Medicinal chemistry

Satoh and Nishida, Med chem 2014, 4:7
DOI: 10.4172/2161-0444.1000189

Review Article Open Access

Cardio-Electopharmacology and Vasodilating Mechanisms of Quercetin

Hiroyasu Satoh* and Seiichiro Nishida

Health Life Science, Shitennoji University, Habikino, Osaka 583-8501, Japan

Abstract

Quercetin, a kind of flavonoids, exerts the cardiovascular actions. In guinea pig ventricular cardiomyocytes, quercetin depresses the action potential duration (APD) and inhibited the underlying ionic currents I_{CaL}.,I_{Krec}. I_{K1} in cardiomyocytes. In rat aorta, quercetin (0.1 to 100 μM) relaxed the contraction induced by pretreatment with 5 μM norepinephrine (NE) in aconcentration-dependent manner. N*G*-monomethyl-L-arginine acetate (L-NMMA) at 100 μM reduced the quercetin (100 μM)-induced vasorelaxation from 97.0 ± 3.7% (n=10, p<0.05) to 78.0 ± 11.6% (n=5, p<0.05). Endothelium removal as well attenuated the vasodilatation. In the presence of both 100 μM L-NMMA and 10 μM indomethacin, the quercetin-induced vasorelaxation was further attenuated by high K (30 mM) or 10 μM tetraethylammonium (TEA). Among K_{Ca} channel inhibitors, the quercetin-induced vasodilatation was attenuated by 0.3 μM apamin (sensitive to SK), but not by 30 nM charybdotoxin (sensitive to BK and IK). Under KCl-induced vasoconstriction, the quercetin-induced vasorelaxation was attenuated by PK-C inhibitors; Gö6983 (α-, β-, γ-, δ and ζ-sensitive) produced stronger than Ro-31-8425 (α-, β-, γ- and ε-sensitive). In rat mesenteric artery, the quercetin-induced vasodilatation was almost resistant to both 100 μM L-N*G*-nitro arginine methyl ester (L-NAME) and 100 μM indomethacin. The L-NAME/indomethacin-resistant quercetin-induced vasodilatation was not modified by TEA (1 mM), but was attenuated by endothelium removal and 100 μM 18α- and 50 μM 18β-glychrrhetinic acids (gap junction inhibitors). Therefore, quercetin dilates the vascular smooth muscle mediated by endothelium-dependent and -independent mechanisms.

Keywords: Quercetin; Vasodilatation; Endothelium; Gap junction; Ionic currents

Introduction

Quercetin, a flavonoid, exists as quercetin glycosides in food, beverages and herbs, and in plasma, as mainly glucuronids or sulfates of quercetin and unconjugated quercetin [1,2]. Quercetin can exhibit significant vasodilatation of rat arteries. In general, flavonoids are vasodilator [3] and scavenger for free radicals [4], and reduce the incidence of cardiovascular diseases and carcinogenesis [5]. Thus, quercetin exerts protective cardiovascular actions through their various pharmacological effects [6,7].

The endothelium dependency of quercetin is predominant in aorta. Most studies (including our previous reports) have shown the endothelium-dependency of the quercetin-induced vasodilatation [7,8]. However, there are several reports that quercetin is less endothelium-dependent or has only a weak endothelium dependency [3,9]. Quercetin can increase the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ in the endothelium [10]. The increase of $[Ca^{2+}]_i$ leads to endogenous nitric oxide synthase (eNOS) activation and NO production [11], and as a result, may involve with endothelium-derived hyperpolarizing factor (EDHF) for the quercetin-induced vasodilatation [12,13]. Plant polyphenols have been reported to induce EDHF-type relaxation [14]. The cardiovascular pharmacological actions of quercetin we have so far investigated are discussed separately in cardiac and vascular pharmacology.

Cardiac Pharmacology

The cells were prepared from tissue taken from the ventricle muscle of guinea pig hearts, using the methods similar to those described previously [15-17]. Under an anesthesia with sodium pentobarbital (30 mg/kg, i.p), the chest was opened and the aorta was cannulated *in situ*. The heart was dissected out and perfused with normal Tyrode solution on the Langendorff apparatus. The heart was washed out by high-K^+ and low-Cl^- solution (KB solution). The temperature of all solutions was maintained at 36.5°C. Effects of quercetin on the action potentials and the ionic currents in the cardiomyocytes were investigated using an Axopatch patch-clamp amplifier (Axon Instruments, Burlingame, CA, U.S.A.) and standard techniques. The composition of the modified Tyrode solution was (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.3, glucose 5, and HEPES 5. The pH was adjusted to 7.4 with NaOH. Quercetin (Tocris Biosci., Northpoint, UK) was dissolved with DMSO. The final concentration of DMSO was diluted 100 to 2000 times, and never caused any responses. The pipette solution contained (in mM): K-aspartate 110, KCl 20, $MgCl_2$ 1, EGTA 10, Mg-ATP 5, creatine phosphate 5, and HEPES 5 (pH 7.2). All values are given as mean ± S.E.M. The differences of mean values were analyzed by Student's t-test and ANOVA for paired data, and a p value of less than 0.05 was considered significant. All the experiments were carried out according to the guidelines laid down by the Nara Medical University Animal Welfare Committee, and also under the terms of the Declaration of Helsinki.

Effects on the action potentials

Under the current-clamp experiments, the isolated single cell was stimulated at 1 Hz. The action potential amplitude (APA) and the maximum rate of depolarization (Vmax) significantly decreased by approximately 12% (n=8) at 1-3 μM (Table 1). The resting potential (RP) was unaffected. Quercetin at 3 μM prolonged the action potential duration at 75% (APD_{75}) by 11.9 ± 3.4% (n=8, p<0.05) and at 90% repolarizations (APD_{90}) by 17.9 ± 3.0% (n=8, p<0.05), but not at the lower concentrations (0.1-1 μM) [6]. The APD means a period for the membrane repolarization, making T wave on ECG. The APD is clinically reflected directly to QT interval, a period between the depolarization and the repolarization of action potential, mainly responsible for the alteration of the delayed rectifier K^+ current (I_{Krec}). Thus, the APD prolongation increases the refractory period

***Corresponding author:** Dr. Hiroyasu Satoh Health Life Science Shitennoji University, 3-2-1 Gakuenmae, Habikino, Osaka 583-8501, Japan, Tel: +81-72-956-3181; Fax: +81-72-956-6011; E-mail: hysat@shitennoji.ac.jp

Received April 29, 2014; **Accepted** June 25, 2014; **Published** June 27, 2014

Citation: Satoh H, Nishida S (2014) Cardio-Electopharmacology and Vasodilating Mechanisms of Quercetin. Med chem 4: 523-530. doi:10.4172/2161-0444.1000189

	n	APA (mV)	RP (mV)	APD_{75} (ms)	APD_{90} (ms)	V_{max} (V/s)
Control	8	117.4 ± 1.2	-80.9 ± 0.5	77.0 ± 2.2	94.1 ± 2.0	182.8 ± 2.5
Quercetin						
0.1 μM	8	117.6 ± 1.2	-80.6 ± 0.6	78.6 ± 2.4	95.0 ± 2.1	182.1 ± 1.2
0.3 μM	8	117.8 ± 1.1	-80.7 ± 1.0	79.8 ± 3.8	97.3 ± 2.0	182.5 ± 2.3
1 μM	8	114.6 ± 1.4	-80.4 ± 2.3	82.9 ± 2.3	100.3 ± 2.6	173.8 ± 2.2 [1)]
3 μM	8	113.7 ± 1.2	-79.7 ± 2.1	86.2 ± 2.0 [1)]	110.9 ± 2.3 [1)]	161.7 ± 2.4 [2)]
Washout	8	110.7 ± 1.0	-80.0 ± 0.6	79.4 ± 2.2	96.8 ± 2.4	172.3 ± 2.2

Values are represented as mean ± S.E.M. [1)]: P<0.05, [2)]: P<0.01, with respect to control value.

Table 1: Modulation of the action potential configurations by quercetin in guinea pig ventricular cardiomyocytes.

and simultaneously elevates $[Ca^{2+}]_i$ [15,17]. A washout for 15-20 min recovered to approximately 90% of the control value.

Effects on the ionic currents

Whole-cell patch voltage-clamp experiments were performed, and test pulses (1 sec duration) were applied to -20 to +60 mV and -40 to -120 mV from a holding potential of -30 mV. The average capacitance was 86.1 ± 2.0 pF (n=44) [17]. Application of quercetin (0.1 to 3 μM) inhibited the L-type Ca^{2+} current (I_{CaL}) (Figure 1). The I_{CaL} at 10 mV decreased by 34.9 ± 3.2% (n=8, p<0.05) at 0.3 μM and by 56.8 ± 3.3% (n=8, p<0.05) at 3 μM. The responses were produced in a concentration-dependent manner. The cells not causing run-down were chose and used for the experiments. Simultaneously, the I_{Krec} at 60 mV increased by 60.4 ± 2.7% (n=8, p<0.001) at 0.3 μM and by 89.7 ± 3.3% (n=8, p<0.001) at 3 μM. In general, the I_{Krec} enhancement may protect a cell due to an APD shortening and a decline of $[Ca^{2+}]_i$, although quercetin prolonged APD. Quercetin decreased the inwardly rectifying K^+ current (I_{K1}). Quercetin did not affect the I_{K1} at lower concentrations, but at 3 μM inhibited it by 12.4 ± 2.1% (n=8, p<0.05). The I_{K1} is closely related with the RP, and is not yet activated in range of -70 to -90 mV of RP. The I_{Na} (as a Vmax) decreased by approximately 18-20% (n=8-9) at higher concentrations (1-3 μM) of quercetin. The effect may produce antiarrhythmic actions. These responses were almost reversible (80-90% of control) after 20 min washout.

Summary

The experiments in guinea pig ventricular cardiomyocytes showed that (1) quercetin prolonged the APD, (2) other action potential parameters were unaffected, (3) quercetin inhibited I_{CaL}, I_{K1}, and Vmax (I_{Na}), but enhanced I_{Krec}, and (4) these responses were almost reversible after a washout. The inhibitions finally lead to decline of $[Ca2+]_i$, resulting in the suppression of the abnormal excitations. Therefore, these electropharmacological effects of quercetin would exert many helpful and protective actions upon cardiac muscle cells under the diseased conditions.

Vascular Pharmacology

Vasodilating effects on rat aorta

Wistar male rats, weighing 150 to 250 g, 7-16 weeks old (n=32), were anesthetized with ether, and euthanized by exsanguination [18,19]. The isolated artery was cut into 1 mm rings in length, suspended between two stainless steel stirrups in a bath filled with 3 ml modified Krebs-Henseleit solution. The modified Krebs-Henseleit solution contained 118 mM NaCl, 4.6 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 11.1 mM glucose, 27.2 mM $NaHCO_3$, 0.03 mM ethylene glycol-O,O'-bis (2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), and 1.8 mM $CaCl_2$. The chamber solution was oxygenated with 95% O_2 and 5% CO_2 at 36.5°C. The removal of endothelial cells was carried

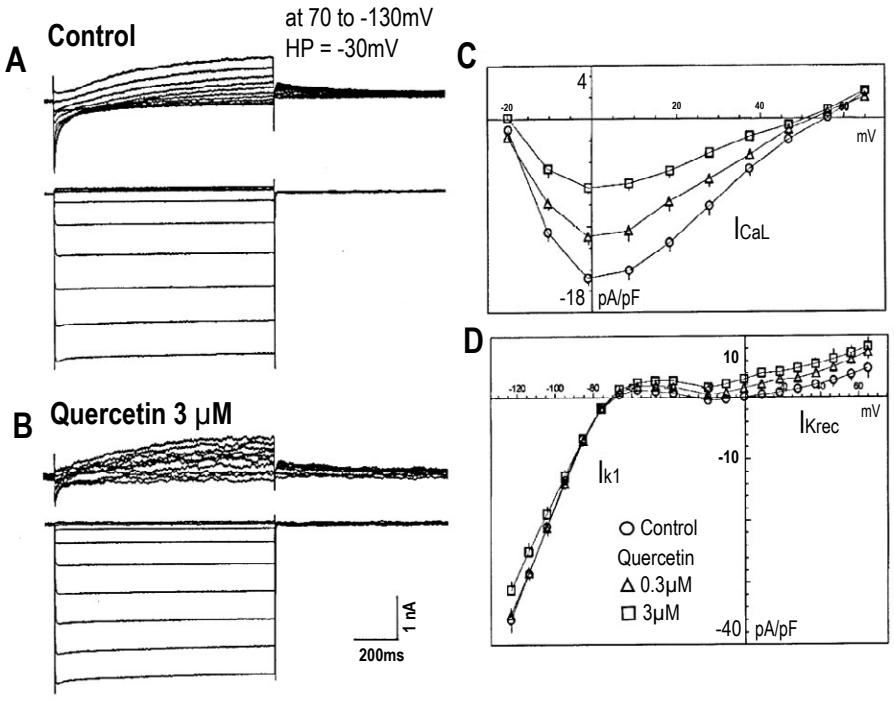

Figure 1: Modulation by quercetin of the ionic currents in guinea pig ventricular cardiomyocytes. A: Control. The test pulses were applied 70 mV and -130 mV from a holding potential of -30 mV. B: Quercetin 3 μM. C: Modulation of I_{CaL} in the absence and the presence of quercetin. Current traces at 30 to 70 mV are superimposed. D: Modulation of I_{K1} by quercetin. Current traces at -130 to -30 mV are presented.

out by usual methods, and the almost absence through microscopy was confirmed after the experiments.

Effects on the endothelial cells

On endothelium-derived releasing factor (EDRF): Rat aorta exhibited a strong contraction by 5 μM NE. Quercetin (0.1 to 100 μM) subsequently administrated exhibited the marked endothelium-dependent actions (Table 2). Prior administration of L-NMMA (100 μM) significantly inhibited the quercetin (100 μM)-induced vasodilatation from 97.8 ± 3.7% (n=10) to 78.0 ± 11.6% (n=5, $p<0.05$). Another NOS inhibitor, L-NAME had the similar effects. This is enforced by the results that removal of endothelium abolished or attenuated the quercetin-induced vasorelaxation. Thus, quercetin decreased the relaxing action by NOS inhibitors. Also, both L-NMMA (100 μM) and indomethacin (10 μM) attenuated the quercetin-induced vasodilatation more than that with L-NMMA alone.

The endothelium-dependency is important in the quercetin-induced vasodilatation. Quercetin causes vasodilatation mainly through NO secretion [19,20], induced by elevating endothelial $[Ca^{2+}]_i$ and eNOS phosphorylation [21,22]. The $[Ca^{2+}]_i$ elevation in endothelium facilitates other actions such as a production of hydroxyl hydrogen [23], endothelial hyperpolarization [24,25] induced by activation of K_{Ca} channels [26], and production of endothelium-derived hyperpolarizing factor (EDHF) [12,27].

On endothelium-derived hyperpolarizing factor (EDHF): Quercetin increases $[Ca^{2+}]_i$ in endothelium, stimulating the synthesis of EDHF [13]. Plant polyphenols have been shown to induce the EDHF-type relaxation [14]. The vasodilatation induced by EDHF is considered to be resistant to both inhibitors of NOS and cyclooxygenase [28]. In the presence of L-NMMA (100 μM) and indomethacin (10 μM), quercetin at 100 μM attenuated by 65.2 ± 6.6% (n=5, $p<0.001$)(Table 3). In aorta, however, the EDHF-related vasorelaxation is told never to observe [29]. So, we examined using rat mesenteric artery as mentioned in Section 4.2.

On Ca^{2+}-activated K^+ (K_{Ca}) channel: The elevation of $[Ca^{2+}]_i$ induced by quercetin may stimulate the K_{Ca} channel. The activation of K_{Ca} channel to hyperpolarize the membrane produces the vasodilatation in rat aorta [19,30]. In endothelium-denuded aorta, TEA significantly decreased the quercetin-induced relaxation from 77.9 ± 2.3% to 62.5 ± 4.9% (n=5, $p<0.05$). The relaxation involved with KCa channel was examined in the presence of indomethacin and L-NMMA. The L-NMMA/indomethacin-resistant relaxation induced by quercetin (100 μM) was significantly reduced by high K^+ (30 mM) to 41.0 ± 5.7% (n=5, $p<0.05$) (Table 3). In high K^+ solution, furthermore, TEA attenuated the L-NMMA/indomethacin-resistant relaxation to 43.8 ± 9.5% (n=5, $p<0.05$). These results indicate that quercetin modulates the KCa channel.

The K_{Ca} channels are classified by their conductances as follows: BK channel (200 pS), IK channel (37 pS), and SK channel (32 pS) [31]. TEA is sensitive to all K_{Ca} channels [32,33], apamine to SK channels [34], and carybdotoxin to BK and IK channels [35]. Apamin (0.3 μM), a SK channel inhibitor, strongly decreased the L-NMMA/indomethacin-resistant relaxation induced by 30 μM quercetin from 30.4 ± 6.2% to 9.4 ± 2.7% (n=5, $p<0.05$), and from 65.2 ± 6.6% to 47.1 ±11.4% (n=5, $p<0.05$) by 100 μM quercetin. But charybdotoxin (30 nM), a BK and IK channel inhibitor, had less or no effect. Therefore, quercetin would possess possibly a selective sensitivity to SK channel, but less to BK and IK channels.

On prostaglandin (PG) I_2: The pretreatment with both indomethacin and L-NMMA reduced the relaxation to a greater extent than the pretreatment with L-NMMA alone (but not significantly). The PGI_2 secretion from endothelium may also partly contribute to the relaxation, as reported previously [20]. Therefore, the endothelium-dependent vasorelaxation induced by quercetin is produced due to EDRF and EDHF, and also partly to PGI_2.

Effects on vascular smooth muscle

On L-type Ca^{2+} channel: L-type Ca^{2+} channel is regulated by the signal transductions such as cAMP, cGMP and PK-C [15,36,37]. 4-β-phorbol-12,13-dibutyrate (PDB, a PK-C activator) has small stimulation of the I_{CaL} in A_7R_5 cells [38,39]. Quercetin at 100 μM significantly decreased the vasorelaxation from 97.8 ± 3.7% (n=10) in normal solution to 67.5 ± 3.7% (n=5) in Ca^{2+}-free solution ($p<0.01$) [7,18]. The quercetin-induced vasorelaxation was also attenuated by nicardipine, and by a switch from normal Krebs' solution (Ca^{2+}=1.8 mM) to Ca^{2+}-free solution. Also, quercetin dilated the KCl-induced vasoconstriction [3,19]. These findings demonstrate the vasorelxation due to its Ca^{2+} channel inhibitory action. Satoh [17] has already reported that quercetin is an inhibitor of I_{CaL} channel in cardiomyocytes. These are summarized in Table 3.

On protein kinase C: The Ca^{2+} mobilization mediated by PK-C stimulation might be dependent on kinds of smooth muscles [40]. PDB caused the vasoconstriction. Quercetin possesses the inhibitory actions of PK-C [3,4]. Quercetin at 100 μM dilated the PDB (300 nM)-induced vasoconstriction by 84.8 ± 6.0% (n=5, $p<0.01$) (Table 3). Under NE-induced vasoconstriction, staurosporine (100 nM), a PK-C inhibitor, decreased the relaxation induced by 100 μM quercetin by approximately 40%. It has been shown that PK-Cα and/or δ are necessary for phorbol ester-mediated constriction of aortic smooth muscle, but are not essential for NE-, vasopressin-, or K^+-induced

	Quercetin							
	n	0.1	0.3	1	3	10	30	100 μM
Control	10	1.7 ± 0.5	3.6 ± 0.9[1)]	6.9 ± 0.9[1)]	12.4 ± 1.1[2)]	32.0 ± 5.7[2)]	54.1 ± 8.4[3)]	97.8 ± 3.7[3)]
Endothelium-denuded	5	1.5 ± 1.3	3.1 ± 1.6	6.9 ± 1.8	11.2 ± 2.4	30.8 ± 4.9	44.1 ± 4.2[a)]	77.9 ± 2.3[a)]
L-NAME 100 μM	5	1.9 ± 0.9	3.6 ± 1.1	7.1 ± 2.1	13.2 ± 3.7	21.1 ± 4.6	33.0 ± 5.3[a)]	69.5 ± 6.1[a)]
L-NMMA 100 μM	5	1.9 ± 0.6	4.1 ± 1.3	7.3 ± 1.8	12.0 ± 2.4	25.4 ± 4.8	38.7 ± 6.0[a)]	78.0 ± 11.6[a)]
L-NMMA 100 μM + Indomethacin 10 μM	5	1.9 ± 0.8	4.1 ± 1.3	6.8 ± 1.3	11.0 ± 2.0	18.2 ± 4.7	30.4 ± 6.2[a)]	65.2 ± 6.6[a)]

Values (%) represent mean ± S.E.M. 1) and a) : $p<0.05$, 2) : $p<0.01$, 3): $p<0.001$. Symbols of 1), 2), and 3) mean significant differences in comparison between the effects of quercetin itself at each concentration and the maximal constriction induced by NE. Symbol of a) indicates significant difference as compared with control (quercetin alone) values.

Table 2: Endothelium-dependent vasodilatation induced by quercetin in rat aorta.

Quercetin								
	n	0.1	0.3	1	3	10	30	100 μM
Ⅰ Constriction induced by Norepinephrine								
A)								
Control (Endothelium+)	10	1.7±0.5	3.6±0.9[1)]	6.9±0.9[2)]	12.4±1.1[2)]	32.0±5.7[3)]	54.1±8.4[3)]	97.8±3.7[3)]
Ca2+-free	5	2.1±1.5	3.1±1.4	9.0±2.0	12.8±3.3	22.6±2.5	45.0±3.3[a)]	67.5±7.7[a)]
Nicardipine	5	0.0±0.0	1.4±1.4	1.4±1.4	1.4 ±1.4[a)]	6.8±3.6[a)]	15.0±7.2[a)]	61.2±16.6[a)]
Ro-31-8425 (0.1μM)	5	1.5±1.0	3.0±1.9	6.6±2.5	12.8±4.0	23.0±4.9	42.8±4.2[a)]	80.8±2.6[b)]
Gö6983 (0.1μM)	5	1.0±0.6	2.0±1.2	2.8±1.7	8.1±2.5	18.8±3.5	40.0±6.2[a)]	84.6±7.7[a)]
B)								
Endothelium-denuded	5	1.5±1.3	3.1±1.6	6.9±1.8	11.2±2.4[1)]	30.8±4.9[2)]	44.1±4.2[2)]	77.9±2.3[3)]
+TEA (100μM)	5	1.0±0.75	3.0±1.3	5.5±2.6	9.2±3.6	17.9±4.2[a)]	31.7±4.9[a)]	62.5±4.9[a)]
C)								
L-NMMA(100μM)+Indomethacin (10μM)	5	1.9±0.8	4.1±1.3	6.8±1.3[1)]	11.0±2.0[2)]	18.2±4.7[2)]	30.4±6.2[3)]	65.2±6.6[3)]
+high K+(30mM)	5	0.5±0.3	2.6±1.5	5.1±1.6	9.4±2.3	13.6±1.9	24.4±3.9[a)]	41.0±5.7[a)]
+TEA (100μM)	5	1.2±0.5	1.8±1.5	1.8±1.5	4.0±4.0	8.5±5.5	16.8±7.0[a)]	43.8±9.5[a)]
+Charybdotoxin	5	2.1±1.3	3.8±2.6	4.2±2.0	4.5±2.3	16.8±3.6	28.2 ±2.7	63.4±11.8
+Apamin	5	0.6±0.6	0.8±0.8	1.6±1.6	3.7±2.7	5.1±1.6	9.4±2.4[b)]	47.1±11.1[b)]
Ⅱ Constriction induced by KCL								
A)								
Control	10	0.0±0.0	0.8±0.4	0.9±0.4	0.9±0.4	8.8±2.2[1)]	29.9±6.0[2)]	92.8±4.0[3)]
Ro-31-8425 (0.1μM)	5	0.0±0.0	0.0±0.0	0.3±0.3	1.0±0.6	9.6±3.1	16.0±3.1[a)]	75.6±7.9[a)]
Gö6983 (0.1μM)	5	0.0±0.0	1.0±1.0	1.1±1.0	1.4±1.2	8.0±1.2	26.8±2.8	49.5±13.7[a)]

Values (%) represent mean ± S.E.M. **1)** and **a)** : $p<0.05$, 2) and b):$p<0.01$, **3)**: $p<0.001$.
Ⅰ: A) The symbols of a), b) indicate significant difference as compared with control (endothelium (+)) values. **B)** The symbol of a) indicate indicates significant difference as compared with endothelium-denuded. **C)** The symbol of a), b) indicates significant difference as compared with L-NAME+indomethacin. **Ⅱ: A)** Symbols of **1)**, **2)**, and **3)** mean significant differences in comparison between effects of quercetin itself at each concentration and the maximal constriction induced by KCl. The symbol of a) indicates significant difference as compared with control (quercetin alone) values.

Table 3: The alteration of endothelium-independent vasodilatation induced by quercetin in rat aorta.

constriction [41]. Furthermore, PK-Cα and δ isozymes are dominant in cultured rat aortic vascular smooth muscle cell, and both isozymes are completely down-regulated by prolonged (16-24 hr) stimulation with the PK-C activator [42]. PK-Cε can modulate phenylephrine-induced contraction in mesenteric artery via calcium-independent pathways [43].

To examine which subtypes of PK-C are related with the quercetin-induced constriction, two different types of PK-C inhibitors were chosen; Gö6983 sensitive to α, β, γ, δ and ζ isozymes, and Ro-31-8425 to α, β, γ and ε isozymes of of PK-C [44]. Ro-31-8425 (0.1 μM) attenuated the quercetin (100 μM)-induced vasorelaxation by 80.8 ± 2.6% (n=5, p<0.001). Gö6983 (0.1 μM), also had similar effects by 84.6 ± 7.6% (n=5, p<0.001). Under the KCl-induced vasoconstriction, both Gö6983 and Ro-31-8425 attenuated the quercetin (100 μM)-induced vasorelaxation by 49.5 ± 7.9% (n=5, p<0.01) and by 75.5 ± 7.9% (n=5, p<0.001), respectively (Table 3). Thus, quercetin might inhibit possibly mediated through PK-Cδ, although it is still difficult to clearly distinguish. In addition, PK-C phosphrylates tyrosin kinase and vasodilator-stimulated phosphoprotein (VASP) as a substrate of cGMP-dependent protein kinase (cGMP-PK) [45]. In this study, however, genistein (tyrosine kinase inhibitor) at 50 μM failed to affect the quercetin-induced constriction (by just 0.7% decrease). The activation of MLCK is abolished by PK-C [40,46]. Furthermore, quercetin inhibits the phosphorylation of mitogen-activated protein kinases (MAPKs); extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK, and c-jun amino-terminal kinase (JNK) in cultured aortic cells and phosphatidylinositol 3-kinase (PI_3-kinase)/protein kinase B (Akt) [47,48].

On Ca^{2+}-activated K^+ (K_{Ca}) channels: Flavonoids have been shown to produce vasodilatation due to the K_{Ca} channel to hyperpolarize the membrane of vascular smooth muscle [30]. The $[Ca^{2+}]_i$ in smooth muscle cells initially increases, and then, facilitates the other physiological actions. The vasorelaxation of quercetin is also due to K_{Ca} activation involved with EDHF [9]. Recently, quercetin has been demonstrated to activate BK channel in coronary arteries via production of H_2O_2 [49]. In addition, glibenclamide (a K_{ATP} channel inhibitor) have not been reported to affect the quercetin-induced vasodilatation in rat aorta [9]. Anyway, the vasodilatation is responsible for SK channel activation (though not distinguish in endothelium or smooth muscle cells) (Table 3).

Summary: The vasodilation mechanisms in rat aorta were due to the inhibitions of (1) NOS by L-NMMA or L-NAME and removal of endothelium, (2) I_{CaL} by Ca^{2+} antagonist, in Ca^{2+} free solution and under the KCl constriction, (3) PK-C activation mediated through possibly the PK-Cδ subtype, and (4) K_{Ca} channel by apamin but not by charybdotoxin. Furthermore, the vasodilation was produced by (5) both L-NMMA and indomethacin, (6) L-NMMA and indomethacin plus TEA during exposure to high K^+ solution, and (7) TEA in endothelium-denuded aorta (Figure 2). Therefore, quercetin's effect is due to endothelium-dependent actions mediated through the NO (EDRF), EDHF and partly PGI_2 syntheses. As well it is due to endothelium-independent actions mediated through the Ca^{2+} channel, the K_{Ca} channels selective to SK channel, and PK-C [7,18].

Vasodilating Effects on Rat Mesenteric Artery

For this investigation, the similar methods to rat aorta experiments were performed. Mesenteric artery was removed from the same Wistar male rats [18,19]. The isolated mesenteric artery was cut into 1 mm rings in length, suspended between two stainless steel stirrups in a bath filled with modified Krebs-Henseleit solution. The isometric force was recorded using the force-displacement transducer (UL-10GR, Minebea Co., Tokyo, Japan). Rat mesenteric artery also caused the contraction by an application of 1 μM NE. Quercetin (0.1-100 μM) caused the significant

vasodilatation in a concentration-dependent manner; at 100μM, the vasodilatation was 99.6 ± 3.0% (n=10, p<0.001). The quercetin-induced vasodilatation was almost consistent with the results in rat aorta [50]. But it was weaker blockade than the results in rat aorta (Table 4). Similarly both L-NAME (100 μM) and indomethacin (100 μM) failed to cause a remarkable change in the quercetin-induced vasodilatation, as compared with that with L-NAME alone. Endothelium-denuded arteries also contracted at 1 μM NE. Endothelium removal strongly decreased the quercetin-induced vasodilatation; at 100 μM quercetin by 77.9 ± 2.4% (n=6, p<0.01). Furthermore, endothelium-removal more strongly reduced the quercetin-induced vasodilatation in the presence of L-NAME and indomethacin, indicating that the relaxation is independent of endothelium. Thus, NO may play a minor role for the quercetin-induced vasodilatation in mesenteric artery, different from aorta.

On K_{Ca} channel

The L-NAME/indomethacin-resistant (NO/prostaglandin-independent) relaxation induced by quercetin was never affected by 100 μM TEA (Table 4). Increasing TEA concentration to 1 mM tended to be reduced the resistant vasodilation at 10 to 30 μM quercetin, but not significantly. At 100 μM quercetin, TEA (1 mM) was almost identical to the L-NAME/indomethacin-resistant vasodilatation (82.3 ± 2.6, n=8, p<0.001) [50]. Since quercetin stimulates SK channel in rat aorta [19], quercetin would similarly activate SK channel in mesenteric artery.

On endothelium-derived hyperpolarizing factor (EDHF)

Since the contribution to EDHF-related vasorelaxation is told never to observe in aorta [29], the experiments using rat mesenteric artery were performed. The hyperpolarization induced by EDHF makes the membrane stable, and depresses the ionic channel activity related to the vasoconstriction. The quercetin-induced relaxation in the presence of indomethacin and L-NMMA was reduced by high K^+ or TEA. Apamin inhibited the quercetin-induced vasorelaxation. These findings strongly demonstrate that the quercetin-induced vasorelaxation is closely involved with EDHF. Therefore, it is possible that quercetin may increases $[Ca^{2+}]_i$, and then facilitates EDHF synthesis as well as NOS in endothelium.

In rat mesenteric artery, quercetin actually produces NO from endothelium. However, endothelium removal attenuated the quercetin-induced vasodilatation to a greater extent than both L-NAME and indomethacin (Table 4). This strongly demonstrates that the endothelium of mesenteric artery plays an important role in the quercetin-induced vasodilatation, mainly dependent on EDHF rather than NO. In resistant vessels than aorta, the EDHF-type relaxation is relatively more predominant [29]. ACh causes the EDHF-type relaxation mediated through K_{Ca} channels in fetal aorta [51]. L-NAME attenuated (though be a weak blockade) the quercetin-induced vasodilatation. Thus, NO plays a minor role for the quercetin-induced vasodilatation of mesenteric artery. As well, it may be considered that quercetin is responsible for EDHF-type relaxation, which which is attenuated by high K+ or TEA [32,52]. Moreover, the EDHF-type relaxation is attenuated by bothapamin and charybdotoxin [53].

EDHF hyperpolarizes to dilate smooth muscle cells. Some candidates for EDHF have already been shown: K^+ [52,53], epoxyeicosatrienoic acids (EETs) [52,54], and H_2O_2 derived from endothelium [23]. In our studies, two mechanisms for the EDHF-induced hyperpolarization have been inferred: (1) EDHF hyperpolarizes smooth muscle cells by stimulating K_{Ca} channels [13,28], and (2) the EDHF-mediated response is due to gap junctions, which is confirmed by the electrical communications between endothelium and smooth muscle cells [27,52]. Furthermore, the gap junction for EDHF-type relaxation has already identified by using antibody analysis [55,56].

On gap junction

In our experiments, the quercetin-induced relaxation in the presence of indomethacin and L-NAME was not reduced by high TEA (1 mM). Additional pretreatment with 100 μM 18α- or 50 μM 18β-glychrrhetinic acids (18α- and 18β-GAs) significantly inhibited the L-NAME/indomethacin-resistant vasodilatation from 82.0 ± 2.4% (n=6) to 63.3 ± 5.5% (n=9, p<0.01) (Table 4). 18α- and 18β-GAs are the selective blockers of gap junctions [57]. Endothelium removal also attenuated the quercetin-induced vasodilatation to the same extent as the presence of the inhibitors such as L-NAME, indomethacin, and 18α- and 18β-GAs. So, these findings indicate that the gap junctions contribute to the quercetin-induced vasodilatation. Both 18α- and 18β-GAs reduced the quercetin-induced vasodilatation to a greater extent, as compared with the effect of TEA.

The discrepancy between the results of TEA and 18α- and 18β-GAs might be due to both mechanisms: (1) quercetin elevates endothelial $[Ca^{2+}]_i$ and then, activates K_{Ca} channels, and (2) quercetin itself has a stimulatory actions on K_{Ca} channels. The elevation of $[Ca^{2+}]_i$ induced by quercetin activates SK channels [19] to hyperpolarize the endothelium [58,59]. Quercetin would activate SK channel in endothelium via both

	Quercetin							
	n	0.1	0.3	1	3	10	30	100 μM
Control A	10	1.6 ± 0.94	8.0 ± 1.7[1)]	23.4 ± 3.2[2)]	33.9 ± 2.7[2)]	55.5 ± 3.2[3)]	69.8 ± 3.4[3)]	99.6 ± 3.0[3)]
Endothelium-denuded	6	1.8 ± 0.55	3.8 ± 1.6[b)]	8.5 ± 1.9[b)]	13.0 ± 2.6[b)]	30.8 ± 4.9[b)]	44.2 ± 4.2[b)]	77.9 ± 2.4[b)]
L-NAME 100 μM	8	1.3 ± 0.9	3.2 ± 1.8	19.2 ± 3.4	31.0 ± 2.8	51.2 ± 2.0	62.3 ± 4.7	86.0 ± 7.0[a)]
B								
L-NAME+Indomethacin (100 μM)	8	1.9 ± 0.75	4.1 ± 1.3	19.0 ± 7.3	31.2 ± 6.3	52.5 ± 7.8	63.2 ± 6.9	82.0 ± 2.2
+TEA(100μM)	6	1.3 ± 0.80	7.5 ± 2.9	20.2 ± 5.7	32.0 ± 3.0	52.5 ± 2.6	67.8 ± 2.4	82.0 ± 2.4
+glycyrrhetinic acids	9	2.2 ± 1.3	5.5 ± 2.8	11.5 ± 5.0	20.5 ± 5.3	35.0 ± 6.0[a)]	51.1 ± 4.3[a)]	63.3 ± 5.5[b)]

Values (%) represent mean ± S.E.M. [1)] and [a)]: $p<0.05$, [2)] and [b)] :$p<0.01$, [3)]: $p<0.001$.
Symbols of [1)], [2)], and [3)] mean significant differences in comparison between effects of quercetin itself at each concentration and the maximal contraction induced by NE. A) The symbol of [a), b)] indicate significant difference as compared with control (quercetin alone) values. B) The symbol of [a), b)] indicate significant difference as compared with L-NAME and indomethacin.

Table 4: Modulation of endothelium-dependent relaxation induced by quercetin in rat mesenteric artery.

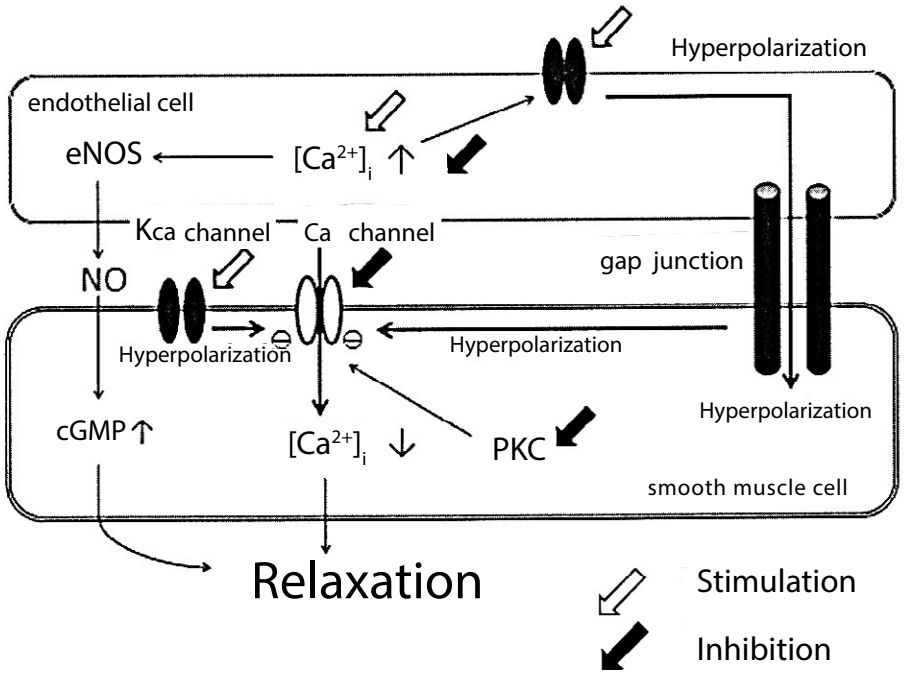

Figure 2: Diagram for the mechanism of quecetin-induced vasorelaxation. Quercetin induces $[Ca^{2+}]_i$ elevation, leading to NO production and K_{Ca} channel activation in endothelium cell. The hyperpolarization induced by the activation of K_{Ca} channel inhibits Ca^{2+} channel in smooth muscle cell. Moreover, modulation of gap junction aggravates the vasodilatation.

mechanisms. Thus, TEA failed to inhibit K_{Ca} channels in endothelium. Although the effect of TEA on the quercetin-induced vasodilatation is affected by both mechanisms, the transduction of the endothelial hyperpolarization to smooth muscle cells is blocked by 18α- and 18β-GAs, and also the endothelial removal. Therefore, the gap junctions involved with EDHF would be responsible for the quercetin-induced vasodilatation [50].

Summary

Rat mesenteric artery possesses almost the similar characteristics to the aorta (Figure 2); (1) quercetin caused a concentration-dependent vasodilatation, (2) the quercetin-induced vasodilation was attenuated by L-NAME, (3) the addition of indomethacin did not cause further modifications, (4) TEA (1 mM) tended to reduce L-NAME/indomethacin-resistant vasodilatation, (5) at high concentrations (100 μM) of quercetin, TEA failed to produce any effects on L-NAME/indomethacin-resistant vasodilatation, (6) L-NAME/indomethacin-resistant vasodilatation was reduced by 18α- and 18β-GAs, (7) endothelium removal strongly attenuated the quercetin-induced vasodilatation, and (8) 18α- and 18β-GAs reduced the quercetin-induced vasodilatation to the same significant extent as the endothelium removal.

The vasodilatation in rat mesenteric artery is also endothelium-dependent. Quercetin produces NO in endothelium, but plays minor role in the quercetin-induced vasodilatation. The vasodilatation induced by quercetin is mainly due to the gap junctions, closely involved with EDHF.

References

1. Manach C, Morand C, Texier O, Favier ML, Agullo G, et al. (1995) Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. J Nutr 125: 1911-1922.
2. Erlund I, Silaste ML, Alfthan G, Rantala M, Kesäniemi YA, et al. (2002) Plasma concentrations of the flavonoids hesperetin, naringenin and quercetin in human subjects following their habitual diets, and diets high or low in fruit and vegetables. Eur J Clin Nutr 56: 891-898.
3. Duarte J, Pérez-Vizcaíno F, Zarzuelo A, Jiménez J, Tamargo J (1993) Vasodilator effects of quercetin in isolated rat vascular smooth muscle. Eur J Pharmacol 239: 1-7.
4. Murota K, Terao J (2003) Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. Arch Biochem Biophys 417: 12-17.
5. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 342: 1007-1011.
6. Satoh H (2003) Effects of ginkgo biloba extract and bilobalide, a main constituent, on the ionic currents in guinea pig ventricular cardiomyocytes. Arzneimittelforschung 53: 407-413.
7. Satoh H, Nishida S (2004) Electropharmacological actions of Ginkgo biloba extract on vascular smooth and heart muscles. Clin Chim Acta 342: 13-22.
8. Satoh H, Nishida S (2010) Cardiovascular pharmacology of Ginkgo biloba extract and its constituents. Basics of Evidences-Based Herbal Medicine. Signpost, Kerala, India, pp. 37-58.
9. Pérez-Vizcaíno F, Ibarra M, Cogolludo AL, Duarte J, Zaragozá-Arnáez F, et al. (2002) Endothelium-Independent Vasodilator effects of the flavonoid quercetin and its methylated metabolites in rat conductance and resistance arteries. J Pharmacol Exp Ther 302: 66-72.
10. Kuhlmann CR, Schaefer CA, Kosok C, Abdallah Y, Walther S, et al. (2005) Quercetin-induced vasodilatation of the NO/cGMP pathway depends on Ca^{2+}-activated K^+ channel-induced hyperpolarization-mediated Ca2+ entry into cultured human endothelial cells. Planta Medica 6: 520-524.
11. Mayer B, Schmidt K, Humbert P, Böhme E (1989) Biosynthesis of endothelium-derived relaxing factor: a cytosolic enzyme in porcine aortic endothelial cells Ca^{2+}-dependently converts L-arginine into an activator of soluble guanylate cyclase. Biochem. Biophys Res Commun 164: 678-685.
12. Chen GF, Suzuki H (1990) Calcium dependency of the endothelium-dependent hyperpolarization in smooth muscle cells of the rabbit carotid artery. J Physiol 421: 521-534.
13. Félétou M, Vanhoutte PM (2002) The Alternative: EDHF. J Mol Cell Cardiol 31: 15-22.

14. Ndiaye M, Chataigneau T, Andriantsitohaina R, Stoclet JC, Schini-Kerth VB (2003) Red wine polyphenols cause endothelium-dependent EDHF-mediated relaxations in porcine coronary arteries via a redox-sensitive mechanism. Biochem Biophys Res Commun 310:371-7.

15. Satoh H (1996) Enhancement of K^+ currents by stimulation of protein kinase C in the uterine smooth muscle cells of the pregnant rat. Gen Pharmacol 27: 455-458.

16. Satoh H (1996) Modulation of Ca(2+)-activated K+ current by isoprenaline, carbachol, and phorbol ester in cultured (and fresh) rat aortic vascular smooth muscle cells. Gen Pharmacol 27: 319-324.

17. Satoh H (2004) Comparative Electropharmacological Actions of Some Constituents from Ginkgo biloba Extract in Guinea-pig Ventricular Cardiomyocytes. Evid Based Complement Alternat Med 1: 277-284.

18. Nishida S, Satoh H (2004) Comparative vasodilating actions among terpenoids and flavonoids contained in Ginkgo biloba extract. Clin Chim Acta 339: 129-133.

19. Nishida S, Satoh H (2009) Possible Involvement of Ca Activated K Channels, SK Channel, in the Quercetin-Induced Vasodilatation. Korean J Physiol Pharmacol 13: 361-365.

20. Ajay M, Gilani AU, Mustafa MR (2003) Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. Life Sci 74: 603-612.

21. Khoo NK, White CR, Pozzo-Miller L, Zhou F, Constance C, et al. (2010) Dietary flavonoid quercetin stimulates vasorelaxation in aortic vessels. Free Radic Biol Med 49: 339-347.

22. Li PG, Sun L, Han X, Ling S, Gan WT, et al. (2012) Quercetin induces rapid eNOS phosphorylation and vasodilation by an Akt-independent and PKA-dependent mechanism. Pharmacology 89: 220-228.

23. Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, et al. (2000) Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. J Clin Invest 106: 1521-1530.

24. Busse R, Fichtner H, Lückhoff A, Kohlhardt M (1988) Hyperpolarization and increased free calcium in acetylcholine-stimulated endothelial cells. Am J Physiol 255: H965-969.

25. Vaca L, Licea A, Possani LD (1996) Modulation of cell membrane potential in cultured vascular endothelium. Am J Physiol 270: C819-824.

26. Dora KA, Gallagher NT, McNeish A, Garland CJ (2008) Modulation of endothelial cell KCa3.1 channels during endothelium-derived hyperpolarizing factor signaling in mesenteric resistance arteries. Circ Res 102: 1247-1255.

27. Ungvari Z, Csiszar A, Koller A (2002) Increases in endothelial Ca(2+) activate K(Ca) channels and elicit EDHF-type arteriolar dilation via gap junctions. Am J Physiol Heart Circ Physiol 282: H1760-1767.

28. Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A (1997) Sources of Ca2+ in relation to generation of acetylcholine-induced endothelium-dependent hyperpolarization in rat mesenteric artery. Br J Pharmacol 120: 1328-1334.

29. Woodman OL, Wongsawatkul O, Sobey CG (2000) Contribution of nitric oxide, cyclic GMP and K+ channels to acetylcholine-induced dilatation of rat conduit and resistance arteries. Clin Exp Pharmacol Physiol 27: 34-40.

30. Calderone V, Chericoni S, Martinelli C, Testai L, Nardi A, et al. (2004) Vasorelaxing effects of flavonoids: investigation on the possible involvement of potassium channels. Naunyn Schmiedebergs Arch Pharmacol 370: 290-298.

31. Brayden JE, Nelson MT (1992) Regulation of arterial tone by activation of calcium-dependent potassium channels. Science 256: 532-535.

32. Chen G, Yamamoto Y, Miwa K, Suzuki H (1991) Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. Am J Physiol 260: H1888-1892.

33. Hill CE, Hickey H, Sandow SL (2000) Role of gap junctions in acetylcholine-induced vasodilation of proximal and distal arteries of the rat mesentery. J Auton Nerv Syst 81: 122-127.

34. Maylie J, Bond CT, Herson PS, Lee WS, Adelman JP (2004) Small conductance Ca2+-activated K+ channels and calmodulin. J Physiol 554: 255-261.

35. Neylon CB, Lang RJ, Fu Y, Bobik A, Reinhart PH (1999) Molecular cloning and characterization of the intermediate-conductance Ca(2+)-activated K(+) channel in vascular smooth muscle: relationship between K(Ca) channel diversity and smooth muscle cell function. Circ Res 85: e33-43.

36. Satoh H (1995) Depression of the spontaneous activity by phorbol esters in young embryonic chick cardiomyocytes. Jpn J Pharmacol 67: 297-304.

37. Satoh H, Hashimoto K (1988) On electrophysiological responses to phorbol esters which stimulate protein kinase C in rabbit sino-atrial node cells. Naunyn Schmiedebergs Arch Pharmacol 337: 308-315.

38. Satoh H, Sperelakis N (1991) Calcium and potassium currents in cultured in rat aortic vascular smooth muscle cell lines, Ion Channels of Vascular Smooth Muscle Cells and Endothelial Cells. Academic Press, New York, USA, pp.55-63.

39. Satoh H, Sperelakis N (1995) Modulation of L-type Ca2+ current by isoprenaline, carbachol and phorbol ester in cultured rat aortic vascular smooth muscle (A7r5) cells. Gen Pharmacol 26: 369-379.

40. Murthy KS, Grider JR, Kuemmerle JF, Makhlouf GM (2000) Sustained muscle contraction induced by agonists, growth factors, and Ca(2+) mediated by distinct PKC isozymes. Am J Physiol Gastrointest Liver Physiol 279: G201-210.

41. Ohanian V, Ohanian J, Shaw L, Scarth S, Parker PJ, et al. (1996) Identification of protein kinase C isoforms in rat mesenteric small arteries and their possible role in agonist-induced contraction. Circ Res 78: 806-812.

42. Busuttil SJ, Morehouse DL, Youkey JR, Singer HA (1996) Antisense suppresssion of protein kinase C-alpha and -delta in vascular smooth muscle. J Surg Res 63: 137-142.

43. Shirasawa Y, Rutland TJ, Young JL, Dean DA, Joseph BN (2003) Modulation of protein kinase C (PKC)-mediated contraction and the possible role of PKC epsilon in rat mesenteric arteries. Front Biosci 8: a133-138.

44. Zhu S, White RE, Barman SA (2006) Effect of PKC isozyme inhibition on forskolin-induced activation of BKCa channels in rat pulmonary arterial smooth muscle. Lung 184: 89-97.

45. Wentworth JK, Pula G, Poole AW (2006) Vasodilator-stimulated phosphoprotein (VASP) is phosphorylated on Ser157 by protein kinase C-dependent and -independent mechanisms in thrombin-stimulated human platelets. Biochem J 393: 555-564.

46. Hagiwara M, Inoue S, Tanaka T, Nunoki K, Ito M, et al. (1988) Differential effects of flavonoids as inhibitors of tyrosine protein kinases and serine/threonine protein kinases. Biochem Pharmacol 37: 2987-2992.

47. Shih CM, Lin H, Liang YC, Lee WS, Bi WF, et al. (2004) Concentration-dependent differential effects of quercetin on rat aortic smooth muscle cells. Eur J Pharmacol 496: 41-48.

48. Lin CW, Hou WC, Shen SC, Juan SH, Ko CH, et al. (2008) Quercetin inhibition of tumor invasion via suppressing PKC delta/ERK/AP-1-dependent matrix metalloproteinase-9 activation in breast carcinoma cells. Carcinogenesis 29: 1807-1815.

49. Cogolludo A, Frazziano G, Briones AM, Cobeño L, Moreno L, et al. (2007) The dietary flavonoid quercetin activates BKCa currents in coronary arteries via production of H2O2. Role in vasodilatation. Cardiovasc Res 73: 424-431.

50. Nishida S, Satoh H (2013) Role of gap junction involved with endothelium-derived hyperpolarizing factor for the quercetin-induced vasodilatation in rat mesenteric artery. Life Sci 92: 752-756.

51. Martínez-Orgado J, González R, Alonso MJ, Marín J (1999) Nitric oxide-dependent and -independent mechanisms in the relaxation elicited by acetylcholine in fetal rat aorta. Life Sci 64: 269-277.

52. Campbell WB, Gebremedhin D, Pratt PF, Harder DR (1996) Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ Res 78: 415-423.

53. Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH (1998) K+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 396: 269-272.

54. Edwards G, Thollon C, Gardener MJ, Félétou M, Vilaine J, et al. (2000) Role of gap junctions and EETs in endothelium-dependent hyperpolarization of porcine coronary artery. Br J Pharmacol 129: 1145-1154.

55. Mather S, Dora KA, Sandow SL, Winter P, Garland CJ (2005) Rapid endothelial cell-selective loading of connexin 40 antibody blockes endothelium-derived hyperpolarizing factor dilation in rat small mesenteric arteries. Circ Res 97: 399-407.

56. Lang NN, Luksha L, Newby DE, Kublickiene K (2007) Connexin 43 mediates endothelium-derived hyperpolarizing factor-induced vasodilatation in subcutaneous resistance arteries from healthy pregnant womem. Am J Physiol Heart Circ Physiol 292: 1026-32.

57. Guan BC, Si JQ, Jiang ZG (2007) Blockade of gap junction coupling by glycyrrhetinic acids in guinea pig cochlear artery: a whole-cell voltage- and current-clamp study. Br J Pharmacol 151: 1049-1060.

58. Murphy ME1, Brayden JE (1995) Apamin-sensitive K+ channels mediate an endothelium-dependent hyperpolarization in rabbit mesenteric arteries. J Physiol 489: 723-734.

59. García-Pascual A, Labadía A, Jimenez E, Costa G (1995) Endothelium-dependent relaxation to acetylcholine in bovine oviductal arteries: mediation by nitric oxide and changes in apamin-sensitive K+ conductance. Br J Pharmacol 115: 1221-1230.