of control responses at the above same doses, respectively, as shown in Fig.15 and 16. Also, in the presence of larger dose of PCRC (10 mg/kg/30min), they were greatly inhibited to 58±8% (P< 0.01), 61±6% (P< 0.01) and 62±7% (P< 0.01) of control responses at the above same doses, respectively (Fig.15 and 16).

IV. DISCUSSION

The present experimental results demonstrate that PCRC causes vasorelaxation in the isolated aortic strips of SHRs at least partly by the increased NO production through the activation of NO synthase of vascular endothelium, but not through the activation of cyclooxygenase. In support of this idea, recently, it has been demonstrated that PCRC inhibits the CA secretory responses evoked by stimulation of cholinergic (both muscarinic and nicotinic) receptors as well as by direct membrane-depolarization from the isolated perfused adrenal gland of the normotensive rats (Kee and Lim, 2007) and spontaneously hypertensive rats (Lim and Hong, 2007). It seems that this inhibitory effect of PCRC is exerted by inhibiting both the Ca^{2+} influx into the rat adrenal medullary chromaffin cells and the uptake of Ca^{2+} into the cytoplasmic calcium store partly through the increased NO production due to the activation of nitric oxide synthase (Kee and Lim, 2007; Lim and Hong, 2007*).* In the present study, PCRC elicited a concentration-dependent inhibition in phenylehrine-induced contractile responses of aortic rings of SHRs with functional endothelium. This effect was greatly abolished in the absence of functional endothelium by treatment with CHAPS, which is a detergent for removal of endothelium, indicating that the vasodilator effect of PCRC is dependent on endothelium-derived relaxing factors. To evaluate the participation of NO in the vasorelaxant activity of PCRC, aortic rings were treated with L-NAME, a classical NO synthase inhibitor. In the present experimental condition, the PCRC-induced vasodilatation was markedly blocked, as similarly observed in

endothelium-denuded aortic rings by CHAPS, suggesting that NO is the main endothelium-derived relaxing factor involved in PCRC activity. The present results are fully in accordance with previous those findings obtained from red wines and grapes. Previously, it has been reported that red wines and grapes exhibit endothelium-dependent relaxation of blood vessels via enhanced generation and/or increased biological activity of NO, leading to the elevation of cGMP levels (Fitzpatrick et al., 1993; Fitzpatrick et al., 1995; Fitzpatrick et al., 2000; Zenebe et al., 2003). *In vivo* the polyphenol compounds of red wine (PCRW) were shown to reduce blood pressure in normotensive and hypertensive rats (Mizutani et al., 1999; Diebolt et al., 2001; Bernátová et al., 2002). The administration of purple grape juice improved the endothelium dependent, flow-mediated vasodilation in coronary artery disease patients with impaired endothelial function (Stein et al., 1999). The amplitude of vasorelaxation changed depending on the variability of wine constituents according to grape varieties, area of cultivation, and vinification methods. Consequently, the vasodilatatory effect does not apply to all wines and the degree of vasorelaxation is correlated to the content and type of phenols. Endothelium-dependent relaxation was greatest for red wines produced "en barrique", a procedure leading to high concentration of phenolic compounds (Flesch et al., 1998). A correlation between the phenolic content with vasodilatatory effect was later confirmed by Burns and his colleagues (2000). While the antioxidant activity was associated with different classes of phenols (gallic acid, resveratrol and catechins), vasodilatation activity was correlated only with the total content of anthocyanosides (ACs) (Burns et al., 2000). Investigations devoted to characterize PCRW responsible for the endothelium-dependent relaxation activity (Fitzpatrick et al., 2000;

Andriambeloson et al., 1997; Freslon et al., 1997; Andriambeloson et al., 1998; Fitzpatrick et al., 2002) agree that monomeric catechins and simple phenols (benzoic acid, gallic acid and hydroxycinnamic acids) are devoid of effect. On the contrary, AC enriched fractions and oligomeric proanthocyanidines (PAs: dimers, trimers and tetramers) were the active compounds. Threshold for relaxation by PAs oligomers was between 0.5 and 4 µg/ml (Fitzpatrick et al., 2000; Fitzpatrick et al., 2002). Much higher concentration (>0.1 mg/l) were required for ACs (Andriambeloson et al., 1998). The endothelium-dependent relaxation activity was lost when higher molecular weight polymers were assayed (Andriambeloson et al., 1998). PCRW enhanced NO synthesis and cGMP accumulation only in the presence of functional endothelium. In denuded aortic rings, PCRW concentration 103-fold higher was necessary to induce relaxation (Ndiaye et al., 2003; Corder et al., 2001). Besides NO, red wine affected the formation of other mediators of vascular tone, such as endothelium-derived hyperpolarizing factor (Ndiaye et al., 2003) and prostacyclin (Derek et al., 1997). The mechanisms underlining NO-dependent vasorelaxation caused by PCRW were investigated (Zenebe et al., 2003; Andriambeloson et al., 1999; Martin et al., 2002). In addition to the increased NO synthase activity, PCRW may prolong the half-life and increase the bioavailability of NO, by reducing its degradation mediated by reactive oxygen species (de aetano and Cerletti, 2001). It has also been that Provinol elicited endothelium-dependent relaxation of rat femoral artery by the Ca^{2+}-induced increase of NO synthase activity and by protecting NO from degradation (Zenebe et al., 2003). Recently, Yu and his colleagues (2008) have found that PCRW inhibits the CA secretory responses evoked by stimulation of cholinergic (both muscarinic and nicotinic) receptors as well as by direct

membrane-depolarization from the isolated perfused adrenal gland of the normotensive rats. It seems that this inhibitory effect of PCRW is mediated by blocking the influx of both ions through Na^+ and Ca^{2+} channels into the rat adrenomedullary chromaffin cells as well as by inhibiting the release of Ca^{2+} from the cytoplasmic calcium store, which are due at least partly to the increased NO production through the activation of nitric oxide synthase.

Consumption of wine polyphenol-, quercetin- or catechin-enriched diets increased aortic NO production in rats (Benito et al., 2002). Oral administration of an alcohol-free hydroalcoholic grape skin extract (from vinifera grape, *Vitis labrusca*) significantly reduced systolic, mean and diastolic arterial pressure in two distinct models of hypertensive Wistar rats (Soares De Moura et al., 2002). Intragastric administration of resveratrol (3 mg/kg/day), red wine (4 ml/kg/day) or even dealcoholized red wine (4 ml/kg/day) for 12 weeks to hypercholesterolemic rabbits improved the endothelial function, reduced plasma endothelin-1 levels and induced a significant elevation in NO levels (Zou et al., 2003). Moreover, in human studies, in healthy volunteers, the coronary flow-velocity reserve was increased 30 min after drinking red wine (1 g/kg ethanol), but not after drinking the same quantity of alcohol in white wine or vodka (Shimada et al., 1999). The endothelium-dependent vasodilation was also improved after acute intake of 500 ml of red wine or red wine without alcohol in men, as determined by ultrasonography of the brachial artery (Hashimoto et al., 2001). Endothelium-derived NO plays an important role in the control of vascular homeostasis. NO modulates the vascular tone, the growth of vascular smooth muscle cells, and decreases platelet adhesion and aggregation. It also decreases the adherence of other blood components (Moncada et al., 1991; Scott-Burden

and Vanhoutte, 1994). A decrease in NO production or bioavailability is closely associated with endothelial dysfunction or injury, which is an important factor in pathologies such as atherosclerosis, restenosis and hypertension (Landmesser and Drexler, 2007). PCRW and a grape skin extract also reduced blood pressure in males in several models of experimental hypertension (Bernatova et al., 2002; Pechanova et al., 2004; Sarr et al., 2006; Soares de Moura et al., 2002; Jiménez et al., 2007), which was related to a combination of vasodilator and antioxidant actions. Pechanova and his colleagues (2004) also provided evidence that Provinols partially prevents L-NAME-induced hypertension, cardiovascular remodeling and vascular dysfunction via the increase of NO-synthase activity and prevention of oxidative stress. Thus, in view of the beneficial effects of plant polyphenols, the present results of PCRC should shed light on the fact that the unique components of PCRC may contribte to the treatment or prevention of hypertension through their complex influence on the NO balance in the cardiovascular system.

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 extracellualr Ca^{2+} (Bolton, 1979; Schwartz & Taira, 1983; Dube et al., 1985; 1988). Kim and his colleagues (1989) have shown that the contractile responses of vascular smooth muscle induced by $CaCl_2$ and KCl may result most likely from the increased influx of extracellular Ca^{2+} through the voltage-dependent calcium channels (VDCCs). VDCCs are activated by depolarization of the plasma membrane when the extracellular K^+ concentration is increased. In the present work, incubation with

PCRC inhibited KCl concentration-dependent contractile response in aortic strips of SHRs. This result is consistent with the effect of 17-β estradiol on a large elastic aorta as in previous report (Li et al., 2002; 2006) and is also supported by another study (Nevala et al., 1998). These findings suggest that PCRC may have Ca^{2+} antagonistic properties and can inhibit extracellular Ca^{2+} influx through VDCCs, which are similar to those of 17-β estradiol or resveratrol. Generally, the mechanism of potassium-induced vasoconstriction has been shown to be through the calcium-influx by the opening of the voltage-dependent calcium channels (Spedding and Paoletti, 1992; Ryman et al., 1989). Voltage-dependent calcium channel blockers such as nifedipine or verapamil have been reported to attenuate potassium-induced vasoconstriction (Cortijo et al., 1986; Triggle et al., 1989). The contractile activity of vascular smooth muscle cells is mainly regulated by control over the cytoplasmic calcium concentration and both intracellular and extracellular calcium pools (Triggle et al., 1989; Johns et al., 1987). Based on these findings, the present results that PCRC inhibited high K^+-evoked contractile responses, and that the inhibitory effect of PCRC on high K^+-evoked contractile responses was enhanced, although their data are not shown here, indicate that PCRC may block the VDCCs in aortic smooth muscle cells.

In the present work, PCRC inhibited the norepinephrine-induced pressor responses as well as phenylephrine-evoked contractile responses in aortic strips isolated from SHRs. These results suggest that PCRC may elicit the antagonistic activity of adrenergic α_1-receptors.

In general, among drugs which interfere with peripheral sympathetic function, adrenergic α-receptor blocking agents alone 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. However, 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) as this agent processes little β_2-agonist activity (Ablad et al., 1975). Based on these findings, the results that phenylephrine-evoked contractile response and norepinephrine-induced hypertensive response were markedly depressed by PCRC, it is thought that the vasorelaxant activity of PCRC may be medated through the adrenergic α-receptor blockade.

On the other hand, vasodilatation was not significantly modified in aortic rings treated with indomethacin, a cyclooxygenase inhibitor, at a concentration which inhibited contraction by arachidonic acid. This finding demonstrates that prostanoids are probably not involved in vasodilatation induced by PCRC. In support of idea, indomethacin inhibits the synthesis of prostaglandins and markedly decreases the vascular relaxation induced by arachidonic acid in sheep isolated coronary artery (Cornish et al., 1983) and in newborn pigs (Leffler et al., 1993). However, in the present study, indomethacin did not affect PCRC-induced relaxation in endothelium-intact aortic strips. This result indicates that the release of vasodilator prostanoids is not involved in PCRC-induced relaxation in aortic strips isolated from SHRs. This finding is also in agreement with the report that incubation with indomethacin, the inhibitor of prostanoid synthesis, did not inhibit the concentration-dependent vasorelaxation induced by resveratrol in porcine

coronary rings with endothelium (Li et al., 2006).

Based on all these results, many studies strongly support the view that polyphenol-rich diet, such as Bokbooja (*Rubus coreanum*) and red wine, could improve endothelial function and that the mechanisms of this beneficial effect found in above discussed *in vitro* studies (especially increased NO) might be involved *in vivo*, both in patients and in animals.

In conclusion, as shown in Fig. 17, the present study provides conclusive data showing for the first time that PCRC elicits the endothelium- and NO-dependent vasorelaxation, which are due to unique polyphenolic constituents of PCRC that may augment eNOS activity and thus facilitates endothelial NO output, and suggesting that PCRC might be helpful in treating or alleviating cardiovascular diseases, such as hypertension and angina pectoris. The identification of the responsible constituents should help in the design of strategies to prevent or to improve cardiovascular diseases.

V. SUMMARY

Recently, Kee and Lim (2007) have demonstrated that polyphenolic compounds (PCRC), isolated from Bokboonja wine (覆盆子酒) which is brewed from *Rubus coreanum* MIQUEL, inhibits the secretory responses of catecholamines (CA) evoked by stimulation of cholinergic (both muscarinic and nicotinic) receptors as well as by direct membrane-depolarization from the isolated perfused adrenal gland of the normotensive rats. It seems that this inhibitory effect of PCRC is exerted by inhibiting both the calcium influx into the rat adrenal medullary chromaffin cells and the uptake of Ca^{2+} into the cytoplasmic calcium store partly through the increased NO production due to the activation of endothelial nitric oxide synthase (eNOS), which are at least relevant to the direct interaction with the nicotinic receptor itself. The purpose of the present study was to investigate whether PCRC may affect the contractility of the aortic strips isolated from spontaneously hypertensive rats (SHRs), and to clarify its mechanism of action. PCRC (200~800 μg/mL) concentration-depenedently blocked phenylephrine (10 μM)-induced contractile responses of the isolated aortic strips of SHRs. PCRC (400 μg/mL), added in to bath medium, also depressed the contractile active tension evoked by both phenylephrine (3 and 10 μM) and high potassium (25 and 56 mM). In the simultaneous presence of PCRC (400 μg/mL) and L-NAME (a selective inhibitor of NO synthase, 300 μM), the contractile responses evoked by phenylephrine and high K^+ were recovered to considerable level of the corresponding control release compared with those effects of PCRC-treatment alone. However, in the simultaneous presence of

indomethacin (10 μM, a selective cyclooxygenase inhibitor) and PCRC (400 μg/mL), they were not affected. In the endothelium-denuded aortic strips by CHAPS-treatment, PCRC did not affect the contractile responses induced by phenylephrine or high potassium. Interestingly, PCRC (1.0, 3.0 and 10.0 mg/kg/ 30 min, i.v., respectively) dose-dependently suppressed intravenous norepiphrine-induced vasopressor responses in anesthetized SHRs. Collectively, the present study provide these results demonstrate for the first time that PCRC causes vascular relaxation in the isolated aortic strips with intact endothelium of SHRs at least partly by the increased NO production through the activation of NO synthase of vascular endothelium, but not through the activation of cyclooxygenase. Based on these results, it seems that PCRC might be helpful to prevent or alleviate cardiovascular diseases, including hypertension and angina pectoris.

REFERENCES

Ablad, B., Borg, K. O., Carlsson, E., Johnson, G., Malmfors, L., and Regardh, C. G., A survey of the pharmacological properties of metoprolol in animals and man. *Acta. Pharmacol. Toxicol. (Copenh),* 36(5), 7-23 (1975).

Aldini, G., Carini, M., Piccoli, A., Rossoni, G., and Facino, R. M., Procyanidins from grape seeds protect endothelial cells from peroxynitrite damage and enhance endothelium-dependent relaxation in human artery: new evidences for cardio-protection. *Life Sci.,* 73, 2883–2898 (2003).

Andriambeloson, E., Kleschyov, A. L., Muller, B., Beretz, A., Stoclet J. C., and Andriantsitohaina, R., Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. *Br. J. Pharmacol.,* 120, 1053–1058 (1997).

Andriambeloson, E., Magnier, C., and Haan-Archipoff, G.*,* Lobstein Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta. *J. Nutr.,* 128, 2324–2333 (1998).

Andriambeloson, E., Stoclet, J.C., and Andriantsitohaina, R., Mechanism of endothelial nitric oxide-dependent vasorelaxation induced by wine polyphenols in rat thoracic aorta. *J. Cardiovasc. Pharmacol.,* 33, 248–254 (1999).

Benito, S., Lopez, D., Saiz, M. P., Buxaderas, S., Sanchez, J., Puig-Parellada, P., and Mitjavila, M. T., A flavonoid-rich diet increases nitric oxide production in rat aorta. *Br. J. Pharmacol.,* 135, 910–916 (2002).

Bernátová, I., Pecháòová, O., Babál, P., Kyselá, S., Stvrtina, S., and

Andriantsitohaina, R., Wine polyphenols improve cardiovascular remodelling and vascular function in NO-deficient hypertension. *Am. J. Physiol.*(*Heart Circ. Physiol.*), 282, H942–H948 (2002).

Bolton, T. M., Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.,* 3, 606-718 (1979).

Bouloumié, A., Bauersachs, J., and Linz, W., Endothelial dysfunction coincides with an enhanced nitric oxide synthase expression and superoxide anion production. *Hypertension,* 30, 934-941 (1997).

Burns, J., Gardner, P. T., O'Neil, J., Crawford, S., Morecroft, I., McPhail, D. B., Lister, C., Matthews, D., MacLean, M. R., Lean, M. E., Duthie, G. G., and Crozier, A., Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. *J. Agric. Food Chem.,* 48, 220–230 (2000).

Caderni, G., De Filippo, C., Luceri, C., Salvadori, M., Giannini, A., Biggeri, A., Remy, S., Cheynier, V., and Dolara, P., Effects of black tea, green tea and wine extracts on intestinal carcinogenesis induced by azoxymethane in F344 rats. *Carcinogenesis,* 21(11), 1965-1969 (2000).

Cho, Y. J., Comparison of physiological activities between hot-water and ethanol extracts of Bokbunja (*Rubus coreanum* F.). *Korean J. Food & Nutr.,* 34(6), 790-796 (2005).

Constantine, J. W., Mcshane, W. K., Scriabine, A. and Hess, H. J., Analysis of the hypotensive action of prazosin. In Onesti, G., Kim, K. E. and Moyer, J. H. (Eds.)s. Hypertension: Mechanisms and Management. New York, Grume & Stratton Inc., pp. 429, (1973).

Corder, R., Douthwaite, J. A., Lees, D. M., Khan, N. Q., Viseu Dos Santos, A. C.,

Wood, E. G., and Carrier, M. J., Endothelin-1 synthesis reduced by red wine. *Nature,* 414(6866), 863–864 (2001).

Cornish, E. J., Goldie, R. G., Krstew, E. V., and Miller, R. C., Effect of indomethacin on responses of sheep isolated coronary artery to arachidonic acid. *Clin. Exp. Pharmacol. Physiol.,* 10(2), 171-517 (1983).

Cortijo, J., Perpina, M., and Esplugues, J., Pharmacological characterization of the effects of verapamil and nifedipine on isolated guinea-pig lung parenchymal strip. *Gen. Pharmacol.,* 17, 211–217 (1986).

de Aetano, G., and Cerletti, C., Wine and cardiovascular disease. *Nutr. Metab. Cardiovasc. Dis.,* 11, 47–50 (2001).

Derek, D., Pearson, D. A., and German, J. B., Endothelial cell basal PGI2 release is stimulated by wine in vitro: one mechanism that may mediate the vasoprotective effects of wine. *J. Nutr. Biochem.,* 8, 647–651 (1997).

Diebolt, M., Bucher, B., and Andriantsitohaina, R., Wine polyphenols decrease blood pressure, improve NO vasodilatation, and induce gene expression. *Hypertension,* 38, 159–165 (2001).

Dube, G. P., Baik, Y. H., and 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, G. P., Baik, Y. H., Van Breemen, C., and Schwartz, A., Effects of isosorbide dinitrate and diltiazem on Ca^{2+} flux and contraction in artery. *European J. Pharmacol.,* 145, 39-47 (1988).

Fitzpatrick, D. F., Bing, B., Maggi, D. A., Fleming, R. C., and O'Malley, R., Vasodilating procyanidins derived from grape seeds. *Ann. N. Y. Acad. Sci.,*

957, 78–89 (2002).

Fitzpatrick, D. F., Fleming, R. C., Bing, B., Maggi, D. A., and O'Malley, R., Isolation and characterization of endothelium-dependent vasorelaxing compounds from grape seeds. *J. Agric. Food Chem.,* 48(12), 6384–6390 (2000).

Fitzpatrick, D. F., Hirschfield, S. L., and Coffey, R. G., Endothelium-dependent vasorelaxing activity of wine and other grape products. *Am. J. Physiol.,* 265, H77-78 (1993).

Fitzpatrick, D. F., Hirschfield, S. L., Ricci, T., Jantzen, P., and Coffey, R. G., Endothelium-dependent vasorelaxation caused by various plant extracts. *J. Cardiovasc. Pharmacol.,* (1), 90–95 (1995).

Flesch, M., Schwarz, A., and Bolun, M., Effects of red and white wine on endothelium-dependent vasorelaxation of rat aorta and human coronary arteries. *Am. J. Physiol.,* 275(4 Pt 2), H1183–H1190 (1998).

Formica, J. V., and Regelson, W., Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol.,* 33, 1061-1080 (1995).

Freis, E. D., Mackay, J. C., and Oliver, W. F., The effect of "sympatholytic" drugs on the cardiovascular responses to epinephrine and norepinephrine in man. Circulation, 3, 254-259 (1951).

Freslon, J. L., Mendes, A., Desgranges, C., and Vercauteren, J., Procyanidin oligomers from grape seed induce NO-dependent relaxation of aortic rings via $P2Y_1$ receptor activation. *Br. J. Pharmacol.,* 122, 200 [abstract] (1997).

Hashimoto, M., Kim, S., Eto, M., Iijima, K., Ako, J., Yoshizumi, M., Akishita, M., Kondo, K., Itakura, H., Hosoda, K., Toba K., and Ouchi, Y., Effect of acute intake of red wine on flow-mediated vasodilatation of the brachial artery. *Am.*

J. Cardiol., 88, 1457–1460 (2001).

Hertog, M. G. L., Feskens, E. J. M., and Kromhout, D., Antioxidant flavonols and coronary heart disease risk: ten year follow-up of the Zutphen elderly study. *Lancet,* 349, 699 (1997).

Jiménez, R., López-Sepúlveda, R., Kadmiri, M., Romero, M., Vera, R., Sánchez, M., Vargas, F., O'Valle, F., Zarzuelo, A., Dueñas, M., Santos-Buelga, C., and Duarte, J., Polyphenols restore endothelial function in DOCA-salt hypertension: role of endothelin-1 and NADPH oxidase. *Free Radic. Biol. Med.,* 43, 462–473 (2007).

Johns, A., Leijten, P., and Yamamoto, H. et al., Calcium regulation in vascular smooth muscle contractility. *Am. J. Cardiol.,* 59, 18A–23A (1987).

Kee, Y. W., and Lim, D. Y., Influence of polyphenolic compounds isolated from *Rubus coreanum* on catecholamine release in the rat adrenal medulla. *Arch. Pharm. Res.,* 30(10), 1240-1251 (2007).

Keli, S. O., Hertog, M. G. L., Feskens, E. J. M., and Kromhout, D., Flavonoids, antioxidant vitamins and risk of stroke. The Zutphen elderly study. *Arch. Intern. Med.,* 156, 637-642 (1996).

Kim, J. M., Park, K. O., and Baik, Y. H., Effects of antiepileptic drugs on contractile responses of vascular smooth muscles. *Chonnam J. Med. Sci.,* 2(1), 50-59 (1989).

Landmesser, U., and Drexler, H., Endothelial function and hypertension. *Curr. Opin. Cardiol.,* 22(4), 316-320 (2007).

Lee, J. W., and Do, J. H., Chemical compound and volatile flavor of *Rubus coreanum. Korean J. Food & Nutr.,* 13(5), 453-459 (2000).

Leffler, C. W., Mirro, R., Shibata, M., Parfenova, H., Armstead, W. M., and

Zuckerman, S., Effects of indomethacin on cerebral vasodilator responses to arachidonic acid and hypercapnia in newborn pigs. *Pediatr. Res.,* 33(6), 609-614 (1993).

Li, H. F., Li, W., Zheng, T. Z., Qu, S. Y., and Zhang, C. L., A study of the mechanisms involved in relaxation induced by 17 β-estradiol in the isolated rabbit aorta. *Arch. Gynecol. Obstet.,* 266, 101-104 (2002).

Li, H. F., Tian, Z. F., Qiu, X. Q., Wu, J. X., Zhang, P, and Jia, Z. J., A Study of mechanisms involved in vasodilatation induced by resveratrol in isolated porcine coronary artery. *Physiol, Res.,* 55(4), 365-372 (2006).

Lim, D. Y., and Hong, S. P., Inhibitory mechanism of polyphenols isolated from *Rubus coreanum* on catecholamine secretion from the adrenal medulla of SHRs. *6th Asian-Pacific Congress of Hypertension, Abstract Book,* 2-345 (2007).

Martin, S., Andriambeloson, E., Takeda, K., and Andriantsitohaina, R., Red wine polyphenols increase calcium in bovine aortic endothelial cells: a basis to elucidate signalling pathways leading to nitric oxide production. *Br. J. Pharmacol.,* 135, 1579–1587 (2002).

Mizutani, K., Ikeda, K., Kawai Y., and Yamori, Y., Extract of wine phenolics improves aortic biomechanical properties in stroke-prone spontaneously hypertensive rats (SHRSP). *J. Nutr. Sci. Vitaminol.(Tokyo),* 45(1), 95–106 (1999).

Moncada, S., Palmer, R. M. J., and Higgs, E. A., Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.,* 43, 109-142 (1991).

Moore, P., Kal-Swayeh, O. A., Chong, N. W., Evans, R. A., and Gibson, A., L-NG-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of

endothelium-dependent vasodilatation *in vitro*. *Br. J. Pharmacol.,* 99(2), 408–412 (1990).

Ndiaye, M., Chataigneau, T., Andriantsitohaina, J. C. S., and Schini-Kerth, V. B., Red wine polyphenols cause endothelium-dependent EDHF-mediated relaxations in porcine coronary arteries via a redox-sensitive mechanism. *Biochem. Biophys. Res. Commun.,* 310, 371–377 (2003).

Nevala, R., Korpela, R., and Vapaatalo, H., Plant derived estrogens relax rat mesenteric artery in vitro. *Life Sci.,* 63(6), 95-100 (1998).

Palmer, R. M. J., Ferrige, A. G., and Moncada, S., Nitric oxide release account for the biology activity of endotheliumderived relaxing factor. *Nature,* 327, 524-526 (1987).

Pechanova, O., Bernatova, I., Babal, P., Martinez, M. C., Kysela, S., Stvrtina, S., and Andriantsitohaina, R., Red wine polyphenols prevent cardiovascular alterations in L-NAME-induced hypertension. *J. Hypertens.,* 22, 1551–1559 (2004).

Radomski, M. W., Palmer, R. M. J., and Moncada, S., The anti-aggregating properties of vascular endothelium adhesion to vascular endothelium. *Lancet,* 2, 1057-1058 (1987).

Ryman, T., Brandt, L., Anderson, K. E. et al., Regional and species differences in vascular reactivity to extracellular potassium. *Acta. Physiol. Scand.,* 136, 151–159 (1989).

Sarr, M., Chataigneau, M., Martins, S., Schott, C., El Bedoui, J., Oak, M. H., Muller, B., Chataigneau, T., and Schini-Kerth, V. B., Red wine polyphenols prevent angiotensin II-induced hypertension and endothelial dysfunction in rats: role of NADPH oxidase. *Cardiovasc. Res.,* 71, 794–802 (2006).

Schwartz, A., and 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).

Scott-Burden, T., Vanhoutte, P. M., Regulation of smooth muscle cell growth by endothelium-derived factors. *Tex. Heart Inst. J.,* 21(1), 91-97 (1994).

Shimada, K., Wanatabe, H., Hosoda, K., Takeuchi K., and Yoshikawa, J., Effect of wine in coronary flow-velocity reserve. *Lancet,* 354, 1002 (1999).

Soares de Moura, R., Costa Viana, F. S., Souza, M. A. V., Kovary, K., Guedes, D. C., Oliviera, E. P., Rubenich, L. M., Carvalho, L. C., Oliveira, R. M., Tano, T., and Gusmao Correia, M. L., Antihypertensive, vasodilator and antioxidant effects of vinifera grape-skin extract. *J. Pharm. Pharmacol.,* 54, 1515–1520 (2002).

Spedding, M., and Paoletti, R., Classification of calcium channels and the sites of action of drugs modifying channel function. *Pharmacol. Rev.,* 44, 363–376 (1992).

Stein, J. H., Keevil, J. G., Wiebe, D. A., Aeschlimann S., and Folts, J. D., Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation,* 100(10), 1050–1055 (1999).

Tallarida, R. J., and Murray, R. B., Manual of pharmacologic calculation with computer programs. (2nd Ed.). Speringer-Verlag, New York, pp. 132, (1987).

Triggle, D. J., Zheng, W., Hawthorn. M. et al., Calcium channels in smooth muscle: properties and regulation. *Ann. NY Acad. Sci.,* 560, 215–229 (1989).

Wada, A., Takara, H., Izumi, F., Kobayashi, H., and Yanagihara, N., Influx of ^{22}Na through acetylcholine receptor-associated Na channels: relationship between

[22]Na influx, [45]Ca influx and secretion of catecholamines in cultured bovine adrenal medullary cells. *Neuroscience,* 15, 283-292 (1985).

Yu, B. S., Ko, W. S., and Lim, D. Y., Inhibitory mechanism of polyphenol compounds isolated from red wine on catecholamine release in the perfused rat adrenal medulla. *Biomolecules & Therapeutics,* 16(2), 147-160 (2008).

Zenebe, W., and Pecháňová, O., Effect of red wine polyphenolic compounds on the cardiovascular system. *Bratisl. Lek. Listy,* 103, 159-165 (2002).

Zenebe, W., Pecháňová, O., and Andriantsitohaina, R., Red wine polyphenols induce vasorelaxation by increased nitric oxide bioactivity. *Physiol. Res.,* 52, 425–432 (2003).

Zou, J. G., Wang, Z. R., Huang, Y. Z., Cao, K. J., and Wu, J. M., Effect of red wine and wine polyphenol resveratrol on endothelial function in hypercholesterolemic rabbits. *Int. J. Mol. Med.,* 11, 317–320 (2003).

Wine of *Rubus coreanum* MIQUEL (覆盆子酒)

Diaion HP-20 column/chromatography

(H$_2$O, 16L; EtOH, 16L)

H$_2$O Eluent

(Discard)

EtOH Eluent

(Polyphenolic Fraction)

Concentration
Lyophilzed & Finely Sprayed

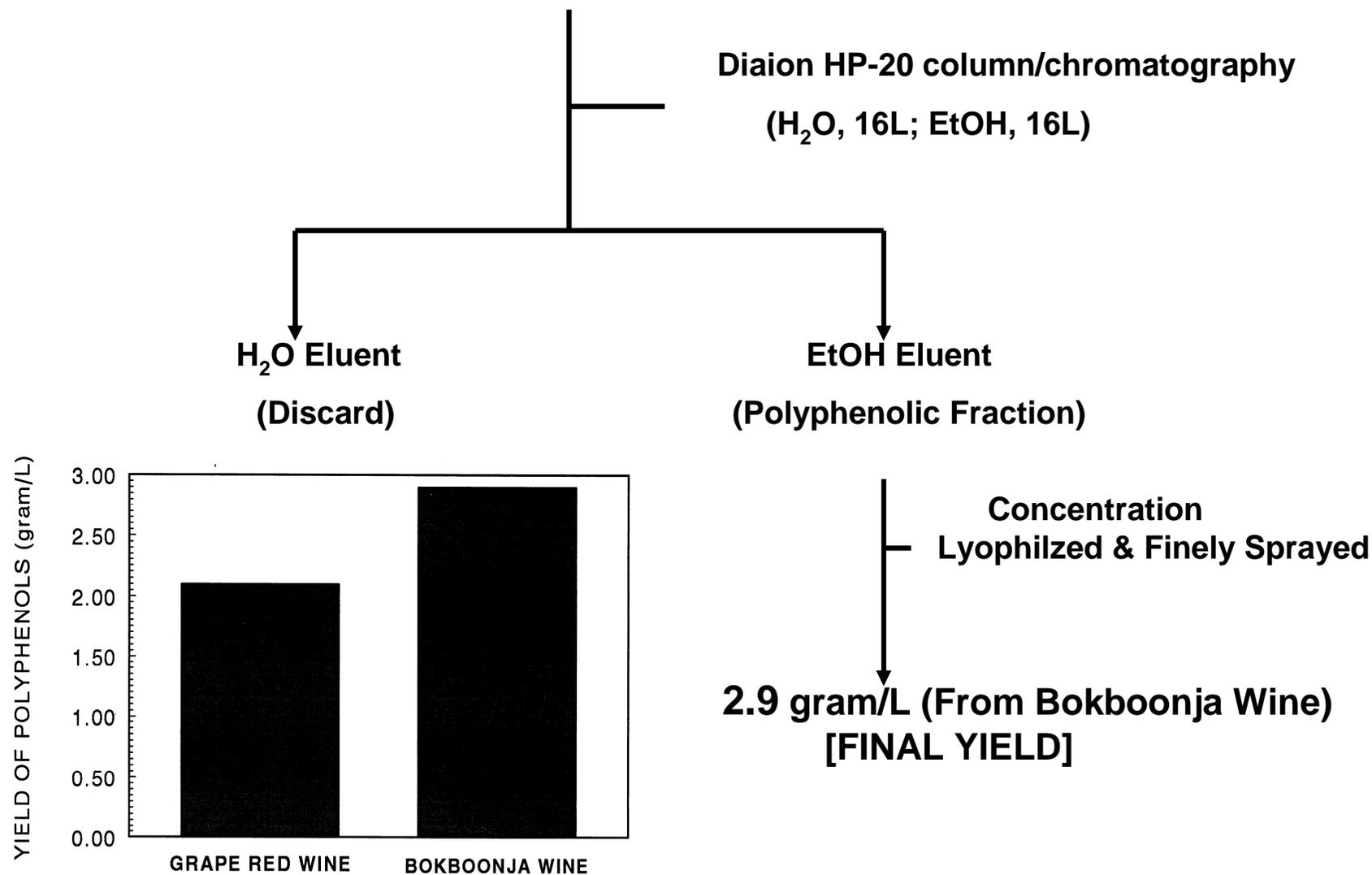**2.9 gram/L (From Bokboonja Wine)**
[FINAL YIELD]

Fig. 1. Preparation of polyphenolic compounds from Bokboonja wine (*Rubus coreanum* MIQUEL, 覆盆子酒).
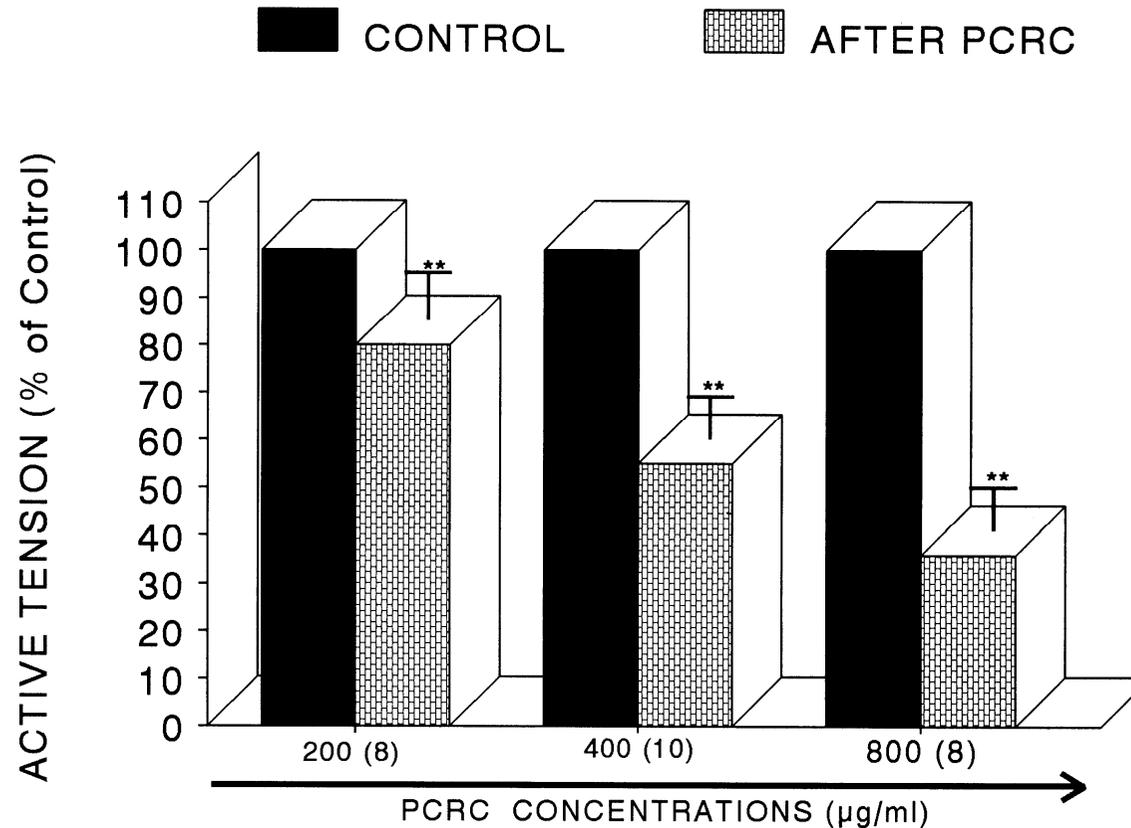
Fig. 2. Dose-dependent inhibitory effects of polyphenolic compounds isolated from *Rusus coreanum* (PCRC) on phenylephrine (PE)-induced contractile responses in the isolated aortic strips of spontaneously hypertensive rats (SHRs). The contractile responses were induced by adding 10 μM of PE at 120 min interval after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol. "CONTROL" and "AFTER" denote active tension induced evoked by PE before (CONTROL) and after adding 200, 400 and 800 μg/ml of PCRC for 20 min. Numeral in the parenthesis indicates number of aortic strips isolated from SHRs. Vertical bars represent the standard error of the mean (S.E.M). Ordinate: the active tension (% of control [PE , 10 μM]). Abscissa: Concentrations of PCRC (μg/ml). Statistical difference was obtained by comparing the control with the PCRC-pretreated group. **: P< 0.01.
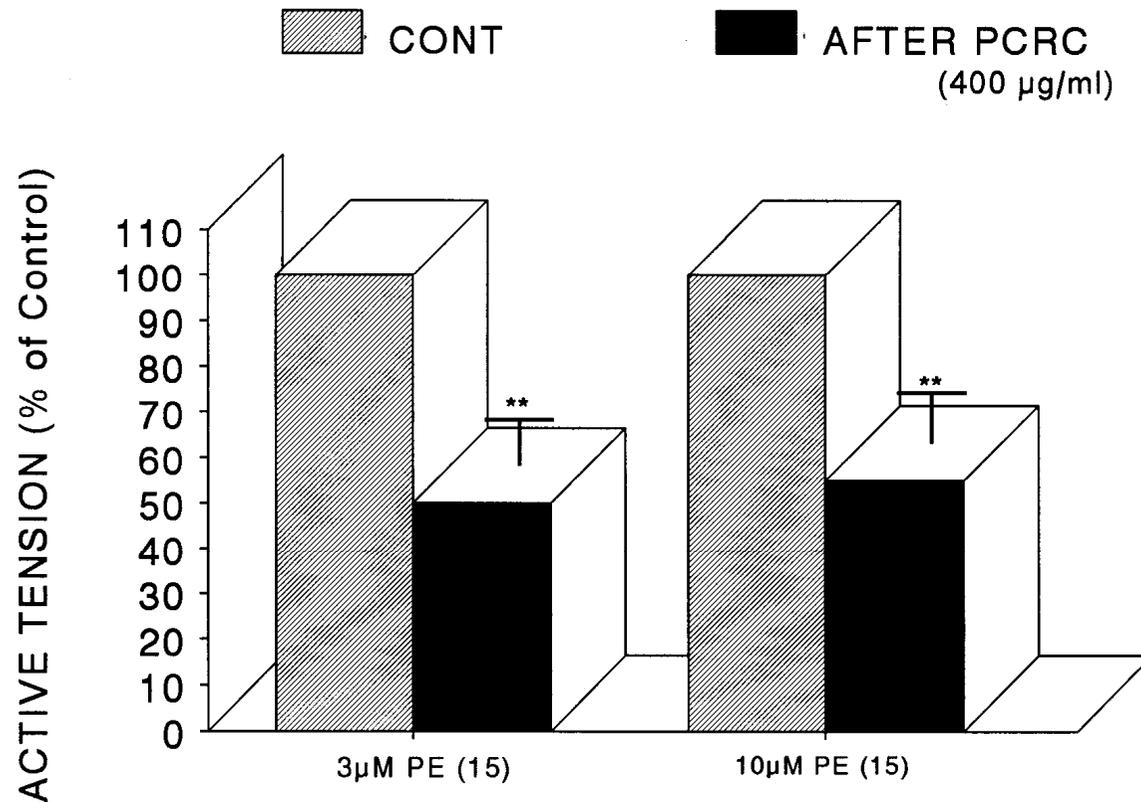
Fig. 3. Influence of polyphenolic compounds isolated from *Rusus coreanum* (PCRC) on phenylephrine (PE)-induced contractile responses in the isolated aortic strips of spontaneously hypertensive rats (SHRs). The contractile responses were induced by adding 3 and 10 μM of PE at 120 min interval after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol, respectively. "CONTROL (CONT)" and "AFTER" denote active tension induced evoked by PE before (CONTROL) and after adding 400 μg/ml of PCRC. Numeral in the parenthesis indicates number of aortic strips isolated from SHRs. Vertical bars represent the standard error of the mean (S.E.M). Ordinate: the active tension (% of control, 0.9 ± 0.1g [3 μM] and 1.0 ± 0.1g [10 μM]). Abscissa: Concentrations of PE (3 and 10 μM). Statistical difference was obtained by comparing the control with the PCRC-pretreated group. **: P< 0.01.
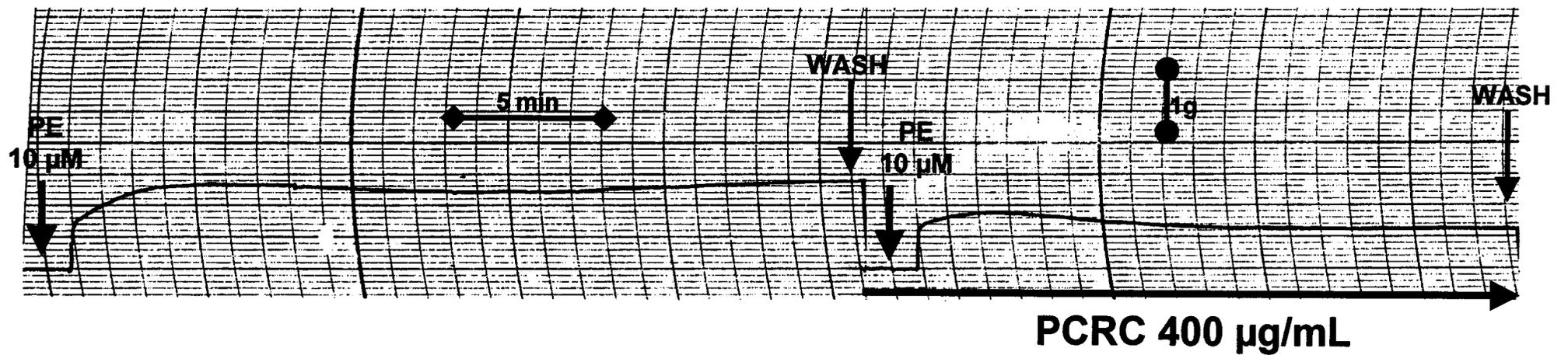
Fig. 4. The typical tracing showing the inhibitory effect of PCRC on phenylephrine (PE)-induced contractile response in the aortic strip OF the SHR. Left: PE-induced contractile response (Control). Right: PE-induced contractile response in the presence of PCRC (400 µg/mL). At arrow mark, the indicated dose (10^{-5} M) of phenylephrine was added to the bath. The chart speed was 5 mm/min.
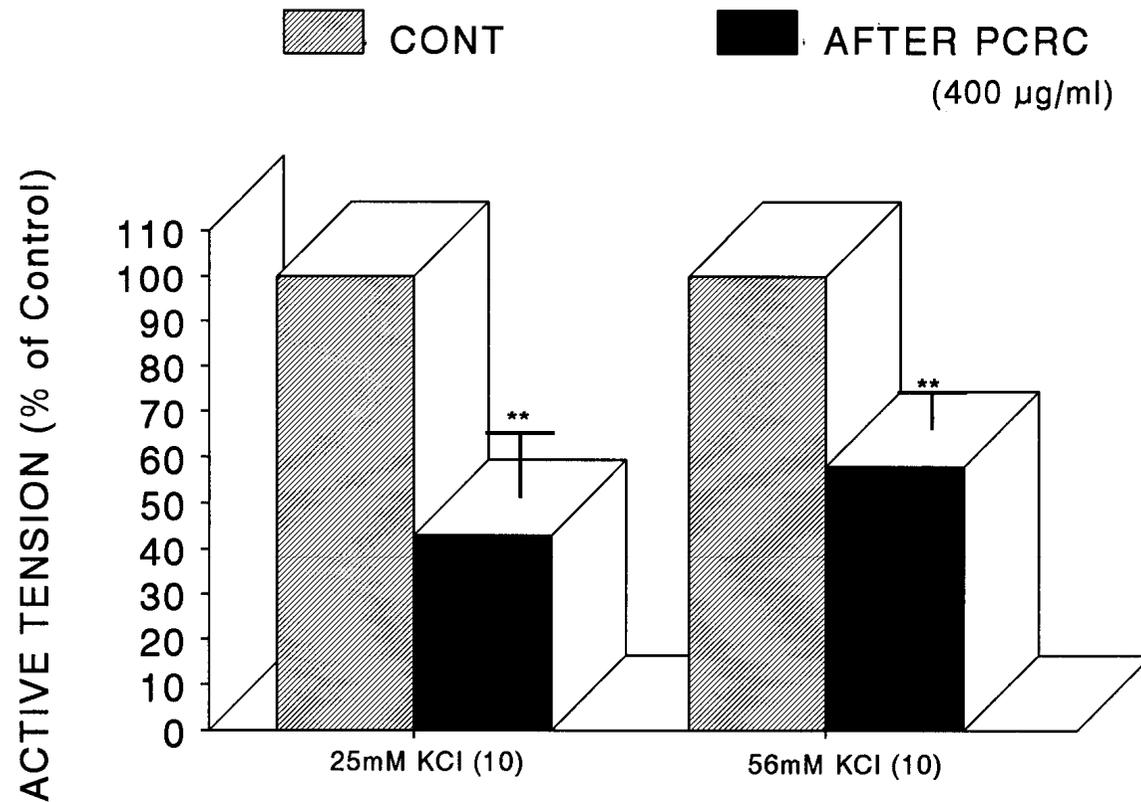
Fig. 5. Influence of PCRC on high potassium (KCl)-induced contractile responses in the isolated aortic strips of SHRs. The contractile responses were induced by adding 25 and 56 mM of KCl at 120 min interval after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol, respectively. Statistical difference was obtained by comparing the control (% of control, 0.7±0.1g [25 mM] and 1.2±0.1g [56 mM]) with the PCRC-pretreated group. Other legends are the same as in Fig. 2. **: P< 0.01.
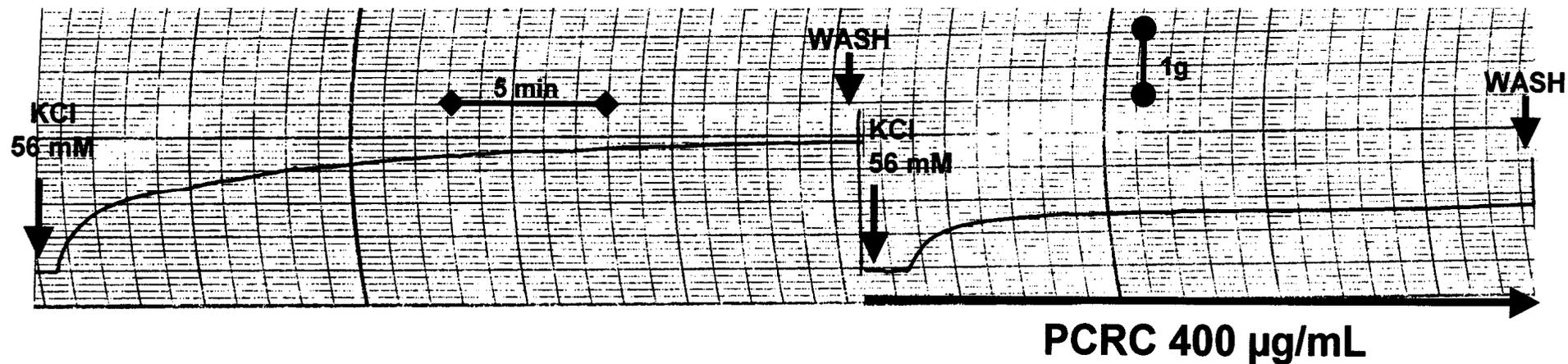
Fig. 6. The typical tracing showing the inhibitory effect of PCRC on high potassium (KCl)-induced contractile response in the aortic strip. Of SHR. Left: KCl-induced contractile response (Control). Right: KCl-induced contractile response in the presence of PCRC (400 µg/mL). At arrow mark, the indicated dose of KCl (56 mM) was added to the bath. The chart speed was 5 mm/min.
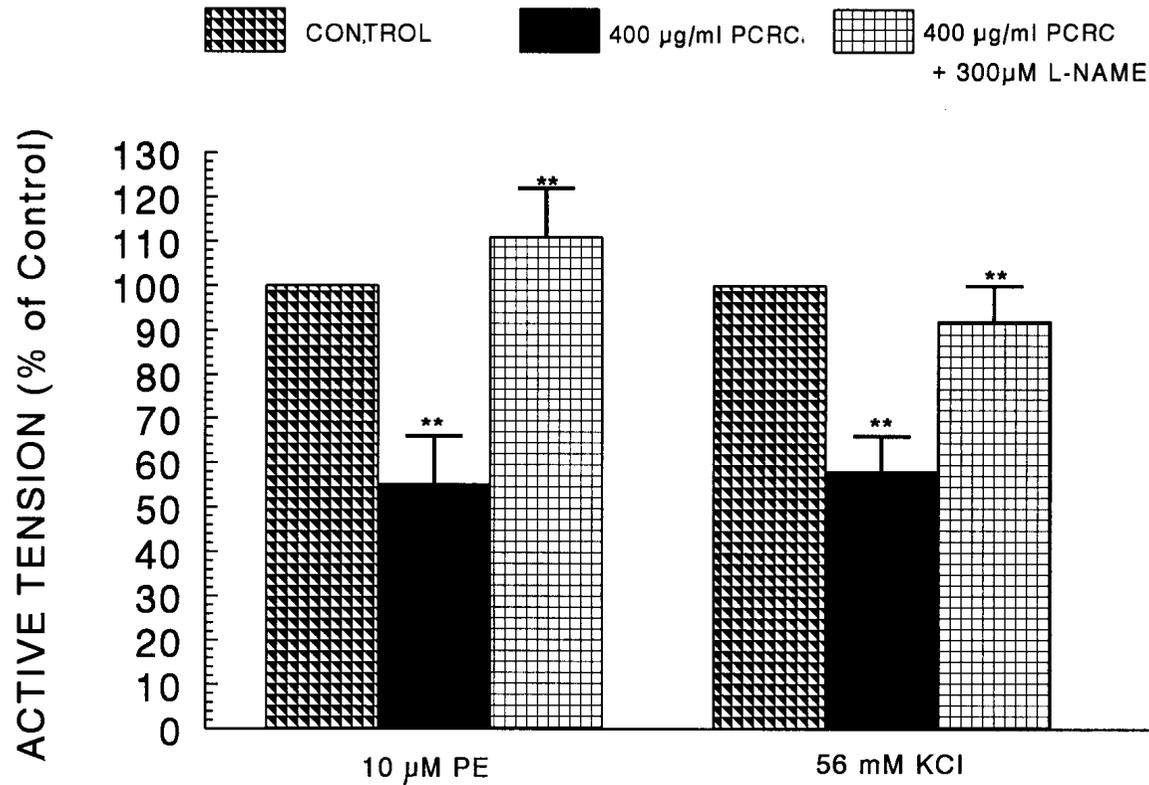
Fig. 7. Influence of PCRC plus L-NAME on PCRC-induced vasodilation to the contractile responses evoked by phenylephrine (PE) and high potassium (KCl) in the isolated aortic strips of SHRs. The contractile responses were induced by adding 10 μM PE and 56 mM KCl after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol, respectively. Statistical difference was obtained by comparing the control (2.9 ± 0.3g [PE] and 1.2 ± 0.1g [KCl]) with the PCRC (400 μg/mL)-pretreated group or PCRC (400 μg/ml) plus L-NAME (300 μM). Other legends are the same as in Fig. 2. **: $P < 0.01$.
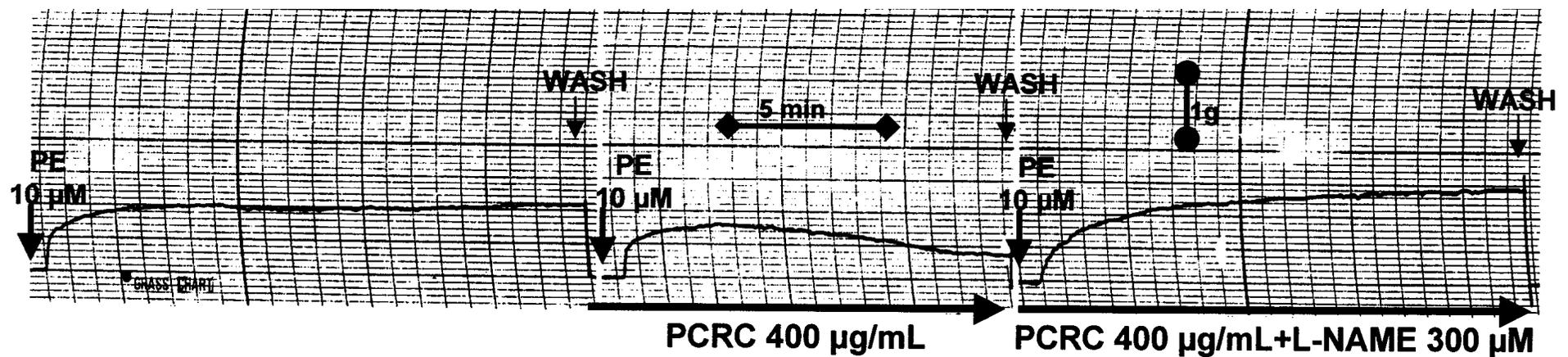
Fig. 8. The typical tracing showing the effect of PCRC plus L-NAME on PCRC-evoked inhibition to phenylephrine (PE)-induced contractile response in the aortic strip of the SHR. Left: PE-induced contractile response (Control). Middle: PE-induced contractile response in the presence of PCRC (400 µg/mL). Right: PE-induced contractile response in the presence of PCRC (400 µg/mL) plus L-NAME (300 µM). At arrow mark, the indicated dose of PE (10 µM) was added to the bath. The chart speed was 5 mm/min.
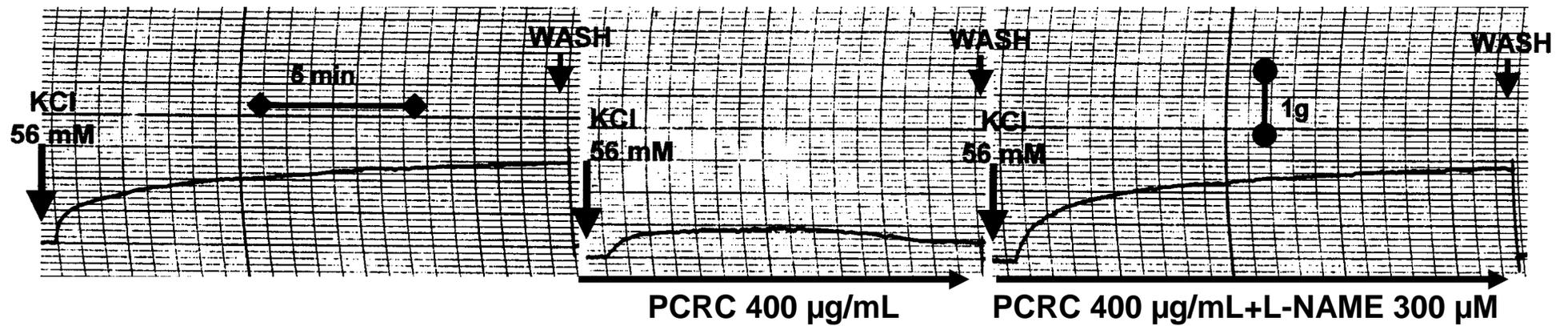
Fig. 9. The typical tracing showing the effect of PCRC plus L-NAME on PCRC-evoked inhibition to high potassium (KCl)-induced contractile response in the aortic strip of SHR. Left: KCl-induced contractile response (Control). Middle: KCl-induced contractile response in the presence of PCRC (400 μg/mL). Right: KCl-induced contractile response in the presence of PCRC (400 μg/mL) plus L-NAME (300 μM). At arrow mark, the indicated dose of KCl (56 mM) was added to the bath. The chart speed was 5 mm/min.
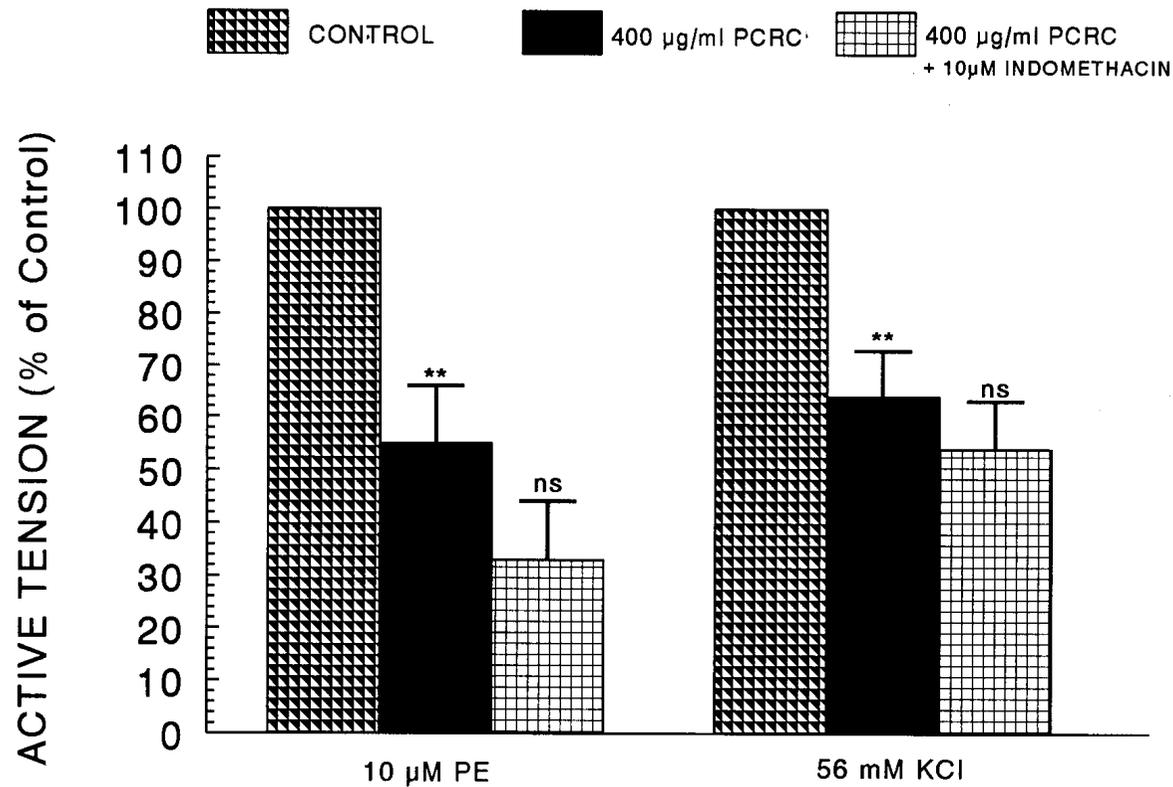
Fig. 10. Influence of PCRC plus indomethacin on PCRC-induced inhibition to the contractile responses evoked by phenylephrine (PE) and high potassium (KCl) in the isolated aortic strips of SHRs. The contractile responses were induced by adding 10 µM PE and 56 mM KCl after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol, respectively. Statistical difference was obtained by comparing the control (0.9±0.1g [PE] and 1.1±0.1g [KCl]) with the PCRC-pretreated group or PCRC (400 µg/ml) plus indomethacin (10 µM) group. Other legends are the same as in Fig. 2. **: P < 0.01.
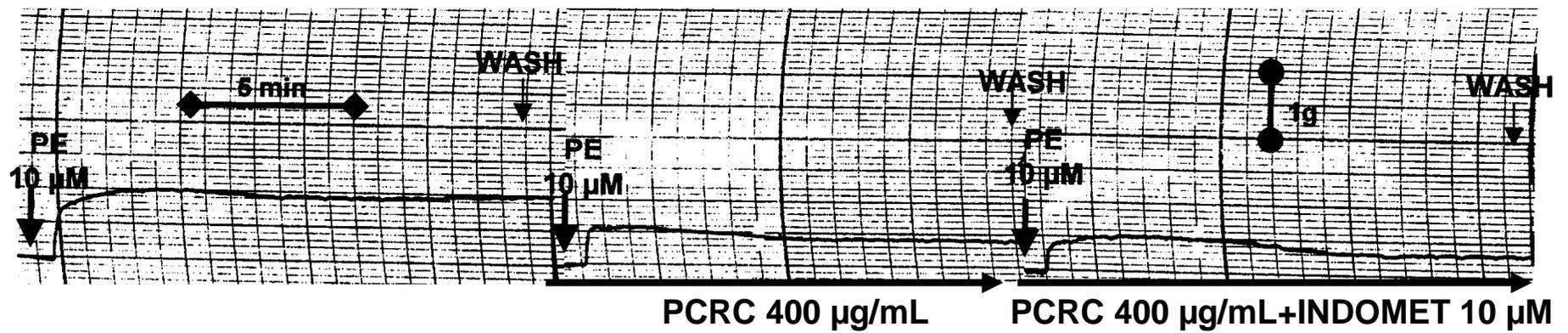
Fig. 11. The typical tracing showing the effect of PCRC plus indomethacin (INDOMET) on PCRC-evoked inhibition to phenylephrine (PE)-induced contractile response in the aortic strip of SHR. Left: PE-induced contractile response (Control). Middle: PE-induced contractile response in the presence of PCRC (400 µg/mL). Right: PE-induced contractile response in the presence of PCRC (400 µg/mL) plus INDOMET (10 µM). At arrow mark, the indicated dose of PE (10 µM) was added to the bath. The chart speed was 5 mm/min.
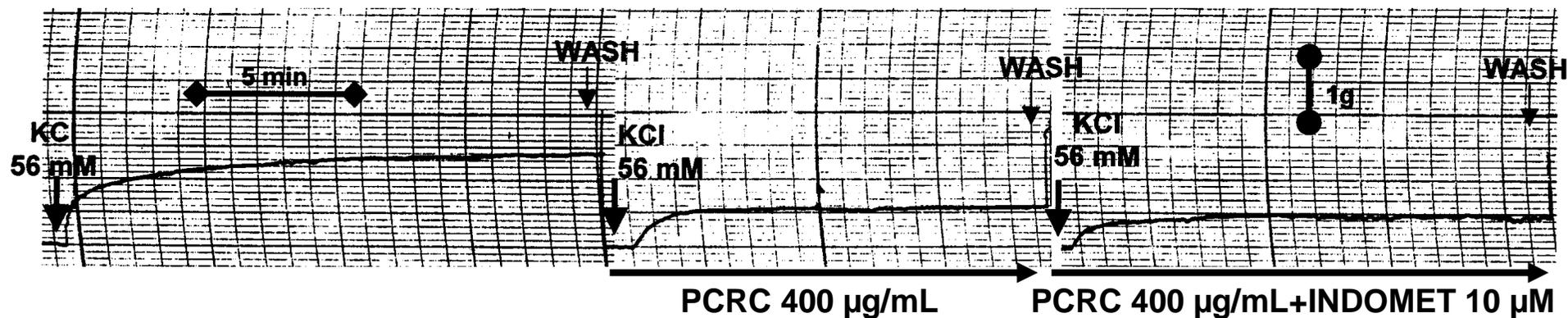
Fig. 12. The typical tracing showing the effect of PCRC plus indomethacin (INDOMET) on PCRC-evoked inhibition to high potassium (KCl)-induced contractile response in the aortic strip of SHR. Left: KCl-induced contractile response (Control). Middle: KCL-induced contractile response in the presence of PCRC (400 µg/mL). Right: KCL-induced contractile response in the presence of PCRC (400 µg/mL) plus INDOMET (10 µM). At arrow mark, the indicated dose of KCl (56 mM) was added to the bath. The chart speed was 5 mm/min.
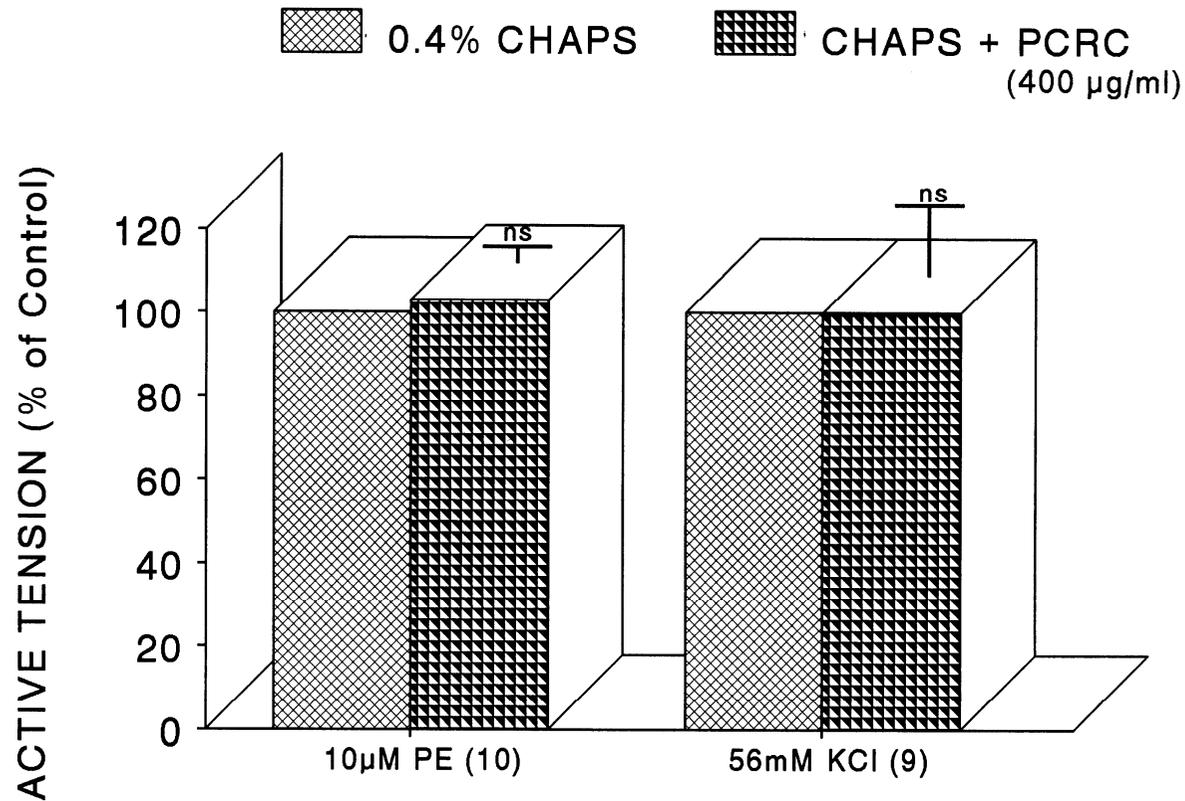
Fig. 13. Influence of CHAPS on contractile responses induced by phenylephrine (PE) and high potassium (KCl) in the isolated aortic strips of SHRs. The contractile responses were induced by adding 10 M PE and 56 mM KCl at 120 min interval after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol, respectively. Statistical difference was obtained by comparing the control (% of control) with the PCRC-treated group after pretreatment of 0.4% CHAPS . Other legends are the same as in Fig. 2. ns: Statistically not significant.
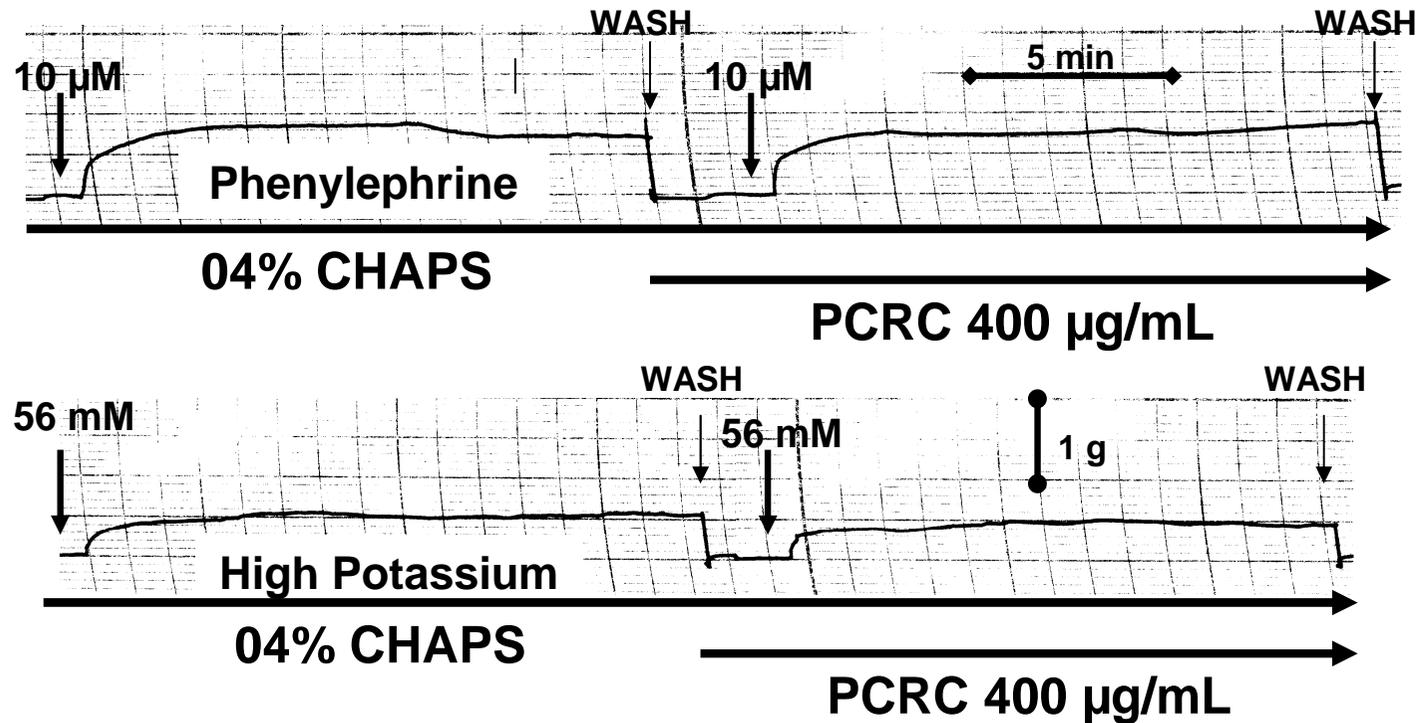
Fig. 14. The representative tracing of CHAPS effect on contractile responses induced by phenylephrine and high potassium in the isolated aortic strips of SHRs. At arrow marks, phenylephrine (10 µM) and high potassium (56 mM) were added into a CHAPS-pretreated aortic strips. Upper: Phenylephrine-induced contractile response after PCRC-treatment in a CHAPS-pretreated aortic strip. Lower: High potassium-induced contractile response after PCRC-treatment in a CHAPS-pretreated aortic strip. The chart speed was 5 mm/min.

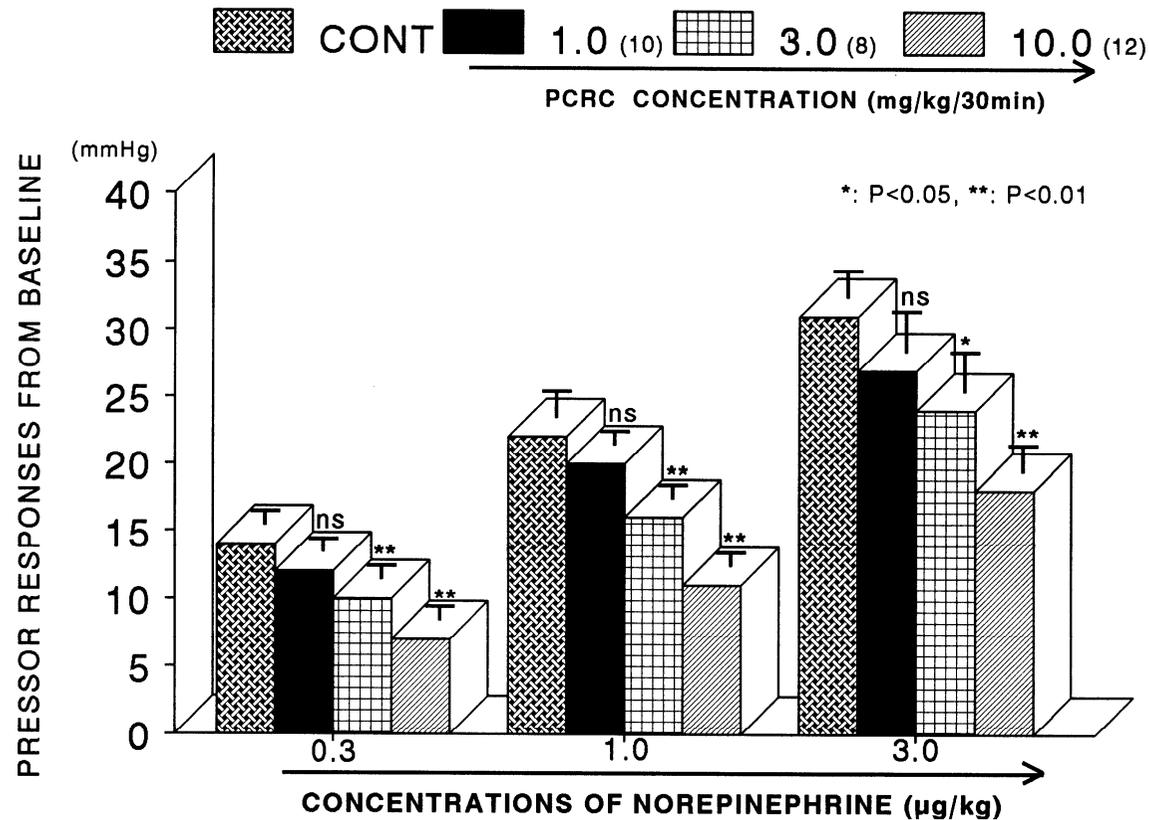Fig. 15. Influence of intravenous PCRC on norepinephrine (NE)-evoked pressor responses in anesthetized SHRs. PCRC (1.0, 3.0 and 10.0 mg/kg/30 min, respectively) was given intravenously after obtaining the corresponding control responses of intravenous norepinephrine (0.3, 1.0 and 3.0 μg/kg). Ordinate: changes of arterial blood pressure in mmHg from 8 rats. Abscissa: intravenous doses of NE in μg/kg. Vertical bars on each column indicate standard error of mean (S.E.M.). There was statistically significant difference in changes of NE-evoked arterial pressor responses from pre-injection level before and after pretreatment of PCRC (1.0, 3.0 and 10.0 μg/kg/30 min). *: P<0.05, **: P< 0.01. ns: Statistically not significant.
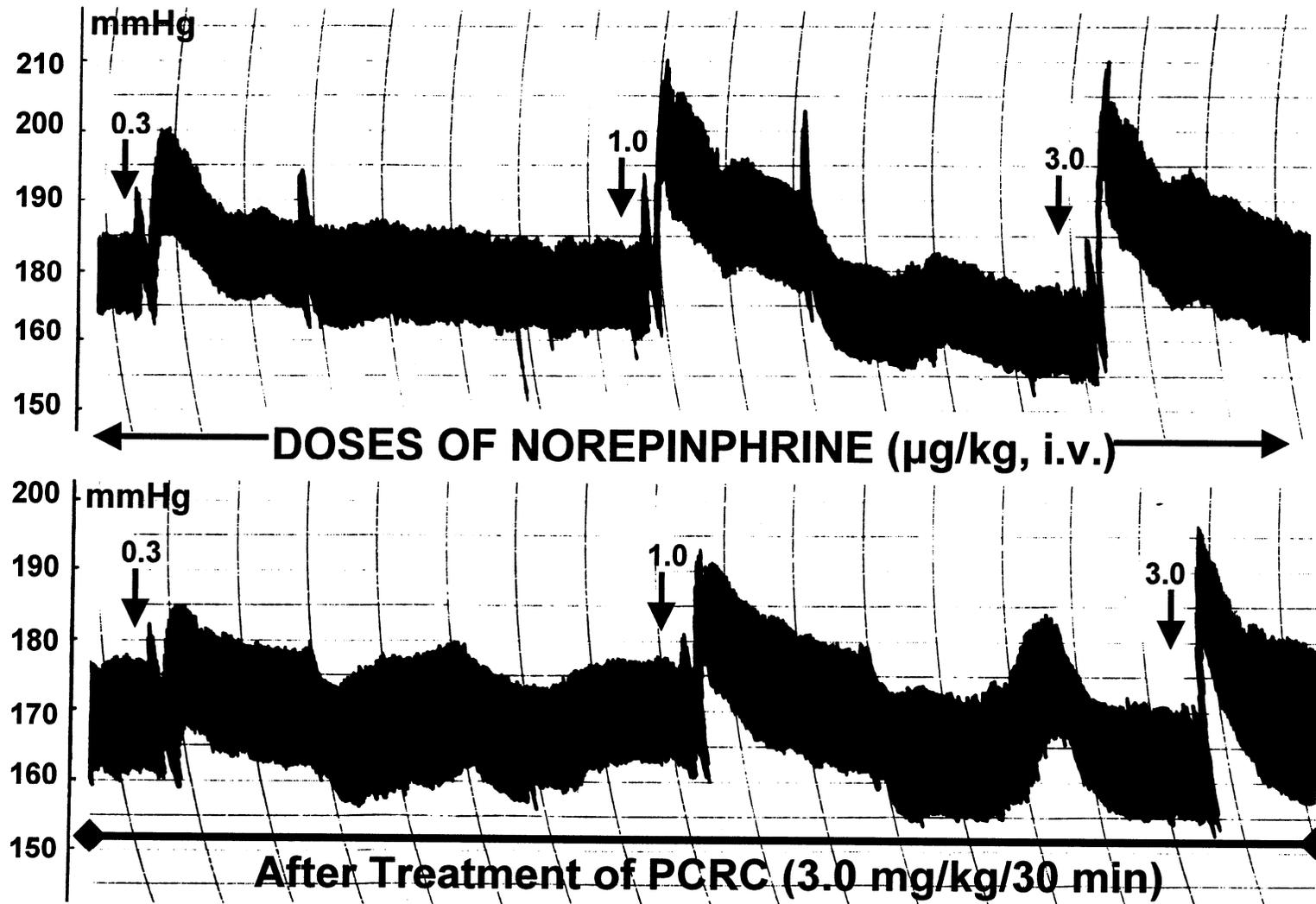
Fig. 16. The representative tracing of PCRC effect on intravenous norepinephrine (NE)-induced pressor responses in an anesthetized SHR. At arrow marks, the indicated doses (0.3, 1.0 and 3.0 μg/kg) of NE were administered into a femoral vein. Upper: NE-induced hypertensive responses in a non-treated rat. Lower: NE-induced hypertensive responses in a PCRC-pretreated rat. PCRC was infused into a femoral vein with a rate of 3 mg/kg/30 min. Arterial blood pressure was expressed in mmHg. The chart speed was 10 mm/min.

Fig. 17. Schematic diagram of possible action site of PCRC in the aortic strips isolated from the SHRs.

저작물 이용 허락서

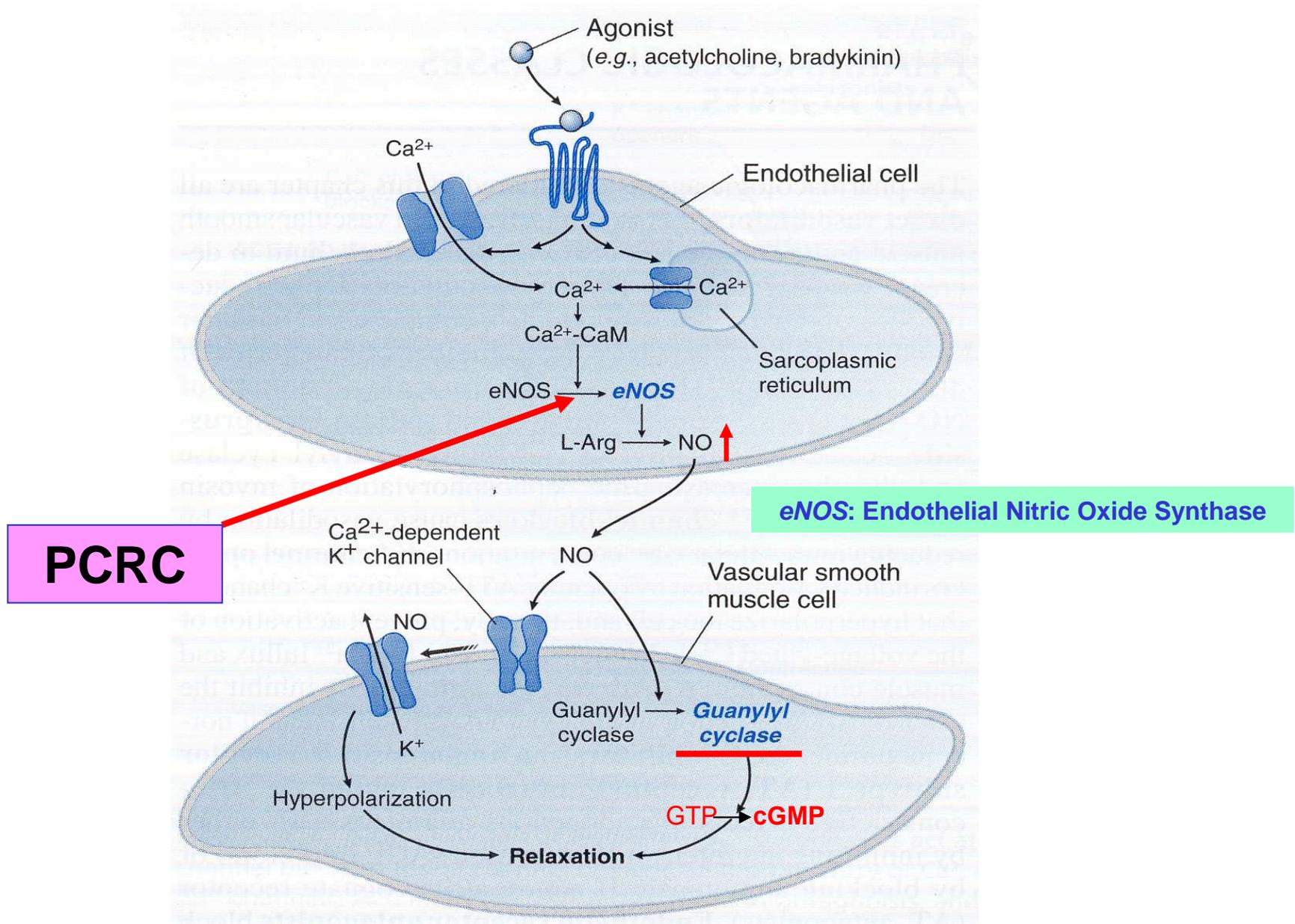| 학 과	의학과	학 번	20067357	과 정	박사
성 명	한글: 민 선 영 한문: 민 선 영 영문: Min, Seon-Young				
주 소	전라남도 해남군 옥천면 영신리251				
연락처	E-MAIL : KOM9194@yahoo.co.kr				
논문제목	한글 : 복분자에서 분리한 폴리페놀이 자연발증 고혈압쥐의 적출 대동맥편의 수축반응에 미치는 영향 영어 : Influence of Polyphenols Isolated from *Rubus coreanum* on Contractile Responses of Aortic Strips Isolated from Spontaneously Hypertensive Rats				

　본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다   음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(O) 반대(   )

2008년 2월

저작자: 민 선 영   (서명 또는 인)

조선대학교 총장 귀하