2007년 2월
박사학위논문

Influence of Resveratrol, a Main Polyphenolic Compound Isolated from Red Wine, on Catecholamine Release in the Perfused Rat Adrenal Medulla

조선대학교 대학원

의학과

나광문

Influence of Resveratrol, a Main Polyphenolic Compound Isolated from Red Wine, on Catecholamine Release in the Perfused Rat Adrenal Medulla

적포도주 폴리페놀화합물의 주성분인 **Resveratrol**이 흰쥐 관류부신수질에서 카테콜아민 유리작용에 미치는 영향

2007년 2월 25일

조선대학교 대학원

의학과

나광문

Influence of Resveratrol, a Main Polyphenolic Compound Isolated from Red Wine, on Catecholamine Release in the Perfused Rat Adrenal Medulla

지도교수 임 동 윤

이 논문을 의학박사 학위 신청논문으로 제출함.

2006 년 10 월 일

조선대학교 대학원

의학과

나광문

나 광 문의 박사학위논문을 인준함

위원장	전남 대학교	교수	인
위 원	조선 대학교	교수	인
위 원	서울 대학교	교수	인
위 원	조선 대학교	교수	인
위 원	조선 대학교	교수	인

2006 년 12 월 일

조선대학교 대학원

CONTENTS OF TABLES

CONTENTS OF FIGURES

CONTENTS

〈국문 초록〉

적포도주 폴리페놀화합물의 주성분인 Resveratrol이 흰쥐 관류부신수질에서 카테콜아민 유리작용에 미치는 영향

나 광 문

(지도교수: 임 동 윤)

조선대학교 대학원 의학과

Resveratrol은 동물에서 강력한 심혈관작용이 있지만 부신의 관류모델에서 카테콜아민(catecholamines, CA)의 분비작용에 대한 resveratrol의 기능적 효과에 대한 보고는 지금까지는 발표된 바가 없다. 따라서 본 연구의 목적은 resveratrol이 정상혈압 흰쥐로부터 분리 적출한 부신의 관류모델에서 CA분비작용에 미치는 영향을 검색하여 그 작용기전을 규명하고, 나아가 적포도주에서 분리한 폴리페놀 화합물(PCRW)과 작용상의 차이유무를 비교 검색코자 본 연구를 시행하여 다음과 같은 결과를 얻었다. Resveratrol (10~100 μM)을 부신정맥 내로 90분간 관류 시 비교적 용량 및 시간 의존적으로 ACh (5.32 mM), 고칼륨 (56 mM, 직접적인 막탈분극제), DMPP (100 μM, 선택성 니코틴수용체 작동제), 및 McN-A-343 (100 μM, 선택성 무스카린수용체 작동제)에 의한 CA 분비반응을 억제하였다. 그러나, resveratrol자체는 기초 CA 분비량에 영향을 미치지 않았다. 또한, resveratrol

(30 μM) 존재 하에서, L형 칼슘통로 활성화제인 Bay-K-8644 (30 μM) 및 세포질에서 Ca^{2+}-ATPase 억제제인 cyclopiazonic acid (10 μM)에 의한 CA 분비반응이 억제되었다. 흥미롭게도, PCRW(60 μg/ml) 존재 하에서 ACh, 고칼륨, DMPP, McN-A-343, Bay-K-8644 및 cyclopiazonic acid에 의한 CA분비작용도 비교적 시간 의존적으로 억제되었다. 또한 resveratrol (30 μM)과 L-NAME (30 μM)을 90분간 동시 처치하였을 때 ACh, 고농도의 K^+, DMPP, 및 Bay-K-8644의 CA 분비효과가 resveratrol 단독처치 시 나타나는 억제효과에 비교하여 상응하는 대조치의 수준까지 회복되었다.

이와 같은 연구결과를 종합하여 보면, 정상혈압 흰쥐의 적출 관류 부신수질에서 resveratrol은 콜린성(니코틴 및 무스카린 수용체)흥분작용 및 막탈분극에 의한 CA 분비작용에 대하여 뚜렷한 억제작용을 나타내었다. 이러한 resveratrol의 억제작용은 흰쥐 적출 부신수질에서 NO Synthase의 활성화에 의한 NO 생성증가로 인하여 크롬친화세포내로 칼슘유입과 세포내 칼슘저장고로부터 칼슘유리를 억제하며, 이는 적어도 니코틴수용체와의 상호작용에 기인되는 것으로 생각된다. 또한 resveratrol의 CA 분비 억제작용은 PCRW와 유사한 기전을 통해서 나타나는 것으로 사료된다.

I. INTRODUCTION

Resveratrol, 3,4',5-trihydroxystilbene, is a naturally occurring polyphenolic compound present in variety of plants, such as *Yucca schidigera* (Uenobe et al., 1997), a South Africa medicinal plant *Erythrophleum lasianthum* (Orisini et al., 1997), and grapes (Sato et al., 1997; Celotti et al., 1996). Interest in resveratrol has expanded in recent years, focusing on its potentially beneficial effects on the cardiovascular, as well as its anti-cancer effects. Recently, it has been shown that resveratrol produced mainly endothelium-dependent and nitric oxide-mediated vasodilation in human internal mammary artery but partially in saphenous vein rings and improved their endothelial reactivity (Rakici et al., 2005). Epidemiological studies indicate an association between moderate consumption of red wine and reduced risk of coronary heart disease (Renaud and de Lorgeril, 1992; German and Walzem, 2000). It has been shown that red wine polyphenolic compounds (PCRW) promote the endothelium-dependent relaxation, activate NO synthase, inhibit platelet aggregation, and prevent oxidation of LDL-cholesterol (Fitzpatrick et al., 1993; Frankel et al., 1993; Demrow and Slane, 1995; Andriambeloson et al., 1997; Flesh et al., 1998; Leikert et al., 2002).

The polyphenolic compound resveratrol contained in red wine is thought to be a responsible factor for its beneficial cardiovascular effects. Since resveratrol has similar effects to PCRW such as promotion of vasodilation, activation of nitric oxide synthase, inhibition of platelet aggregation and leukocyte activation, prevention of oxidation of LDL-cholesterol and reduction of cholesterol synthesis (Frankel et al., 1993; Pace-Asciak et al., 1995; Chen and Pace-Asciak, 1996;

Rotondo et al., 1998; Wallerath et al., 2002).

On the other hand, it has been observed that these natural polyphenols, like a number of antidepressant drugs (Slotkin et al., 1986; Gareri et al., 2000; To et al., 2005), inhibit the uptake of 5-hydroxytryptamine (5-HT) by human platelets. In recent studies (Yáñez et al., 2006), both *cis*-resveratrol and *trans*-resveratrol (5–200 μM) concentration-dependently inhibited the uptake of [^{3}H]NA and [3H]5-HT by synaptosomes from rat brain and the uptake of [^{3}H]5-HT by human platelets. Both *cis*-resveratrol and *trans*-resveratrol (5–200 μM) concentration-dependently inhibited the enzymatic activity of commercial (human recombinant) MAO (monoamine oxidase) isoform (MAO-A and MAO-B) activity.

Resveratrol clearly has various potent cardiovascular effects in animals but there are so far no reports on the functional effect of resveratrol on the secretion of catecholamines (CA) in the perfused model of the adrenal gland. Therefore, the aim of the present study was to investigate the ability of resveratrol on the CA secretion in the isolated perfused model of the rat adrenal gland, to establish its mechanism of action, and additionally to compare its effect with that of PCRW.

II. MATERIALS AND METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 180 to 300 grams, were anesthetized with thiopental sodium (40 mg/kg) intraperitoneally. The adrenal gland was isolated by the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by placing 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 (Fig.1).

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 $\pm 1^{o}$C (Fig.1).

Perfusion of adrenal gland

The adrenal glands were perfused by means of ISCO pump (WIZ Co.) at a rate

of 0.33 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.

Drug administration

The perfusions of DMPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 minutes and/or a single injection of ACh (5.32 x 10^{-3} M) and KCl (5.6 x 10^{-2} M) in a volume of 0.05 ml were made into perfusion stream via a three-way stopcock, respectively. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) 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 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

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 resveratrol on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing quinine for 20 min, 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 resveratrol, 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

CA content of 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 content of CA in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Isolation of polyphenolic compounds

Polyphenolic compounds were prepared as described by Caderni et al. (2000), using adsorption chromatography from 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. 2): 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 2.1 g PCRW was obtained from 1 L red grape wine. The working solution of this PCRW was prepared by dissolving in 0.9% NaCl solution on the day of each experiment and filtered before administration.

Statistical analysis

The statistical difference between the control and pretreated 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: PCRW (gifted from professor Young-Hong Baik, Department of Pharmacology, College of Medicine, Chonnam National University,

Gwangju, Korea), resveratrol hydrochloride, 1.1-dimethyl-4 -phenyl piperazinium iodide (DMPP), acetylcholine chloride (ACh), 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-car boxylate (BAY-K-8644), cyclopiazonic acd, (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 PCRW (μg/mL) used are expressed in terms of molar base.

III. RESULTS

Effect of resveratrol on CA secretion evoked by ACh, excess K^+, DMPP and McN-A-343 from the perfused rat adrenal glands

After the perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 23.1 ± 2.2 ng/2 min (n=6). Since some papers demonstrate that resveratrol relaxes isolated vascular arteries (Fitzpatrick et al., 1993; Jager and Nguyen-Duong, 1999; Naderali et al., 2000; Naderali et al., 2001), it was attempted initially to examine the effects of resveratrol itself on CA secretion from the perfused model of the rat adrenal glands. However, in the present study, resveratrol (10^{-5} ~ 10^{-4} M) itself did not produce any effect on basal CA output from perfused rat adrenal glands (data not shown). Therefore, it was decided to investigate the effects of resveratrol on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion. Secretagogues were given at 15 min-intervals. Resveratrol was present 15 min before initiation of stimulation.

When ACh (5.32 x 10^{-2} M) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was 1227±79 ng for 4 min. However, the pretreatment with resveratrol in the range of 10^{-5} ~ 10^{-4} M for 90 min concentration- and time-dependently inhibited ACh-evoked CA secretion. As shown in Fig. 3 and Table 1~3, in the presence of resveratrol, ACh-evoked CA releasing responses were inhibited by 60% of the corresponding control release. Also, it has been found that depolarizing agent like KCl stimulates markedly CA secretion (605±30 ng for 0-4 min). However, in the presence of resveratrol (10^{-5}

M ~ 10^{-4} M), excess K^+ (5.6 x 10^{-2} M)-stimulated CA secretion was significantly inhibited by 59% of the control release (Fig. 4 and Table 1~3). When perfused through the rat adrenal gland, DMPP (10^{-4} M), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (1189±54 ng for 0-8 min). However, as shown in Fig. 5, DMPP-stimulated CA secretion after pretreatment with resveratrol was greatly reduced to 63% of the control release (Table 1~3). McN-A-343 (10^{-4} M), 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 (4894±21 ng for 0-4 min). However, McN-A-343-stimulated CA secretion in the presence of resveratrol was markedly depressed to 55% of the corresponding control secretion (100%) as depicted in Fig. 6 and Table 1~3.

Effect of resveratrol on CA secretion evoked by Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

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 resveratrol on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. Bay-K-8644 (10^{-5} M)-stimulated CA secretion under the presence of resveratrol was greatly blocked to 72% of the control except for the early 45 min as compared to the corresponding control release (461±21 ng for 0-4 min) from 10 glands as shown in Fig. 7 and Table 4.

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 resveratrol on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 8 and Table 4. In the presence of resveratrol in 12 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was significantly depressed by 71% of the control secretory response (448±19 ng for 0-4 min).

Effect of PCRW on CA secretion evoked by ACh, excess K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

As shown in Fig. 3 ~ 8, resveratrol significantly inhibited the CA secretory responses evoked by cholinergic stimulation and membrane depolarization form the perfused rat adrenal glands. Therefore, in order to compare resveratrol with PCRW, polyphenolic compounds isolated from red wine, on the inhibitory effect of CA release, it was likely of interest to examine the effect of PCRW on CA secretion evoked by cholinergic stimulation and membrane depolarization from the isolated perfused rat adrenal glands. In order to test the effect of PCRW on cholinergic receptor-stimulated CA secretion as well as membrane depolarization-mediated secretion, PCRW (60 μg/ml) was loaded into the adrenal medulla for 90 min. PCRW itself also did not give any effects on basal CA output from perfused rat adrenal glands (data not shown). Therefore, in the subsequent experiments, the effects of PCRW on the CA seretory responses evoked by ACh, high K^+, DMPP and McN-A-343 were examined. As illustrated in Fig. 9 and table

5, ACh (5.32 x 10^{-3} M)-evoked CA release prior to the perfusion with PCRW was 1328±68 ng (0-4 min). In the presence of PCRW (60 µg/ml) for 60 min, it was significantly inhibited by 81% of the control release (100%). High potassium (56 mM KCl), a direct membrane-depolarizing agent, stimulates CA secretion (691±21 ng, 0-4 min). In the present work, high K^+ (5.6 x 10^{-2} M)-evoked CA release in the presence of PCRW (60 µg/ml) for 90 min was also reduced by 81% of the corresponding control secretion (100%) after 30 min period, as shown in Fig. 10 and table 5. DMPP (10^{-4} M), a selective nicotinic receptor agonist in autonomic sympathetic ganglia, when perfused through the rat adrenal gland, evoked a sharp increase in CA secretion. As shown in Fig. 11 and table 5, DMPP (10^{-4} M)-stimulated CA secretion following the loading with PCRW (6x10^{-5} M) was inhibited by 89% compared to the corresponding control secretion (1109±29 ng, 0-8 min). As illustrated in Fig. 12, McN-A-343 (10^{-4} M), which is a selective muscarinic M_1-receptor agonist (Hammer and Giachetti, 1982), perfused into an adrenal vein for 4 min caused an increased CA secretion to 555±18 ng (0-4 min). However, in the presence of PCRW (60 µg/ml), McN-A-343-evoked CA secretion was significantly reduced by 77% of the corresponding control release (Table 5).

Bay-K-8644 (10^{-5} M)-stimulated CA secretion in the presence of PCRW was greatly inhibited to 73% of the corresponding control release (480±21 ng for 0-4 min) from 8 rat adrenal glands, as shown in Fig. 13 and table 6.

As depicted in Fig. 14 and table 6, in the presence of PCRW from 12 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was reduced to 71% of the control response (448±24 ng for 0-4 min).

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

It has also been found that, in this study, resveratrol inhibits the CA secretory response evoked by cholinergic stimulation in the perfused rat adrenal gland. Therefore, to study the relationship between NO and resveratrol-induced inhibitory action on the CA release from the rat adrenal glands, the effect of L-NAME on resveratrol-induced inhibitory responses of CA secretion evoked by cholinergic receptor-stimulation as well as membrane depolarization was examined. In the present study, ACh (5.32 mM)-evoked CA release before perfusion with resveratrol plus L-NAME was 1152±57 ng (0-4 min) from 10 rat adrenal glands. In the simultaneous presence of resveratrol (30 μM) and L-NAME (30 μM) for 90 min, it was initially not affected at 0-34 min, but later rather inhibited by 85% of the corresponding control release for the period of 45-94 min as illustrated in Fig. 15 and table 7. High K^+ (56 mM)-evoked CA release in the presence of resveratrol (30 μM) and L-NAME (30 μM) for 90 min was also not changed for 0-49 min, but later rather inhibited to 88~79% of the corresponding control release only for 45-94 min period in comparison to the control secretion (603±37 ng, 0-4 min) from 7 glands (Fig. 16 and Table 7). As shown in Fig. 17 and table 7, DMPP-evoked CA release prior to the perfusion with resveratrol and L-NAME was 1216±37 ng (0-8 min). The simultaneous perfusion of resveratrol and L-NAME for 90 min no longer inhibited DMPP-evoked CA release for the period of 0-28 min from 12 experiments while later rather depressed to 98~88% of the control release at the period of 40-84 min. As illustrated in Fig. 18,

McN-A-343 (10^{-4} M) perfused into an adrenal vein for 4 min caused an increased CA secretion to 530±18 ng (0-4 min). However, in the simultaneous presence of resveratrol (30 μM) and L-NAME (30 μM) for 90 min, McN-A-343-evoked CA secretion was initially not affected for the period of 0-49 min, but later rather reduced by 83~76% of the corresponding control release only for the period of 60-94 min (Table 7).

As shown in Fig. 19 and table 8, the simultaneous perfusion of resveratrol (30 μM) 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 8 experiments, but later rather depressed to 75% of the control release at the last period of 75-94 min in comparison to their corresponding control responses (512±10 ng, 0-4 min). Also, in the simultaneous presence of resveratrol (30 μM) and L-NAME (30 μM) for 90 min from 10 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was reduced to 88~77% of the control response (448±24 ng for 0-4 min) only for the period of 75-94 min (Fig. 20 and table 8).

IV. DISCUSSION

In the present study, it has been shown that resveratrol as well as 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 resveratrol is exerted by inhibiting both the calcium influx into the rat adrenomedullary chromaffin cells and the release of Ca^{2+} from the cytoplasmic calcium store partly through the activation of NO production, which are likely at least relevant to the direct interaction with the nicotinic receptors.

In support of this idea, it has been reported that PCRW from two different sources, with a similar pharmacological profile in different blood vessels, are able to produce endothelium-dependent relaxation of the rat thoracic aorta (Andriambeloson et al., l997; l999). This effect was mediated by an increase in NO content due to enhancement of NO synthesis rather than protection against its breakdown by oxygen radicals associated with the antioxidant properties of PCRW (Andriambeloson et al., l997). Furthermore, studies in bovine aortic endothelial cells (BAEC) have shown that an increase in NO-synthase activity is associated with an increase in intracellular calcium concentration ($[Ca2+]_i$) and the activation of tyrosine kinases (Martin et al., 2002). It has been shown that oral treatment with Provinols^{TM} (polyphenol-contained product) accelerated the regression of blood pressure and prevents the development of cardiovascular remodeling, myocardial fibrosis and aortic stiffness in NO-deficient hypertensive rats. Furthermore, it has been found that oral administration of Provinols^{TM}

produced a decrease of systolic blood pressure in normotensive rats, which was accompanied with an enhanced endothelium-dependent relaxation and induction of gene expression within the arterial wall (Diebolt et al., 200l). This effect probably involved a NO pathway, inasmuch N^{ω}-nitro-L-arginine-methyl-ester (L-NAME) treatment plus PCRW abolished the decrease in blood pressure and the improvement of endothelial function (Bernátová et al., 2002).

In the present study, in the simultaneous presence of resveratrol and L-NAME (NOS inhibitor), the CA secretory responses evoked by ACh, DMPP, high K^+, McN-A-343, Bay-K-8644 and cyclopiazonic acid were considerably recovered to the extent of the corresponding control secretion compared to those of resveratrol treatment alone. This result is well consistent with report that PCRW 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). Of different anthocyanins identified in wine, only delphinidin caused endothelium-dependent relaxation, although it was slightly less potent than PCRW (Andriambeloson et al., 1998).

Taking into account these findings, in this study it is likely that resveratrol inhibits the CA secretory response evoked by various secretagogues through increasing NO production in adrenal chromaffin cells since resveratrol-induced inhibitory responses of CA secretion were significantly reduced in the presence of L-NAME, an inhibitor of NO synthase. Furthermore, some epidemiological studies indicate that there is an association between moderate consumption of red wine and reduced risk of coronary heart disease (Renaud and de Lorgeril, 1992;

German and Walzem, 2000). It has also been shown that PCRW promotes the endothelium-dependent relaxation, activates NO synthase, inhibits platelet aggregation, and prevents oxidation of LDL-cholesterol (Fitzpatrick, et al., 1993; Andriambeloson, et al., 1997; Flesh, et al., 1998; Leikert, et al., 2002; Demrow and Slane, 1995; Frankel, et al., 1993). The polyphenolic compound resveratrol present in red wine is thought to be a responsible factor for its beneficial cardiovascular effects. Since resveratrol has similar effects to PCRW such as promotion of vasodilation, activation of nitric oxide synthase, inhibition of platelet aggregation and leukocyte activation, prevention of oxidation of LDL-cholesterol and reduction of cholesterol synthesis (Chen and Pace-Asciak, 1996; Wallerath, et al., 2002; Pace-Asciak, et al., 1995; Rotondo, et al., 1998; Frankel, et al., 1993). These effects are in agreement with the present result that resveratrol inhibits the CA secretory responses evoked by cholinergic stimulation and membrane depolarization at least by activation of nitric oxide synthase in the isolated perfused rat adrenal medulla, since this inhibitory effect of resveratrol on the CA secretory responses was significantly attenuated in the presence of L-NAME, an inhibitor of nitric oxide synthase.

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. The adrenal medulla possesses characteristic postganglionic sympathetic neurons, and the presence of nNOS has been demonstrated (Marley, et al., 1995; Oset-Gasque, et al., 1994; Palacios, et al., 1989; 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 resveratrol on the CA secretion were recovered to the considerable extent of the control secretion compared with the inhibitory effects of resveratrol alone. This result demonstrates that resveratrol can inhibit the CA release at least partly through the activation of nNOS in the rat adrenal medulla. 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 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 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 resveratrol possesses the ability partly to activate nNOS in the rat adrenal medullary chromaffin cells, in addition to the direct inhibitory effects on the CA secretion.

In general, the adrenal medulla has been employed as a model system to study numerous cellular functions involving not only noradrenergic nerve cells but also neurons. 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 secretion of CA. Based on this fact, the present findings that resveratrol inhibited the CA secretory responses evoked by nicotinic receptor stimulation as well as by membrane depolarization in the rat adrenal medulla 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., 1993; Fitzpatrick et al., 1995; Fitzpatrick et al., 2000; Zenebe et al., 2003).

These experimental results indicate that resveratrol-induced inhibitory activity of CA secretory response evoked by stimulation of nicotinic receptors might contribute at least partly to its hypotensive mechanism. ACh, the physiological presynaptic transmitter at the adrenal medulla, which is released by depolarizing splanchnic nerve terminals and then activates nicotinic receptors, releases CA, and induces dopamine β-hydroxylase by calcium dependent secretory process

(Dixon et al., 1975; Viveros et al., 1968). In terms of this fact, the present results suggest that resveratrol may inhibit CA secretion evoked by nicotinic stimulation from the splanchnic nerve ending through the blockade of nicotinic receptors. The release of epinephrine from the adrenal medulla in response to splanchnic nerve stimulation or nicotinic agonist is mediated by activation of nicotinic receptors located on the chromaffin cells. The exocytotic CA release from the chromaffin cells appears to be essentially similar to that occuring in noradrenergic axons (Douglas, 1968; Sorimachi & Yoshida, 1979). 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 & Hwang, 1991).

In the present study, both resveratrol and PCRW also time-dependently depressed the CA secretory response evoked by Bay-K-8644, which is known to activate L-type voltage-dependent Ca^{2+} channels (Garcia et al., 1984; Schramm et al., 1983). This result indicates that resveratrol may inhibit Ca^{2+} influx to the rat adrenomedullary cells. In support of this idea, in cultured bovine adrenal medullary cells, nicotinic (but not muscarinic) receptors mediate the Ca^{2+}-dependent secretion of CA (Fisher et al., 1981; Yanagihara et al., 1979). It has also been known that the activation of nicotinic receptors stimulates CA secretion by increasing Ca^{2+} entry through receptor-linked and/or voltage-dependent Ca^{2+} channels in both perfused rat adrenal glands (Wakade & Wakade, 1983; Lim & Hwang, 1991) and isolated bovine adrenal chromaffin cells (Kilpatrick et al., 1981; 1982; Knight & Kesteven, 1983). Wada and his coworkers (1985) 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 resveratrol indicates that this inhibitory effect of resveratrol is exerted through the direct inhibition of calcium influx into the rat adrenal chromaffin cells. Furthermore, slight elevation in the extracellular potassium concentration increases both the frequency of spontaneous action potentials and the secretion of CA (Kidokoro & Ritchie, 1980), suggesting that the influx of calcium that occurs during action potentials is directly linked to the rate of secretion. These findings that resveratrol inhibited CA secretion evoked by Bay-K-8644 as well as by high K^+ suggest that resveratrol 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 (Burgoyne, 1984; Oka et al., 1979). Therefore, it seems that resveratrol inhibits the DMPP-evoked CA secretion by inhibiting Ca^{2+} influx through voltage-dependent Ca^{2+} channels activated by nicotinic ACh receptors.

The present study has also shown that resveratrol 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 (Geoger & Riley, 1989; Siedler 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 can be speculated that the inhibitory effect of resveratrol on 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 resveratrol 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 secretion of CA. 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 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 resveratrol-induced depression of the CA secretion evoked by McN-A-343 and cyclopiazonic acid may be due to the inhibition of Ca^{2+} release from the intracellular pools induced by stimulation of muscarinic ACh receptors. Martin and his coworkers (2002) showed that PCRW stimulated a

Ca^{2+}-dependent release of nitric oxide (NO) from bovine aortic endothelial cells accounting for the relaxation of endothelium-denuded rat aortic rings as shown by cascade bioassay. PCRW, Provinols^{TM} and delphinidin also increased cytosolic free calcium ($[Ca^{2+}]_i$), by releasing Ca^{2+} from intracellular stores and by increasing Ca^{2+} entry. However, in the present study, it is uncertain whether the inhibitory effect of resveratrol on Ca^{2+} movement from intracellular pools is due to its direct effect on the PI response or the indirect effects.

On the other hand, the present findings disagree with those obtained by Yáñez and his coworkers (2006), who reported that both *cis*-resveratrol and *trans*-resveratrol (5–200 μM) concentration-dependently inhibited the uptake of [^{3}H]NA and [^{3}H]5-HT by synaptosomes from rat brain and the uptake of [^{3}H]5-HT by human platelets. Both *cis*-resveratrol and *trans*-resveratrol (5–200 μM) also concentration-dependently inhibited the enzymatic activity of commercial (human recombinant) MAO isoform (MAO-A and MAO-B) activity.

It has also been observed that these natural polyphenols, like a number of antidepressant drugs (Slotkin et al., 1986; Gareri et al., 2000; To et al., 2005), inhibit the uptake of 5-HT by human platelets. Also, it is well known that another classes of drugs used to treat major depressive disorders are the inhibitors of monoamine oxidase (MAO) activity (Gareri et al., 2000; To et al., 2005). To date, however, the effects of resveratrol isomers on MAO isoform (MAO-A and MAO-B) activity have not been studied. Only Zhou and his coworkers (2001), in a preliminary structure–activity relationship study of a number of stilbenoids, have previously shown that *trans*-resveratrol exhibits a selective inhibitory effect on MAO-A activity but not on MAO-B.

In conclusion, the results of the present study have suggest that both resveratrol and PCRW inhibit the CA secretion by stimulation of cholinergic nicotinic receptors as well as by membrane depolarization in the isolated perfused adrenal glands of the normotensive rats. It seems that this inhibitory effect of resveratrol is exerted by blocking both the calcium influx into the rat adrenal medullary chromaffin cells and the release of Ca^{2+} into the cytoplasmic calcium store at least partly via the increased NO production due to the activation of nitric oxide synthase, which are relevant to the direct interaction with the nicotinic receptors. These experimental results may contribute partly to the hypotensive effect of resveratrol components, through inhibition of CA secretion from adrenomedullary chromaffin cells and consequent reduction of the CA level in the circulation. It seems likely that there is no difference in inhibitory action of the CA release between resveratrol and PCRW, based on the concentrations examined in the present work.

V. SUMMARY

Resveratrol clearly has various potent cardiovascular effects in animal but there are no reports on the functional effect of resveratrol on the secretion of catecholamines (CA) in the perfused model of the adrenal gland. Therefore, the aim of the present study was to investigate the ability of resveratrol on the CA secretion in the isolated perfused model of the normotensive rat adrenal gland, to establish its mechanism of action, and additionally to compare its effect with that of polyphenolic compounds isolated from red grape wine (PCRW).

Resveratrol (10~100 μM) perfused into an adrenal vein for 90 min inhibited the CA secretory responses evoked by ACh (5.32 mM), high K^+ (a direct membrane-depolarizer, 56 mM), DMPP (a selective neuronal nicotinic N_n receptor agonist, 100 μM) and McN-A-343 (a selective muscarinic M_1 receptor agonist, 100 μM). Also, in the presence of resveratrol (30 μM), the secretory responses of CA evoked by 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 resveratrol (30 μM) and L-NAME (an inhibitor of NO synthase, 30 μM), the inhibitory responses of resveratrol on the CA secretion evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid were considerably recovered to the extent of the corresponding control secretion compared with the inhibitory effect of resveratrol alone. Interestingly, PCRW (60 μg/mL) perfused into the adrenal medulla also time-dependently caused the inhibitory effect on the

CA secretory responses evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644, and cyclopiazonic acid.

Taken together, these experimental results demonstrate that resveratrol 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 evoked. It seems that this inhibitory effect of resveratrol is exerted by inhibiting both the calcium influx into the adrenal medullary chromaffin cells of the normotensive rats and the release of Ca^{2+} into the cytoplasmic calcium store partly through the increased NO production due to the activation of nitric oxide synthase, which are at least relevant to the direct interaction with the nicotinic receptors. It is also thought that resveratrol inhibits the CA secretion through the similar mechanism with PCRW.

REFERENCES

Andriambeloson, E., Kleschyov, A.L., Muller, B., Beretz, A., Stoclet J.C., 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. *et al..* “Lobstein Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta.” *J Nutr* ***128***: 2324–2333. 1998.

Andriambeloson, E., Stoclet, J.C., 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, A.H., Sayre, D.F.. "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.

Bernátová, I., Pecháòová, O., Babál, P., Kyselá, S., Stvrtina, S., Andriantsitohaina, R.. “Wine polyphenols improve cardiovascular remodelling and vascular function in NO-deficient hypertension.” *Am. J. Physiol.: Heart Circ. Physiol.* ***282*****:** H942–H948, 2002.

Breslow, M.J., Tobin, J.R., Bredt, D.S., Ferris, C.D., Snyder, S.H., Traystman, R.J.. “Nitric oxide as a regulator of adrenal blood flow.” *Am J Physiol* ***264*** (*Heart Circ Physiol* 33): H464-H469, 1993.

Breslow, M.J., Tobin, J.R., Bredt, D.S., Ferris, C.D., Snyder, S.H., Traystman.

R.J.. “Role of nitric oxide in adrenal medullary vasodilation during catecholamine secretion.” *Eur J Pharmacol* ***210***: 105-106, 1992.

Burgoyne, R.D.. “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(11)***: 1965-1969, 2000.

Celotti, E., Ferrarini, R., Zironi, R., Conte, L. S.. “Resveratrol content of some wines obtained from dried Valpolicella grapes: Recioto and Amarone.” *J. Chromatogr. A.* ***730****:* 47–52, 1996.

Challiss, R.A.J., Jones, J.A., Owen, P.J., Boarder, M.R.. “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, T.R., O'Sullivan, A.J., Moreton, R.B., Berridge, M.J., Burgoyne, R.D.. “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, C.K., Pace-Asciak, C.R.. “Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta.” *Gen Pharmacol* ***27(2)***: 363-366, 1996.

Demrow, H.S., Slane, P.R.. “Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries.” *Circulation* ***91***: 1182–1188, 1995.

Diebolt, M., Bucher, B., Andriantsitohaina, R.. “Wine polyphenols decrease blood

pressure, improve NO vasodilatation, and induce gene expression." *Hypertension* ***38***: 159–165, 2001.

Dixon, Wr., Garcia, A.G., Kirkekar, S.M.. "Release of catecholamines and dopamine-beta-hydroxylase from the rat adrenal gland of the cat." *J Physiol* ***244***: 805-824, 1975.

Douglas, W.W.. "Stimulus-secretion coupling: The concept and clues from chromaffin and other cells." *Br J Pharmacol* ***34***: 451-474, 1968.

Fisher, S.K., Holz, R.W., Agranoff, B.W.. "Muscarinic receptors in chromaffin cell culture mediate enhanced phospholipid labeling but not catecholamine secretion." *J Neurochem* ***37***: 491-487, 1981.

Fitzpatrick, D.F., Fleming, R.C., Bing, B., Maggi D.A., 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., Coffey, R.G.. "Endothelium-dependent vasorelaxing activity of wine and other grape products." *Am J Physiol* ***265***: H77-8, 1993.

Fitzpatrick, D.F., Hirschfield, S.L., Ricci, T., Jantzen, P., Coffey, R.G.. "Endothelium-dependent vasorelaxation caused by various plant extracts." *J Cardiovasc Pharmacol* ***26(1)***: 90–95, 1995.

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(4 Pt 2)***: H1183–H1190, 1998.

Frankel, E.N., Waterhouse, A.L., Kinsella, J.E.. "Inhibition of human DL oxidation by resveratrol." *Lancet* ***341***: 1103–1104, 1993.

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

Gareri, P., Falconi, U., De Fazio, P., De Sarro, G.. “Conventional and new antidepressant drugs in the elderly.” *Prog. Neurobiol.* ***61*****:** 353–396, 2000.

German, J.B., Walzem, R.L.. “The health benefits of wine,” *Annu Rev Nutr* **20:** 561–593, 2000.

Goeger, D.E., Riley, R.T.. "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.

Jager, U., Nguyen-Duong, H.. “Relaxant effect of trans-resveratrol on isolated porcine coronary arteries.” *Arzneimitlelforschung* ***49***: 207-211, 1999.

Jager, U., Nguyen-Duong, H.. “Relaxant effect of trans-resveratrol on isolated porcine coronary arteries.” *Arzneimitlelforschung* ***49***: 207-211, 1999.

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

Kilpatrick, D.L., Slepetis, R.J., Corcoran, J.J., Kirshner, N.. “Calcium uptake and catecholamine secretion by cultured bovine adrenal medulla cells.” *J Neurochem* ***38***: 427-435, 1982.

Kilpatrick, D.L., Slepetis, R.J., Kirshner, N.. “Ion channels and membrane

potential in stimulus-secretion coupling in adrenal medulla cells." *J Neurochem* ***36***: 1245-1255, 1981.

Knight, D. E., and Kesteven, N.T.. "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, J.F., Rathel, T.R., Wohlfart, P.V.. "Cheynier, A.M. Vollmar and V.M. Dirsch *et al.*, Red wine polyphenols enhance endothelial nitric oxide release from endothelial cells." *Circulation* ***106***: 1614–1617, 2002.

Lim, D.Y., Hwang, D.H.. "Studies on secretion of catecholamines evoked by DMPP and McN-A-343 in the rat adrenal gland." *Korean J Pharmacol* ***27(1)***: 53-67, 1991.

Lim, D.Y., Kim, C.D., Ahn, K.W.. "Influence of TMB-8 on secretion of catecholamines from the perfused rat adrenal glands." *Arch Pharm Res* ***15(2)***: 115-125, 1992.

Marley, P.D., McLeod, J., Anderson, C., Thomson, K.A.. "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.

Martin, S., Andriambeloson, E., Takeda, K., 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., 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.

Naderali, E.K., Doyle, P.J., Wlliams, G.. “Resveratrol induces vasorelaxation of mesenteric and uterine ateries from female guinea-pigs.” *Clin Sci* ***98***: 537–543, 2000.

Naderali, E.K., Smith, S.L., Doyle, P.J., Wlliams, G.. “The mechanism of resveratrol-induced vasorelaxation differs in the mesenteric resistance arteries of lean and obese rats.” *Clin Sci* ***100*****:** 55–60, 2001.

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.

Oka, M., Isosaki, M., Yanagihara, N.. “Isolated bovine adrenal medullary cells: studies on regulation of catecholamine synthesis and release.” In: Catecholamines: Basic and Clinical frontiers (Eds. Usdin, E., Kopin, I.J., Brachas, J.), *Pergamon Press, Oxford*, pp. 70-72, 1979.

Orsini, F., Pelizzoni, F., Verotta, L., Aburjai, T.. “Isolation, synthesis, and antiplatelet aggregation activity of resveratrol 3-O-b-D-Glucopyranoside and related compounds.” *J. Nat. Prod.* ***60***: 1082–1087, 1997.

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

O'Sullivan, A. J., Burgoyne, R. D.. “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

Pace-Asciak, C.R., Hahn, S.E., Diamandis, E.P., Soleas, G., Goldberg, D.M.. "The red wine phenolics *trans*-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implication for protection against coronary heart disease." *Clin Chim Acta* ***235***: 207–219, 1995.

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

Palmer, R.M., Ashton, D.S., 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(2)****:* 209-115, 2005.

Renaud, S., de Lorgeril, M. "Wine alcohol, platelet and the French paradox for coronary heart disease," *Lancet* ***339*****;** 1523–1526, 1992.

Rodriguez-Pascual, F., Miras-Portugal, M.T., 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.

Rotondo, S., Rajtar, G., Manarinis, S.. "Effect of *trans*-resveratrol, a natural polyphenolic compound, on human polymorphonuclear leukocyte function." *Br J Pharmacol* ***123***: 1691–1699, 1998.

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

Sato, M., Suzuki, Y., Okuda, T., Yokotsuka, K.. "Content of resveratrol, piceid and

their isomers in commercially available wines made from grapes cultivated in Japan.” *Biosci. Biotechnol. Biochem.* ***61:*** 1800–1805, 1997.

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, P.M., 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, N.W., 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.

Slotkin, T.A., Whitmore, W.L., Dew, K.L., Kilts, C.D.. “Uptake of serotonin into rat platelets and synaptosomes: comparative structure-activity relationships, energetics and evaluation of the effects of acute and chronic nortriptyline administration.” *Brain Res. Bull.* ***17*****:** 67–73, 1986.

Sorimachi, M., Yoshida, K.. “Exocytotic release of catecholamines and dopamine-beta-hydroxylase from the perfused adrenal gland of the rabbit and cat.” *Br J Pharmacol* ***65(1)***: 117-125, 1979.

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, R.J., Murray, R.B.. "Manual of pharmacologic calculation with computer programs." 2nd Ed New York Speringer-Verlag p.132, 1987.

To, S.E., Zepf, R.A., Woods, A.G.. “The symptoms, neurobiology, and current pharmacological treatment of depression.” *J. Neurosci. Nurs.* ***37*****:** 102–107, 2005.

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(1):*** 73-77, 1994.

Uenobe, F., Nakamura, S., Miyazawa, M.. “Antimutagenic effects of resveratrol against Trp-P-1.” *Mutat. Res.* ***373:*** 197–200, 1997.

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, O.H.. “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.

Viveros, O.H., Arqueros, L.C., Kirshner, N.. “Release of catecholamines and dopamine beta-hydroxylase from the adrenal medulla.” *Life Sci* ***7***: 609-618, 1968.

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, 1985.

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

Wakade, A.R., Wakade, T.D.. “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., Wine, K. *et al..* “Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase.” *Circulation* ***106***: 1652–1658, 2002.

Yanagihara, N., Isosaki, M., Ohuchi, T., Oka, M.. “Muscarinic receptor-mediated increase in cyclic GMP level in isolated bovine adrenal medullary cells.” *FEBS Lett* ***105***: 296-298, 1979.

Yáñez, M., Fraiz, N., Cano, E., Orallo, F.. “Inhibitory effects of *cis*- and *trans*-resveratrol on noradrenaline and 5-hydroxytryptamine uptake and on monoamine oxidase activity.” *Biochem Biophys Res Commun* 344(2): 688-695, 2006.

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

Zhou, C.X., Kong, L.D., Ye, W.C., Cheng, C.H., Tan, R.X.. “Inhibition of xanthine and monoamine oxidases by stilbenoids from *Veratrum taliense.” Planta Med.* ***67*****:** 158–161, 2001.