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Polyphenols of *Rubus coreanum* Inhibit Catecholamine Secretion from the Perfused Adrenal Medulla of SHRs

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The present study was attempted to investigate whether polyphenolic compounds isolated from wine, which is brewed from *Rubus coreanum* Miquel (PCRC), may affect the release of catecholamines (CA) from the isolated perfused adrenal medulla of the spontaneously hypertensive rats (SHRs), and to establish its mechanism of action. PCRC (20~180 μg/ml) perfused into an adrenal vein for 90 min relatively dose-dependently inhibited the CA secretory responses to ACh (5.32 mM), high K^+ (56 mM), DMPP (100 μM) and McN-A-343 (100 μM). PCRC itself did not affect basal CA secretion (data not shown). Also, in the presence of PCRC (60 μg/ml), the CA secretory responses to veratridine (a selective Na^+ channel activator (10 μM), Bay-K-8644 (a L-type dihydropyridine Ca^{2+} channel activator, 10 μM), and cyclopiazonic acid (a cytoplasmic Ca^{2+}-ATPase inhibitor, 10 μM) were significantly reduced, respectively. In the simultaneous presence of PCRC (60 μg/ml) and L-NAME (an inhibitor of NO synthase, 30 μM), the inhibitory responses of PCRC on the CA secretion evoked by ACh, high K^+, DMPP, and Bay-K-8644 were considerably recovered to the extent of the corresponding control secretion compared with that of PCRC-treatment alone. The level of NO released from adrenal medulla after the treatment of PCRC (60 μg/ml) was greatly elevated compared with the corresponding basal level. Taken together, these results demonstrate that PCRC inhibits the CA secretion from the isolated perfused adrenal medulla of the SHRs evoked by stimulation of cholinergic receptors as well as by direct membrane-depolarization. It seems that this inhibitory effect of PCRC is mediated by blocking the influx of calcium and sodium into the adrenal medullary chromaffin cells of the SHRs as well as by inhibition of Ca^{2+} release from the cytoplasmic calcium store at least partly through the increased NO production due to the activation of NO synthase.

Key Words: PCRC, Catecholamine secretion, Adrenal medulla, Cholinergic receptors, Nitric oxide

INTRODUCTION

Bokboonja wine is also the principal products of Gochang county, Chonbuk province, Korea, where is famous for wine brewed from *Rubus coreanum* Miquel. It has been presently used in treating the disease of the aged, spermatorrhea and impotence in oriental medicine. *Rubus coreanum* has been found to possess several polyphenolic compounds, such as (−)-epicatechin, (+)-catechin, proanthocyanidin, etc. There are no reports about its cardiovascular effects so far, while there are many reports about those of polyphenolic compound isolated from red wine.

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., 2000; Zenebe et al., 2003). In *in vivo* studies, red wine polyphenolic compounds (PCRWs) were shown to reduce blood pressure in normotensive and hypertensive rats (Mizutani et al., 1999; Diebolt et al., 2001). 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). PCRWs enhanced NO synthesis and cGMP accumulation only in the presence of functional endothelium. In denuded aortic rings, 103-fold higher concentration of PCRWs was necessary to induce relaxation (Ndiaye et al., 2003). Resveratrol, one of the polyphenolic compounds presented in red wine is thought to be responsible factor for its beneficial cardiovascular effects. Since resveratrol has similar effects to PCRW such as promotion of vasodilation, activation of NO synthase, inhibition of pla-

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ABBREVIATIONS: ACh, acetylcholine; BAY-K8644, 6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate; CA, catecholamines; DMPP, 1.1-dimethyl-4-phenyl piperazinium iodide, methyl-1,4-dihydro-2; McN-A-343, 3-(m-chlloro-phenyl-carbamoyl-oxy-2-butynyl-trimethyl ammonium chloride; NO, nitric oxide; PCRC, polyphenolic compounds isolated from wine, which is brewed from *Rubus coreanum* Miquel.

telet aggregation and leukocyte activation, prevention of oxidation of LDL-cholesterol and reduction of cholesterol synthesis (Chen and Pace-Asciak, 1996; Rakici et al., 2005).

Recently, it has been shown that green tea extract inhibits the secretory responses of catecholamines (CA) evoked by cholinergic (nicotinic and muscarinic) stimulation and direct membrane-depolarization in the perfused adrenal medulla isolated from the rat (Lim et al., 2003) and the rabbit (Lim, 2005). However, epigallocatechin-3-gallate, one of potent catechins isolated from green tea, did not affect the CA secretion evoked by the above serectagogues. As aforementioned, there are many reports about the effects of red wine on cardiovascular system, but no reports on *in vitro* functional effects of polyphenolic compounds (PCRC) from wine, which is brewed from *Rubus coreanum* MIQUEL, on the cardiovascular system. Therefore, the aim of the present study was to investigate the ability of PCRC on secretion of CA in the perfused adrenal gland of SHRs, and to establish its mechanism of action.

METHODS

Experimental procedure

Mature male spontaneously hypertensive rats (purchased from DAMOOL SCIENCE, International Customer Service, Seoul, Korea), weighing 200 to 300 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 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.

Isolation of adrenal glands: The adrenal gland was isolated by the modification of previous method (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by the placement of three-hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations. A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Heparin (400 IU/ml) was injected into vena cava to prevent blood coagulation before ligating vessels and cannulations. A small slit was made into the adrenal cortex just opposite entrance of adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in adrenal cortex. Then the adrenal gland, along with ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at 37±1°C.

Perfusion of adrenal gland

The adrenal glands were perfused by means of peristaltic pump (Isco, St. Lincoln, NE, U.S,A.) at a rate of 0.32 ml/min. The perfusion was carried out with Krebs-bicarbonate solution of following composition (mM): 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 solution was constantly bubbled with 95% O_2+5% CO_2 and the final pH of the solution was maintained at 7.4~7.5. The solution contained disodium EDTA (10 μg/ml) and ascorbic acid (100 μg/ml) to prevent oxidation of catecholamines.

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 at the Research Institute of Bokboonja, Gochang County, Cheollabukdo Province, Korea or a 2-year-old red a 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. 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.

Drug administration

The perfusions of DMPP (100 μM) for 1 minutes and/or a single injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 ml were made into perfusion stream via a three-way stopcock, respectively. McN-A-343 (100 μM), veratridine (100 μM), Bay-K-8644 (10 μM) and cyclopiazonic acid (10 μM) were also perfused for 4 min, respectively. In the preliminary experiments, it was found that upon administration of the above drugs, secretory responses to ACh, KCl, McN-A-343, Bay-K-8644 and cyclopiazonic acid

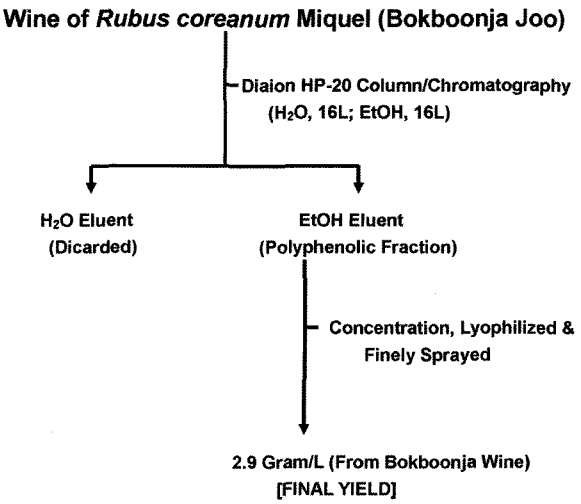

Fig. 1. Preparation of polyphenolic compounds from *Rubus coreanum* Miquel (PCRC). Yield of polyphenolic compounds from Bokboonja wine expressed with grams per liter.

returned to preinjection level in about 4 min, but the responses to DMPP in 8 min.

Collection of perfusate

As a rule, prior to stimulation with various secretagogues, the perfusate was collected for 4 min to determine the spontaneous secretion of CA (background sample). Immediately after the collection of the background sample, collection of the perfusates was continued in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Stimulated sample's perfusate was collected for 4 to 8 min. The amounts secreted in the background sample have been subtracted from that secreted from the stimulated sample to obtain the net secretion value of CA, which is shown in all of the figures. To study the effect of PCRC on the spontaneous and evoked secretion, the adrenal gland was perfused with normal Krebs solution for 90 min, and then the perfusate was collected for a certain period (background sample). Then the medium was changed to the one containing the stimulating agent or along with PCRC, and the perfusates were collected for the same period as that for the background sample. The adrenal gland's perfusate was collected in chilled tubes.

Measurement of catecholamines

The content of CA in perfusate was measured directly by the fluorometric method of Anton and Sayre (Anton and Sayre, 1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981) using fluorospectrophotometer (Kontron Co., Milano, Italy). A volume of 0.2 ml of the perfusate was used for the reaction. The CA content in the perfusate of stimulated glands by secretagogues used in the present work was high enough to obtain readings several folds greater than the reading of control samples (unstimulated). The sample blanks were also lowest for perfusates of stimulated and non-stimulated samples. The CA content in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Measurement of NO release

NO release was measured using a NO-selective microelectrode (ami700, Innovative Instruments Inc) and an amplifier (inNo meter, Innovative Instruments Inc). Adrenomedullary NO production was quantified as the integrated signal detected by the microelectrode after perfusion of PCRC into adrenal medulla of SHRs, as previously described (McVeigh et al., 2002). The electrode was calibrated by producing standardized concentrations of NO in 0.5% (wt/vol) KI in 0.1 mol/l H_2SO_4 from $NaNO_2$ standards. NO release was quantitated as the current detected at the electrode after loading PCRC into adrenal medulla. NO release was calculated as picomoles.

Statistical analysis

The statistical difference between the control and the pretreated groups was determined by the Student's paired *t*-test. 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: PCRC (isolated in our lab), 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), acetylcholine chloride, norepinephrine bitartrate, potassium chloride (KCl), N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate (BAY-K-8644), cyclopiazonic acid (Sigma Chemical Co., U.S.A.), and 3-(m-cholro-phenyl-carbamoyl-oxy)-2-butynyltrimethyl ammonium chloride [McN-A-343] (RBI, U.S.A.). Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required except Bay-K-8644, which was dissolved in 99.5% ethanol and diluted appropriately with Krebs-bicarbonate solution (final concentration of alcohol was less than 0.1%). Concentrations of all drugs except PCRC used were expressed in terms of molar base.

RESULTS

Influence of PCRC on the CA secretion evoked by ACh, high K^+, DMPP and McN-A-343 from the perfused adrenal glands of the SHRs

After the perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 21±2 ng for 2 min (n=12). Since, in *in vivo* study, PCRW was shown to reduce blood pressure in normotensive and hypertensive rats (Mizutani et al., 1999; Diebolt et al., 2001), it was attempted initially to examine the effects of PCRC itself on CA secretion from the perfused model of the adrenal glands of the SHRs. However, in the present study, PCRC (20~180 μg/ml) itself did not produce any effect on basal CA output from the perfused rat adrenal glands (data not shown). Therefore, it was decided to investigate the effects of PCRC on cholinergic receptor stimulation- as well as membrane depolarization-evoked CA secretion. Secretagogues were given at 15 to 20 min-intervals. PCRC was present for 90 minutes after the establishment of the control release.

When ACh (5.32 mM) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was 1,589±77 ng for 4 min. However, the pretreatment with PCRC in the range of 20~180 μg/ml for 90 min relatively concentration-dependently inhibited ACh-stimulated CA secretion. As shown in Fig. 2 (Upper), in the presence of PCRC, the CA releasing responses were inhibited by 69% of the corresponding control release (100%). Also, it has been found that depolarizing agent like KCl stimulates markedly CA secretion (718±38 ng for 0~4 min). High K^+ (56 mM)-stimulated CA secretion after the pretreatment with 20 μg/ml PCRC was not affected for the first 45 min period as compared with its corresponding control secretion (Fig. 2-lower). However, following the pretreatment with higher concentrations of PCRC (60~180 μg/ml), high K^+ (5.6×10^{-2} M)-stimulated CA secretion was maximally inhibited to 68% of the control after 75 min period, although it was not initially affected at 60 μg/ml of PCRC. DMPP (100 μM), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (1,449±60 ng for 0~8 min). However, as shown in Fig. 3 (Upper), DMPP-stimulated CA

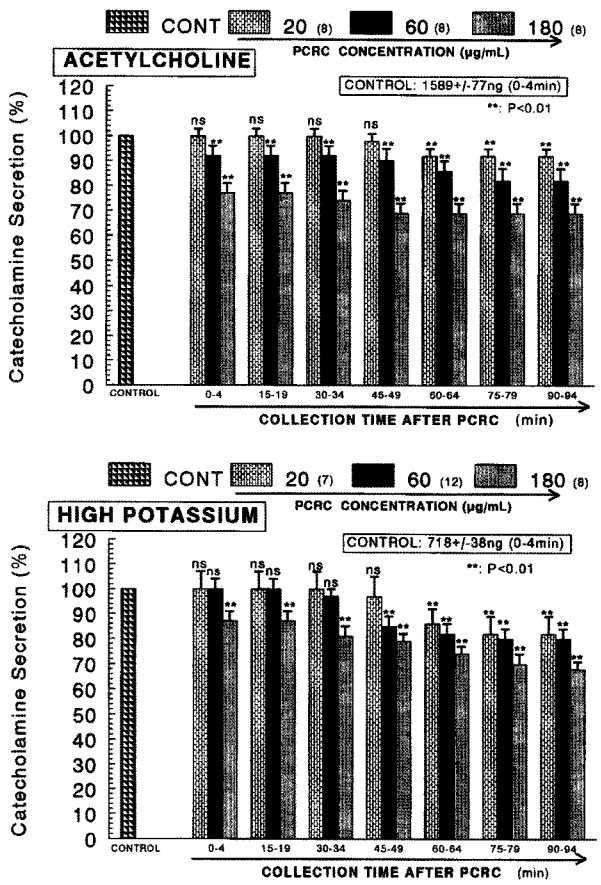

Fig. 2. Dose-dependent effects of PCRC on the secretory responses of catecholamines (CA) evoked by acetylcholine (upper) and high potassium (lower) from the perfused rat adrenal medulla. The CA secretion by a single injection of ACh (5.32 mM) and K^+ (56 mM) in a volume of 0.05 ml was evoked at 15 min intervals during loading with 20, 60 and 180 μg/ml of PCRC for 90 min as indicated by the arrow marks, respectively. The numbers in parentheses indicate the number of rat adrenal glands. Vertical bars on the columns represent the standard error of the mean (S.E.M.). Ordinate: the amounts of CA secreted from the adrenal gland (% of control). Abscissa: collection time of perfusate (min). Statistical difference was obtained by comparing the corresponding control (CONTROL) with each concentration-treated group of PCRC. ACh- and high K^+-induced perfusates were collected for 4 minutes, respectively. $^{**}p<0.01$. ns: Not statistically significant.

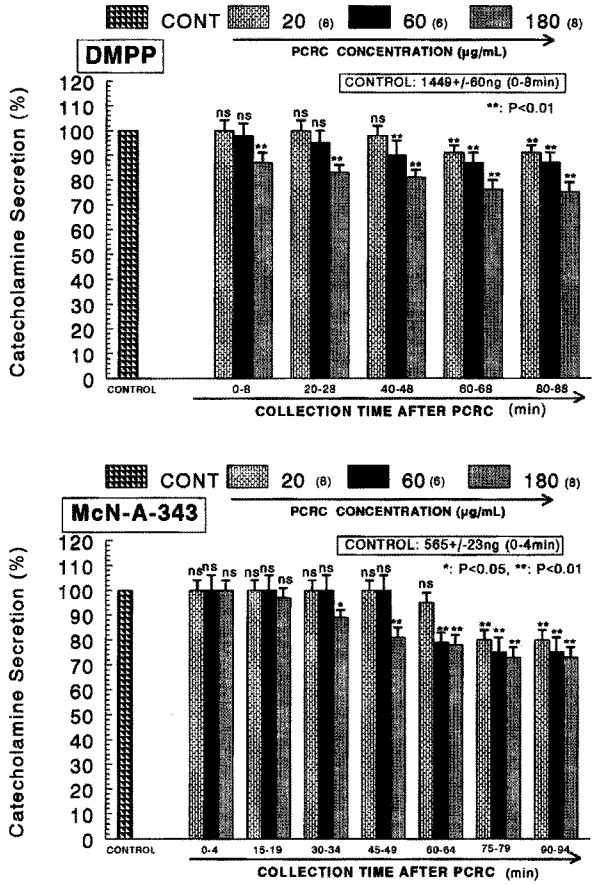

Fig. 3. Dose-dependent effects of PCRC on the CA secretory responses evoked by DMPP (upper) and McN-A-343 (lower) from the perfused rat adrenal medulla. The CA secretion by perfusion of DMPP (100 μM) for 1 min and McN-A-343 (100 μM) for 4 min was induced at 20 and 15 min intervals during loading with 20, 60 and 180 μg/ml of PCRC for 90 min, respectively. Statistical difference was obtained by comparing the corresponding control (CONTROL) with each concentration-pretreated group of PCRC. DMPP- and McN- A-343-induced perfusates were collected for 8 and 4 minutes, respectively. Other legends are the same as in Fig. 2. $^*p<0.05$, $^{**}p<0.01$. ns: Not statistically significant.

secretion after the pretreatment with PCRC was greatly reduced to 75% of the control release. McN-A-343 (100 μM), which is a selective muscarinic M_1-agonist (Hammer and Giachetti, 1982), perfused into an adrenal gland for 4 min caused an increased CA secretion (565±23 ng for 0~4 min). However, McN-A-343-stimulated CA secretion in the presence of PCRC was markedly depressed to 73% of the corresponding control secretion (Fig. 3-lower).

Influence of PCRC on the CA secretion evoked by Bay-K-8644, cyclopiazonic acid and veratridine from the perfused adrenal glands of the SHRs

Since Bay-K-8644 is known to be a calcium channel activator, which enhances basal Ca^{2+} uptake (Garcia et al., 1984) and CA release (Lim et al., 1992), it was of interest to determine the effects of PCRC on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. Bay-K-8644 (10 μM)-stimulated CA secretion in the presence of PCRC was greatly blocked to 75% of the control except for the early 15 min period as compared with the corresponding control release (512±28 ng for 0~4 min) from 7 adrenal glands of SHRs, as shown in Fig. 4 (Upper).

Cyclopiazonic acid, a mycotoxin from *Aspergillus* and *Penicillium*, has been described as a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Goeger and Riley, 1989; Seidler et al., 1989). The inhibitory action of PCRC on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 4 (Lower). However, in the presence of PCRC in 8 adrenal glands of SHRs, cyclopiazonic acid (10 μM)-evoked CA secretion was also inhibited to 73% of the control response (480±21 ng for 0~4 min).

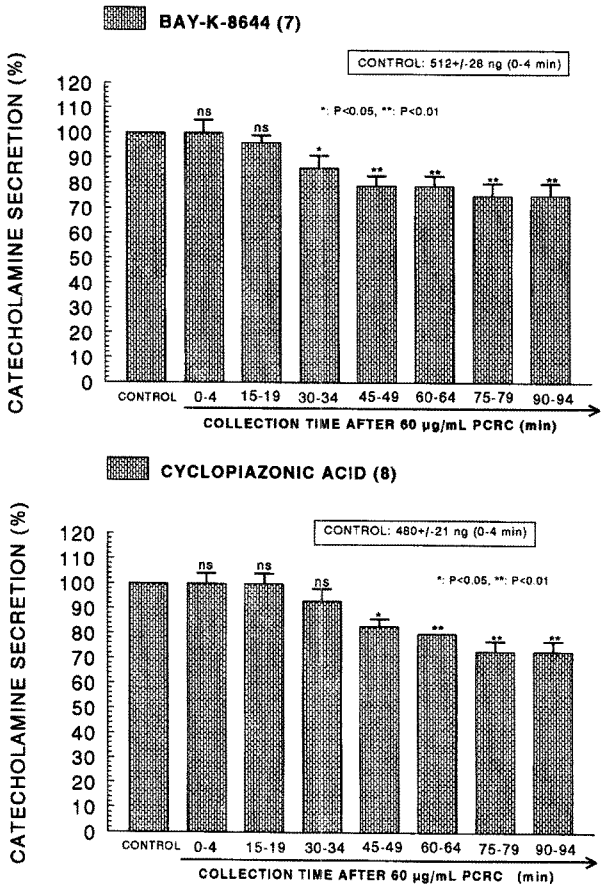

Fig. 4. Time-course effects of PCRC on the CA secretion evoked by Bay-K-8644 (upper) and cyclopiazonic acid (lower) from the perfused rat adrenal medulla. Bay-K-8644 (10 μM) and cyclopiazonic acid (10 μM) were perfused into an adrenal vein for 4 min at 15 min intervals during loading with PCRC (60 μg/ml) for 90 min. Other legends are the same as in Fig. 2. *$p<0.05$, **$p<0.01$. ns: Not statistically significant.

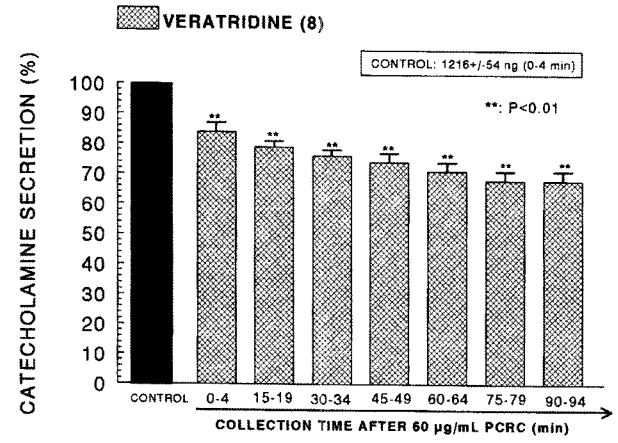

Fig. 5. Time-course effects of PCRC on the CA secretion evoked by veratridine from the perfused rat adrenal medulla. Veratridine (100 μM) was perfused into an adrenal vein for 4 min at 15 min intervals during loading with PCRC (60 μg/ml) for 90 min. Other legends are the same as in Fig. 2. **$p<0.01$.

It has been known that veratridine-induced Na^+ influx mediated through Na^+ channels increased Ca^{2+} influx via activation of voltage-dependent Ca^{2+} channels and produced the exocytotic CA secretion in cultured bovine adrenal medullary cells (Wada et al., 1985a). As shown in Fig. 5, veratridine greatly produced CA secretion (1,216±54 ng for 0~4 min). PCRC (60 μg/ml) also attenuated veratridine-induced CA secretion by 68% of the corresponding control release.

Influence of PCRC plus L-NAME on the CA release evoked by ACh, high K^+, DMPP, McN-A-343, BAY-K-8644 and cyclopiazonic acid from the perfused adrenal glands of the SHRs

It has also been found that, in this study, PCRC inhibited the CA secretory response evoked by cholinergic stimulation in the perfused adrenal glands of SHRs. Therefore, to study the relationship between NO and PCRC-induced inhibitory effects on the CA release from the adrenal glands of SHRs, the effect of L-NAME on PCRC-induced inhibitory responses of CA secretion evoked by cholinergic receptor-stimulation

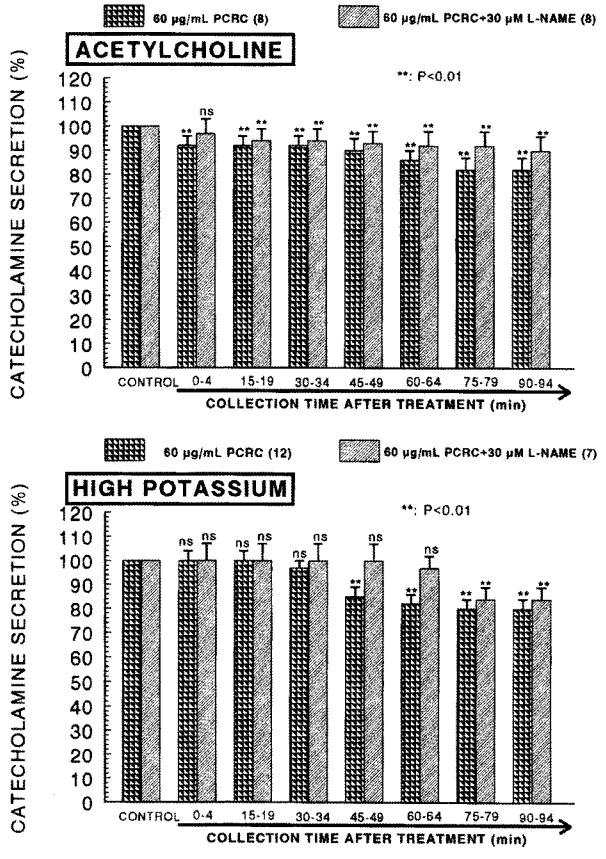

Fig. 6. Influence of PCRC plus L-NAME on the CA secretory responses evoked by acetylcholine (upper) and high potassium (lower) from the perfused rat adrenal medulla. The CA secretion by a single injection of ACh (5.32 mM) and K^+ (56 mM) in a volume of 0.05 ml was evoked at 15 min intervals during simultaneous loading with PCRC (60 μg/ml) plus L-NAME (30 μM) for 90 min. Statistical difference was obtained by comparing the corresponding control (CONTROL) with PCRC-treated group or group treated with PCRC+L-NAME. Other legends are the same as in Fig. 2. **$p<0.01$. ns: Not statistically significant.

as well as membrane depolarization was examined. In the present study, in the simultaneous presence of PCRC (60 μg/ml) and L-NAME (30 μM) for 90 min from 8 adrenal glands of SHRs, ACh (5.32 mM)-evoked CA release was initially not affected at first 4 min, but later rather recovered to 90% of the corresponding control release at the period of 90~94 min compared with that of only PCRC (60 μg/ml)-treated group, as illustrated in Fig. 6 (Upper). High K^+ (56 mM)-evoked CA release in the presence of PCRC (60 μg/ml) and L-NAME (30 μM) for 90 min was also not changed for 0~64 min, and then recovered to 84% of the corresponding control release at the last period of 90~94 min period in comparison to that of only PCRC (60 μg/ml)-treated group from 7 glands (Fig. 6-lower). As shown in Fig. 7 (Upper), the simultaneous perfusion of PCRC and L-NAME for 90 min no longer inhibited DMPP-evoked CA release for the period of 0~68 min from 10 adrenal glands while later rather recovered to 92% of the control release at the period of 80~88 min. Moreover, in the simultaneous presence of PCRC and L-NAME for 90 min, McN-A-343-evoked CA secretory responses was also recovered to 81% of the control secretion compared with that of only PCRC (60 μg/ml)-treated group from 8 glands as shown in Fig. 7 (Lower), although they were not affected at period of 0~64 min.

As shown in Fig. 8 (Upper), the simultaneous perfusion of PCRC (60 μg/ml) and L-NAME (30 μM) for 90 min no longer inhibited the CA release evoked by Bay-K-8644 for the period of 0~64 min from 10 glands, and then also recovered to 80% of the control release at the last period of 90~94 min in comparison to that of PCRC (60 μg/ml)-treated group only. As shown in Fig. 8 (Lower), in the presence of PCRC (60 μg/ml) and L-NAME (30 μM) for 90 min in 10 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was recovered to 78% of the control response (100%) at the period of 90~94 min in comparison to that of PCRC (60 μg/ml)-treated group only.

Influence of PCRC on the level of nitric oxide released from the perfused adrenal medulla of the SHRs

As shown in Fig. 6~8, the inhibitory effects of PCRC on cholinergic stimulation- and direct membrane depolarization-evoked CA secretory responses were significantly reduced in the presence of L-NAME. Therefore, it was decided directly to determine the level of NO released from adrenal

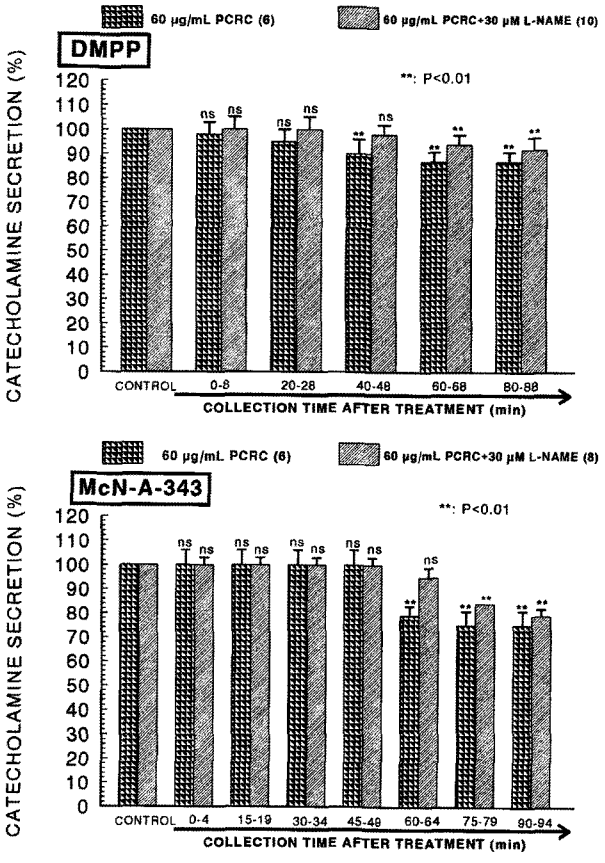

Fig. 7. Influence of PCRC plus L-NAME on the CA secretory responses evoked by DMPP (upper) and McN-A-343 (lower) from the perfused rat adrenal medulla. The CA secretion by perfusion of DMPP (100 μM) for 1 min and McN-A-343 (100 μM) for 4 min was induced at 20 and 15 min intervals after preloading with PCRC (60 μg/ml) plus L-NAME (30 μM) for 90 min, respectively. Other legends are the same as in Fig. 2 and 6. **p<0.01. ns: Not statistically significant.

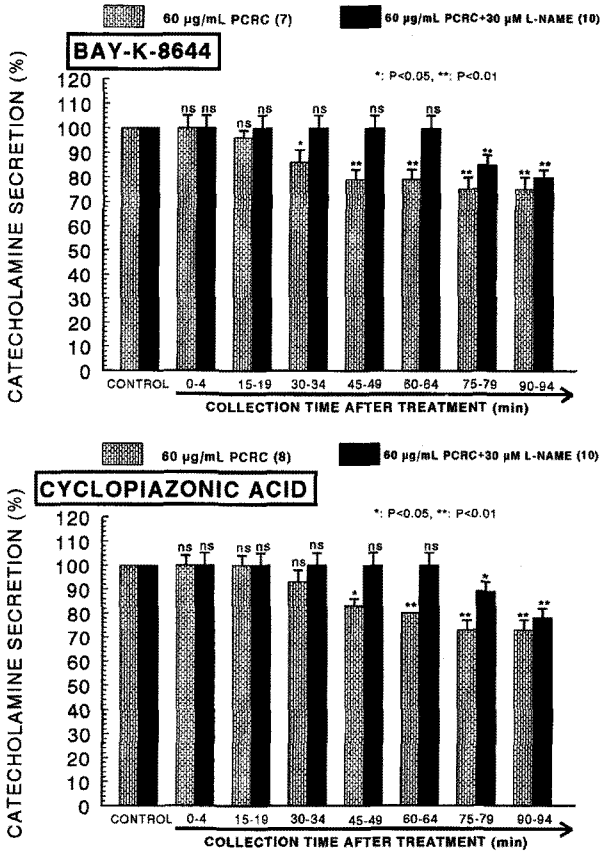

Fig. 8. Influence of PCRC plus L-NAME on the CA secretory responses evoked by Bay-K-8644 (upper) and cyclopiazonic acid (lower) from the perfused rat adrenal medulla. Bay-K-8644 (10 μM) and cyclopiazonic acid (10 μM) were perfused into an adrenal vein for 4 min at 15 min intervals during simultaneous loading with PCRC (60 μg/ml) for 90 min. Other legends are the same as in Fig. 2 and 6. *p<0.05, **p<0.01. ns: Not statistically significant.

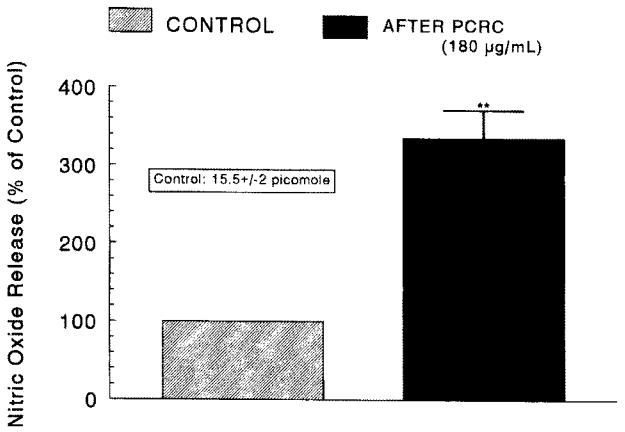

Fig. 9. Influence of of PCRC on nitric oxide (NO) production in the perfused rat adrenal medulla. Perfusate sample was taken for 8 min after loading the perfusion of PCRC (180 μg/ml) at a rate of 0.31 ml/min. Ordinate: the amounts of NO released from the adrenal medulla (% of control). Abscissa: Treatment (before and after PCRC). Statistical difference was made by comparing the control with PCRC-treated group. **$p<0.01$.

medulla of SHRs after the treatment of PCRC. Moreover, it is found 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., 2000; Zenebe et al., 2003). In 10 adrenal glands, the basal amount of NO released from medulla prior to administration of PCRC was 15.5±2.3 picomoles. However, 8 min after loading with PCRC it was greatly elevated to 49.3±8.2 picomoles, which was 328% of the basal release, as shown in Fig. 9.

DISCUSSION

The present experimental results have suggested that PCRC inhibits the CA secretory responses from the isolated perfused adrenal gland of the SHRs evoked by stimulation of cholinergic (both muscarinic and nicotinic) receptors as well as by direct membrane-depolarization. It seems that this inhibitory effect of PCRC is mediated by blocking influx of both calcium and sodium through their channels into the adrenal medullary chromaffin cells of the SHRs as well as by inhibition of Ca^{2+} release from the cytoplasmic calcium store, which are induced partly by the increased NO production due to activation of neuronal NO synthase.

In the present study, in the simultaneous presence of PCRC and L-NAME (NO synthase inhibitor), the CA secretory responses evoked by ACh, DMPP, high K^+ and Bay-K-8644 were considerably recovered to the extent of the corresponding control secretion compared with those of PCRC-treatment alone. This result is well consistent with the report that polyphenolic compounds isolated from red wine produced the endothelium-NO-dependent relaxation through an extracellular Ca^{2+}-dependent mechanism (Andriambeloson et al., 1999). Amongst the different classes of polyphenolic compounds present in PCRW, anthocyanins and oligomeric condensed tannins had the same pharmacological profile as PCRW (Andriambeloson et al., 1998). Moreover, In the present study, following treatment of PCRC into adrenal medulla of SHRs, NO production was greatly elevated as shown in Fig. 9. Taking into account these findings, it is likely that PCRC inhibits the CA secretory response evoked by various secretagogues through increasing NO production in adrenal chromaffin cells since PCRC-induced inhibitory responses of CA secretion were significantly reduced in the presence of L-NAME, an inhibitor of NO synthase, and PCRC also practically enhanced NO release from adrenal medulla of SHRs. It has been found that PCRW promote the endothelium-dependent relaxation and activate NO synthase (Andriambeloson et al., 1997; Flesh et al., 1998; Leikert et al., 2002). The polyphenolic compound resveratrol presented in red wine is thought to be responsible factor for its beneficial cardiovascular effects, since resveratrol has similar effects to RWPC such as promotion of vasodilation, and activation of nitric oxide synthase (Chen and Pace-Asciak, 1996; Wallerath et al., 2002). Furthermore, these effects of resveratrol and PCRW are agreement with the present result that PCRC can inhibit the CA secretory responses evoked by cholinergic stimulation and membrane depolarization at least partly by activation of nitric oxide synthase in the isolated perfused adrenal medulla of SHRs, because this inhibitory effect of PCRC on the CA secretory responses was significantly attenuated in the presence of L-NAME, an inhibitor of nitric oxide synthase. In support of this idea, generally, NO is produced enzymatically from the terminal guanidino nitrogen of L-arginine by the action of NO synthase (NOS) (Palmer et al., 1988; Sakuma et al., 1988). There are at least three isoforms of NOS: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). The adrenal medulla possesses characteristic postganglionic sympathetic neurons, and the presence of nNOS has been demonstrated (Palacios et al., 1989; Oset-Gasque et al., 1994; Marley et al., 1995; Schwarz et al., 1998). *In vitro* studies using NOS inhibitors and NO donors were performed to examine the role of NO in modulating CA secretion from the adrenal medulla but the results remain controversial. In the present work, in presence of L-NAME, the inhibitory responses of PCRC on the CA secretion were recovered to the considerable extent of the control secretion compared with the inhibitory effects of PCRC-treatment alone. This result demonstrates that PCRC can inhibit the CA release at least partly through the activation of nNOS in the adrenal medulla of SHRs. In the support of this finding, it has been reported that the NOS inhibitor, L-NAME enhances K^+-stimulated CA secretion in cultured bovine chromaffin cells (Torres et al., 1994) and that sodium nitroprusside (SNP) inhibits ACh-induced CA secretion in bovine chromaffin cells (Rodriguez-Pascual et al., 1996). These studies suggest that NO may play an inhibitory role in the control of the CA secretion. Moreover, the presence of endothelial cells has been reported to inhibit the K^+-induced or the nicotinic receptor agonist DMPP-induced CA secretion in cultured bovine chromaffin cells (Torres et al., 1994), suggesting that not only nNOS but also eNOS may play roles in modulating adrenal CA secretion. In contrast, it has been reported that L-NAME inhibits acetylcholine (ACh)-induced CA secretion in bovine chromaffin cells (Uchiyama et al., 1994) and that the NO donor SNP enhances nicotine-induced CA secretion in cultured bovine chromaffin cells (O'Sullivan and Burgoyne, 1990). These findings suggest that NO may facilitate cholinergic agonist-induced CA secretion. On the other hand, a few *in vivo* studies have suggested that NO does not play

a role in regulation of adrenal CA secretion (Breslow et al., 1992; Breslow et al., 1993). Based on these reports, the present studies suggest that PCRC possesses the ability partly to activate nNOS in the adrenomedullary chromaffin cells of SHRs, in addition to the direct inhibitory effects on the CA secretion.

In general, during neurogenic stimulation of the adrenal medulla, ACh is released from splanchnic nerve endings and activates cholinergic receptors on the chromaffin cell membrane (Viveros, 1975). This activation initiates a series of events known as stimulus-secretion coupling, culminating in the exocytotic release of CA and other components of the secretory vesicles into the extracellular space. Usually, two mechanisms are involved in the secretion of adrenal medullary hormones. Upon excitation of splanchnic nerves, ACh is released from the nerve terminals, and then activates nicotinic the CA secretion. Based on this fact, the present findings that PCRC inhibited the CA secretory responses evoked by nicotinic receptor stimulation as well as by membrane depolarization in the adrenal medulla of SHRs seem to be able to support the fact that, in *in vivo* studies, PCRW lowers blood pressure in normotensive and hypertensive rats (Mizutani et al., 1999; Diebolt et al., 2001). 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., 2000; Leikert et al., 2002; Zenebe et al., 2003).

These experimental results indicate that PCRC-induced inhibitory activity of the CA secretory response evoked by stimulation of nicotinic receptors might contribute at least partly to its hypotensive mechanism. ACh-evoked CA secretion has shown to be caused through stimulation of both nicotinic and muscarinic receptors in guinea-pig adrenal gland (Nakazato et al., 1988) as well as in the perfused rat adrenal glands (Lim and Hwang, 1991). In support of this idea, it has been found that green tea extract inhibits the CA secretory responses evoked by cholinergic stimulation and membrane depolarization in the adrenal medulla isolated from the rat (Lim et al., 2003) and the rabbit (Lim, 2005). In this study, PCRC inhibited the CA secretory responses evoked by ACh, DMPP, McN-A-343 and high K^+. It suggests that PCRC can produce the similar effect with that of green tea extract in adrenal medulla of the normotensive rats and rabbits.

In the present study, PCRC also depressed the CA secretory response evoked by Bay-K-8644, which is known to activate L-type voltage-dependent Ca^{2+} channels (Schramm et al., 1983; Garcia et al., 1984). This result indicates that PCRC may inhibit Ca^{2+} influx to the adrenomedullary cells of SHRs. In support of this idea, in cultured bovine adrenal medullary cells, nicotinic (but not muscarinic) receptors mediate the Ca^{2+}-dependent CA secretion (Fisher et al., 1981). It has also been known that the activation of nicotinic receptors stimulates the CA secretion by increasing Ca^{2+} entry through receptor-linked and/or voltage-dependent Ca^{2+} channels in both perfused rat adrenal glands (Wakade and Wakade, 1983; Lim and Hwang, 1991) and isolated bovine adrenal chromaffin cells (Kilpatrick et al., 1981; Kilpatrick et al., 1982; Knight and Kesteven, 1983). Wada and his coworkers (1985b) have found that the adrenomedullary chromaffin cells have (i) nicotinic receptor-associated ionic channels, responsible for carbachol-induced Na^+ influx, (ii) voltage-dependent Na^+ channels, responsible for veratridine-induced Na^+ influx and (iii) voltage-dependent Ca^{2+} channels, suggesting that the influx of Na^+ caused either by carbachol or by veratridine leads to activate voltage-dependent Ca^{2+} channels by altering membrane potentials, whereas high K^+ directly activates voltage-dependent Ca^{2+} channels without increasing Na^+ influx. In the present study, the finding that high K^+-induced CA secretory response was depressed by pretreatment with PCRC indicates that this inhibitory effect of PCRC is mediated through the direct inhibition of calcium influx into the adrenal chromaffin cells of SHRs. Furthermore, slight elevation in the extracellular potassium concentration increases both the frequency of spontaneous action potentials and the CA secretion (Kidokoro and Ritchie, 1980), suggesting that the influx of calcium that occurs during action potentials is directly linked to the rate of secretion. These findings that PCRC inhibited the CA secretion evoked by Bay-K-8644 as well as by high K^+ suggest that PCRC inhibits directly the voltage-dependent Ca^{2+} channels. In the bovine chromaffin cells, stimulation of nicotinic, but not muscarinic ACh receptors is known to cause CA secretion by increasing Ca^{2+} influx largely through voltage-dependent Ca^{2+} channels (Oka et al., 1979; Burgoyne, 1984). Therefore, it seems that these inhibitory effects of PCRC on the CA secretion evoked by DMPP and veratridine may be mediated by inhibiting Ca^{2+} influx through voltage-dependent Ca^{2+} channels due to activation of nicotinic receptor-associated ionic channels, responsible for carbachol-induced Na^+ influx, as well as of voltage-dependent Na^+ channels, responsible for veratridine-induced Na^+ influx, respectively.

The present study has also shown that PCRC inhibits the CA secretion evoked by cyclopiazonic acid. Cyclopiazonic acid is known to be a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Goeger and Riley, 1989; Seidler et al., 1989) and a valuable pharmacological tool for investigating intracellular Ca^{2+} mobilization and ionic currents regulated by intracellular Ca^{2+} (Suzuki et al., 1992). Therefore, it is felt that the inhibitory effect of PCRC on the CA secretion evoked by cholinergic stimulation as well as by membrane-depolarization may be associated with the mobilization of intracellular Ca^{2+} from the cytoplasmic calcium store. This indicates that the PCRC has an inhibitory effect on the release of Ca^{2+} from the intracellular pools induced by stimulation of muscarinic ACh receptors, which is weakly responsible for the CA secretion. It has been shown that Ca^{2+}-uptake into intracellular storage sites susceptible to caffeine (Ilno, 1989) is almost completely abolished by treatment with cyclopiazonic acid during the proceeding of Ca^{2+} load (Suzuki et al., 1992). This is consistent with the findings obtained in skinned smooth muscle fibers of the longitudinal layer of the guinea-pig ileum, where Ca^{2+}-uptake was also inhibited by cylopiazonic acid (Uyama et al., 1992). Suzuki and his coworkers (1992) have shown that cyclopiazonic acid easily penetrates into the cytoplasm through the plasma membrane and reduces Ca^{2+}-ATPase activity in sarcoplasmic/endoplasmic reticulum, resulting in an increase in the subsequent Ca^{2+} release from those storage sites. Moreover, in bovine adrenal chromaffin cells, stimulation of muscarinic ACh receptors is also proposed to cause activation of phosphoinositide metabolism, resulting in the formation of inositol 1,4,5-trisphosphate, which induces the mobilization of Ca^{2+} from the intracellular pools (Cheek et al., 1989; Challis et al., 1991). The present results suggest that PCRC-induced depression of the CA secretion evoked by McN-A-343 and cyclopiazonic acid may be due to the inhibition of Ca^{2+} re-

lease from the intracellular pools induced by stimulation of muscarinic ACh receptors. However, in the present study, it is uncertain whether the inhibitory effect of PCRC on Ca^{2+} movement from intracellular pools is due to its direct effect on the PI response or the indirect effects.

In conclusion, the results of the present study have demonstrated that PCRC inhibits the CA secretion by stimulation of cholinergic nicotinic receptors as well as by membrane depolarization in the isolated perfused adrenal glands of SHRs. It seems that this inhibitory effect of PCRC is exerted by blocking influx of sodium and calcium through each ionic channel into the adrenal medullary chromaffin cells of SHRs as well as by inhibition of Ca^{2+} release from the cytoplasmic calcium store, which are at least partly mediated to the increased NO production due to the activation of nitric oxide synthase. Based on these experimental results, the ingestion of PCRC may be helpful to prevent or alleviate the cardiovascular diseases, such as hypertension and angina pectoris, through inhibition of CA secretion from adrenomedullary chromaffin cells and consequent reduction of the CA level in the circulation.

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REFERENCES

Andriambeloson E, Kleschyov AL, Muller B, Beretz A, Stoclet JC, 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, Haan-Archipoff G, Lobstein A, Anton R, Beretz A, Stoclet JC, Andriantsitohaina R. Lobstein Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta. *J Nutr* 128: 2324−2333, 1998.

Andriambeloson E, Stoclet JC, Andriantsitohaina R. Mechanism of endothelial nitric oxide-dependent vasorelaxation induced by wine polyphenols in rat thoracic aorta. *J Cardiovasc Pharmacol* 33: 248−254, 1999.

Anton AH, Sayre DF. A study of the factors affecting the aluminum oxide trihydroxy indole procedure for the analysis of catecholamines. *J Pharmacol Exp Ther* 138: 360−375, 1962.

Breslow MJ, Tobin JR, Bredt DS, Ferris CD, Snyder SH, Traystman RJ. Nitric oxide as a regulator of adrenal blood flow. *Am J Physiol* 264: H464−469, 1993.

Breslow MJ, Tobin JR, Bredt DS, Ferris CD, Snyder SH, Traystman RJ. Role of nitric oxide in adrenal medullary vasodilation during catecholamine secretion. *Eur J Pharmacol* 210: 105−106, 1992.

Burgoyne RD. Mechanism of secretion from adrenal chromaffin cells. *Biochem Biophys Acta* 779: 201−216, 1984.

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

Challiss RAJ, Jones JA, Owen PJ, Boarder MR. Changes in inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate mass accumulations in cultured adrenal chromaffin cells in response to bradykinin and histamine. *J Neurochem* 56: 1083−1086, 1991.

Cheek TR, O'Sullivan AJ, Moreton RB, Berridge MJ, Burgoyne RD. Spatial localization of the stimulus-induced rise in cytosolic Ca^{2+} in bovine adrenal chromaffin cells: Distinct nicotinic and muscarinic patterns. *FEBS Lett* 247: 429−434, 1989.

Chen CK, Pace-Asciak CR. Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta. *Gen Pharmacol* 27: 363−366, 1996.

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

Fisher SK, Holz RW, Agranoff BW. Muscarinic receptors in chromaffin cell culture mediate enhanced phospholipid labeling but not catecholamine secretion. *J Neurochem* 37: 491−487, 1981.

Fitzpatrick DF, Fleming RC, Bing B, Maggi DA, O'Malley R. Isolation and characterization of endothelium-dependent vasorelaxing compounds from grape seeds. *J Agric Food Chem* 48: 6384−6390, 2000.

Flesch M, Schwarz A, Bolun M. Effects of red and white wine on endothelium-dependent vasorelaxation of rat aorta and human coronary arteries. *Am J Physiol* 275: H1183−1190, 1998.

Garcia AG, Sala F, Reig JA, Viniegra S, Frias J, Fonteriz R, Gandia L. Dihydropyridine Bay-K-8644 activates chromaffin cell calcium channels. *Nature* 309: 69−71, 1984.

Goeger DE, Riley RT. Interaction of cyclopiazonic acid with rat skeletal muscle sarcoplasmic reticulum vesicles. Effect on Ca^{2+} binding and Ca^{2+} permeability. *Biochem Pharmacol* 38: 3995−4003, 1989.

Hammer R, Giachetti A. Muscarinic receptor subtypes: M_1 and M_2 biochemical and functional characterization. *Life Sci* 31: 2992−2998, 1982.

Ilno M. Calcium-induced calcium release mechanism in guinea pig taenia caeci. *J Gen Physiol* 94: 363−383, 1989.

Kidokoro Y, Ritchie AK. Chromaffin cell action potentials and their possible role in adrenaline secretion from rat adrenal medulla. *J Physiol* 307: 199−216, 1980.

Kilpatrick DL, Slepetis RJ, Corcoran JJ, Kirshner N. Calcium uptake and catecholamine secretion by cultured bovine adrenal medulla cells. *J Neurochem* 38: 427−435, 1982.

Kilpatrick DL, Slepetis RJ, Kirshner N. Ion channels and membrane potential in stimulus-secretion coupling in adrenal medulla cells. *J Neurochem* 36: 1245−1255, 1981.

Knight DE, Kesteven NT. Evoked transient intracellular free Ca^{2+} changes and secretion in isolated bovine adrenal medullary cells. *Proc R Soc Lond Biol Sci* 218: 177−199, 1983.

Leikert JF, Räthel TR, Wohlfart P, Cheynier V, Vollmar AM, Dirsch VM. Red wine polyphenols enhance endothelial nitric oxide release from endothelial cells. *Circulation* 106: 1614−1617, 2002.

Lim DY. Comparison of green tea extract and epigallocatechin gallate on secretion of catecholamines from the rabbit adrenal medulla. *Arch Pharm Res* 28: 914−922, 2005.

Lim DY, Hwang DH. Studies on secretion of catecholamines evoked by DMPP and McN-A-343 in the rat adrenal gland. *Korean J Pharmacol* 27: 53−67, 1991.

Lim DY, Kim CD, Ahn KW. Influence of TMB-8 on secretion of catecholamines from the perfused rat adrenal glands. *Arch Pharm Res* 15: 115−125, 1992.

Lim DY, Park HG, Lee BR. Green tea extract, not epigallocatechin gallate inhibits catecholamine release from the rat adrenal medulla. *J Applied Pharmacol* 11: 33−40, 2003.

Marley PD, McLeod J, Anderson C, Thomson KA. Nerves containing nitric oxide synthase and their possible function in the control of catecholamine secretion in the bovine adrenal medulla. *J Auton Nerv Syst* 54: 184−194, 1995.

McVeigh GE, Hamilton P, Wilson M, Hanratty CG, Leahey WJ, Devine AB, Morgan DG, Dixon LJ, McGrath LT. Platelet nitric oxide and superoxide release during the development of nitrate tolerance: effect of supplemental ascorbate. *Circulation* 106: 208−213, 2002.

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

Nakazato Y, Ohga A, Oleshansky M, Tomita U, Yamada Y. Voltage-independent catecholamine release mediated by the activation of muscarinic receptors in guinea-pig adrenal glands. *Br J Pharmacol* 93: 101−109, 1988.

Ndiaye M, Chataigneau T, Andriantsitohaina JCS, Schini-Kerth VB. 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.

Oka M, Isosaki M, Yanagihara N. Isolated bovine adrenal medullary cells: studies on regulation of catecholamine synthesis and release. In: Usdin E, Kopin IJ, Brachas J ed, Catecholamines: *Basic and Clinical frontiers*. Pergamon Press, Oxford, p 70−72, 1979.

Oset-Gasque MJ, Parramon M, Hortelano S, Bosca L, Gonzalez MP. Nitric oxide implication in the control of neurosecretion by chromaffin cells. *J Neurochem* 63: 1693−1700, 1994.

O'Sullivan AJ, Burgoyne RD. Cyclic GMP regulates nicotine-induced secretion from cultured bovine adrenal chromaffin cells: effects of 8-bromo-cyclic GMP, atrial natriuretic peptide, and nitroprusside (nitric oxide). *J Neurochem* 54: 1805−1808, 1990.

Palacios M, Knowles RG, Palmer RM, Moncada S. Nitric oxide from L-arginine stimulates the soluble guanylate cyclase in adrenal glands. *Biochem Biophys Res Commun* 165: 802−809, 1989.

Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 333: 664−666, 1988.

Rakici O, Kiziltepe U, Coskun B, Aslamaci S, Akar F. Effects of resveratrol on vascular tone and endothelial function of human saphenous vein and internal mammary artery. *Int J Cardiol* 105: 209−115, 2005.

Rodriguez-Pascual F, Miras-Portugal MT, Torres M. Effect of cyclic GMP-increasing agents nitric oxide and C-type natriuretic peptide on bovine chromaffin cell function: inhibitory role mediated by cyclic GMP-dependent protein kinase. *Mol Pharmacol* 49: 1058−1070, 1996.

Sakuma I, Stuehr DJ, Gross SS, Nathan C, Levi R. Identification of arginine as a precursor of endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* 85: 8664−8667, 1988.

Schramm M, Thomas G, Towart R, Franckowiak G. Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. *Nature* 303: 535−537, 1983.

Schwarz PM, Rodriguez-Pascual F, Koesling D, Torres M, Förstermann U. Functional coupling of nitric oxide synthase and soluble guanylyl cyclase in controlling catecholamine secretion from bovine chromaffin cells. *Neuroscience* 82: 255−265, 1998.

Seidler NW, Jona I, Vegh N, Martonosi A. Cyclopiazonic acid is a specific inhibitor of the Ca^{2+}-ATPase of sarcoplasmic reticulum. *J Biol Chem* 264: 17816−17823, 1989.

Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* 100: 1050−1055, 1999.

Suzuki M, Muraki K, Imaizumi Y, Watanabe M. Cyclopiazonic acid, an inhibitor of the sarcoplasmic reticulum Ca^{2+}-pump, reduces Ca^{2+}-dependent K^+ currents in guinea-pig smooth muscle cells. Br *J Pharmacol* 107: 134−140, 1992.

Tallarida RJ, Murray RB. Manual of pharmacologic calculation with computer programs. 2nd ed. Speringer-Verlag, New York, p 132, 1987.

Torres M, Ceballos G, Rubio R. Possible role of nitric oxide in catecholamine secretion by chromaffin cells in the presence and absence of cultured endothelial cells. *J Neurochem* 63: 988−996, 1994.

Uchiyama Y, Morita K, Kitayama S, Suemitsu T, Minami N, Miyasako T, Dohi T. Possible involvement of nitric oxide in acetylcholine-induced increase of intracellular Ca^{2+} concentration and catecholamine release in bovine adrenal chromaffin cells. *Jpn J Pharmacol* 65: 73−77, 1994.

Uyama Y, Imaizumi Y, Watanabe M. Effects of cyclopiazonic acid, a novel Ca^{2+}-ATPase inhibitor on contractile responses in skinned ileal smooth muscle. *Br J Pharmacol* 106: 208−214, 1992.

Viveros OH. Mechanism of secretion of catecholaminies from adrenal medulla. In handbook of physiology, Endocrinology. Vol VI, Sect 7, The adrenal gland. American physiological society, Washington DC, p 389−426, 1975.

Wada Y, Satoh K, Taira N. Cardiovascular profile of Bay-K-8644, a presumed calcium channel activator in the dog. *Naunyn-Schmiedebergs Arch Pharmacol* 328: 382−387, 1985a.

Wada A, Takara H, Izumi F, Kobayashi H, 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, 1985b.

Wakade AR. Studies on secretion of catecholamines evoked by acetylcholine or transmural stimulation of the rat adrenal gland. *J Physiol* 313: 463−480, 1981.

Wakade AR, Wakade TD. Contribution of nicotinic and muscarinic receptors in the secretion of catecholamines evoked by endogenous and exogenous acetylcholine. *Neuroscience* 10: 973−978, 1983.

Wallerath T, Deckert G, Ternes T, Anderson H, Li H, Witte K, Förstermann U. Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. *Circulation* 106: 1652−1658, 2002.

Zenebe W, Pecháòová O, Andriantsitohaina R. Red wine polyphenols induce vasorelaxation by increased nitric oxide bioactivity. *Physiol Res* 52: 425−432, 2003.