

Cardio-Electropharmacology and Vasodilating Mechanisms of Quercetin

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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 μ M) relaxed the contraction induced by pretreatment with 5 μ M norepinephrine (NE) in a concentration-dependent manner. NG-monomethyl-L-arginine acetate (L-NMMA) at 100 μ M reduced the quercetin (100 μ M)-induced vasorelaxation from $97.0 \pm 3.7\%$ ($n=10$, $p<0.05$) to $78.0 \pm 11.6\%$ ($n=5$, $p<0.05$). Endothelium removal as well attenuated the vasodilatation. In the presence of both 100 μ M L-NMMA and 10 μ M indomethacin, the quercetin-induced vasorelaxation was further attenuated by high K (30 mM) or 10 μ M tetraethylammonium (TEA). Among K_{Ca} channel inhibitors, the quercetin-induced vasodilatation was attenuated by 0.3 μ M 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 μ M L-NG-nitro arginine methyl ester (L-NAME) and 100 μ M indomethacin. The L-NAME/indomethacin-resistant quercetin-induced vasodilatation was not modified by TEA (1 mM), but was attenuated by endothelium removal and 100 μ M 18 α - and 50 μ M 18 β -glycyrrhetic 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 \pm 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 (V_{max}) significantly decreased by approximately 12% ($n=8$) at 1-3 μ M (Table 1). The resting potential (RP) was unaffected. Quercetin at 3 μ M prolonged the action potential duration at 75% (APD_{75}) by $11.9 \pm 3.4\%$ ($n=8$, $p<0.05$) and at 90% repolarizations (APD_{90}) by $17.9 \pm 3.0\%$ ($n=8$, $p<0.05$), but not at the lower concentrations (0.1-1 μ M) [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

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	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 [Ca²⁺]_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 Ca²⁺ 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 [Ca²⁺]_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 MgSO₄, 1.2 mM KH₂PO₄, 11.1 mM glucose, 27.2 mM NaHCO₃, 0.03 mM ethylene glycol-O,O'-bis (2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), and 1.8 mM CaCl₂. The chamber solution was oxygenated with 95% O₂ and 5% CO₂ at 36.5°C. The removal of endothelial cells was carried

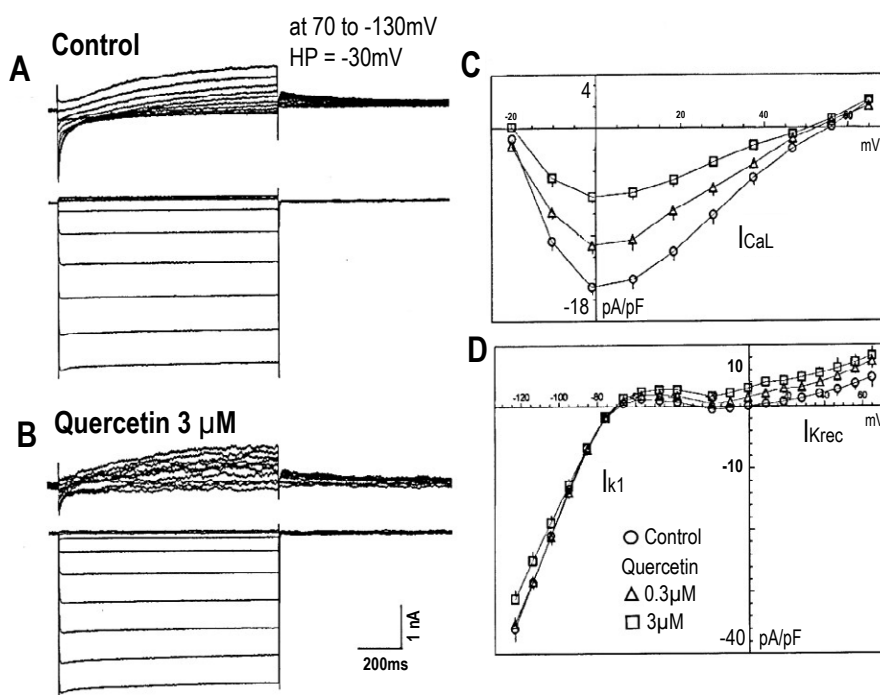


Figure 1: Modulation by quercetin of the ionic currents in guinea pig ventricular cardiomyocytes. A: Control. The test pulses were applied 70 mV and -130 mV from a holding potential of -30 mV. B: Quercetin 3 μM. C: Modulation of I_{CaL} in the absence and the presence of quercetin. Current traces at 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 μ M NE. Quercetin (0.1 to 100 μ M) subsequently administrated exhibited the marked endothelium-dependent actions (Table 2). Prior administration of L-NMMA (100 μ M) significantly inhibited the quercetin (100 μ M)-induced vasodilatation from $97.8 \pm 3.7\%$ (n=10) to $78.0 \pm 11.6\%$ (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 μ M) and indomethacin (10 μ M) 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 μ M) and indomethacin (10 μ M), quercetin at 100 μ M attenuated by $65.2 \pm 6.6\%$ (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 $77.9 \pm 2.3\%$ to $62.5 \pm 4.9\%$ (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 μ M) was significantly reduced by high K^+ (30 mM) to $41.0 \pm 5.7\%$ (n=5, p<0.05) (Table 3). In high K^+ solution, furthermore, TEA attenuated the L-NMMA/indomethacin-resistant relaxation to $43.8 \pm$

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 μ M), a SK channel inhibitor, strongly decreased the L-NMMA/indomethacin-resistant relaxation induced by 30 μ M quercetin from $30.4 \pm 6.2\%$ to $9.4 \pm 2.7\%$ (n=5, p<0.05), and from $65.2 \pm 6.6\%$ to $47.1 \pm 11.4\%$ (n=5, p<0.05) by 100 μ M quercetin. But carybdotoxin (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 μ M significantly decreased the vasorelaxation from $97.8 \pm 3.7\%$ (n=10) in normal solution to $67.5 \pm 3.7\%$ (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 vasorelaxation 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 μ M dilated the PDB (300 nM)-induced vasoconstriction by $84.8 \pm 6.0\%$ (n=5, p<0.01) (Table 3). Under NE-induced vasoconstriction, staurosporine (100 nM), a PK-C inhibitor, decreased the relaxation induced by 100 μ M quercetin by approximately 40%. It has been shown that PK-Ca and/or δ are necessary for phorbol ester-mediated constriction of aortic smooth muscle, but are not essential for NE-, vasopressin-, or K^+ -induced

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

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

Values (%) represent mean \pm 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-C α 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-C ϵ 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 PK-C [44]. Ro-31-8425 (0.1 μ M) attenuated the quercetin (100 μ M)-induced vasorelaxation by 80.8 \pm 2.6% (n=5, $p < 0.001$). Gö6983 (0.1 μ M), also had similar effects by 84.6 \pm 7.6% (n=5, $p < 0.001$). Under the KCl-induced vasoconstriction, both Gö6983 and Ro-31-8425 attenuated the quercetin (100 μ M)-induced vasorelaxation by 49.5 \pm 7.9% (n=5, $p < 0.01$) and by 75.5 \pm 7.9% (n=5, $p < 0.001$), respectively (Table 3). Thus, quercetin might inhibit possibly mediated through PK-C δ , although it is still difficult to clearly distinguish. In addition, PK-C phosphorylates 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 μ M 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 (PI₃-kinase)/protein kinase B (Akt) [47,48].

On Ca²⁺-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 [Ca²⁺]_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 H₂O₂ [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 Ca²⁺ antagonist, in Ca²⁺ free solution and under the KCl constriction, (3) PK-C activation mediated through possibly the PK-C δ 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 PGI₂ syntheses. As well it is due to endothelium-independent actions mediated through the Ca²⁺ 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 μ M NE. Quercetin (0.1-100 μ M) caused the significant

vasodilatation in a concentration-dependent manner; at 100 μ M, the vasodilatation was 99.6 \pm 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 μ M) and indomethacin (100 μ M) 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 μ M NE. Endothelium removal strongly decreased the quercetin-induced vasodilatation; at 100 μ M quercetin by 77.9 \pm 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 μ M TEA (Table 4). Increasing TEA concentration to 1 mM tended to be reduced the resistant vasodilatation at 10 to 30 μ M quercetin, but not significantly. At 100 μ M quercetin, TEA (1 mM) was almost identical to the L-NAME/indomethacin-resistant vasodilatation (82.3 \pm 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 increase [Ca²⁺]_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 is attenuated by high K⁺ or TEA [32,52]. Moreover, the EDHF-type relaxation is attenuated by both apamin 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 H₂O₂ 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 μ M 18 α - or 50 μ M 18 β -glycylrrhethinic acids (18 α - and 18 β -GAs) significantly inhibited the L-NAME/indomethacin-resistant vasodilatation from 82.0 \pm 2.4% (n=6) to 63.3 \pm 5.5% (n=9, p<0.01) (Table 4). 18 α - and 18 β -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 18 α - and 18 β -GAs. So, these findings indicate that the gap junctions contribute to the quercetin-induced vasodilatation. Both 18 α - and 18 β -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 18 α - and 18 β -GAs might be due to both mechanisms: (1) quercetin elevates endothelial [Ca²⁺]_i and then, activates K_{Ca} channels, and (2) quercetin itself has a stimulatory actions on K_{Ca} channels. The elevation of [Ca²⁺]_i induced by quercetin activates SK channels [19] to hyperpolarize the endothelium [58,59]. Quercetin would activate SK channel in endothelium via both

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

Values (%) represent mean \pm 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.

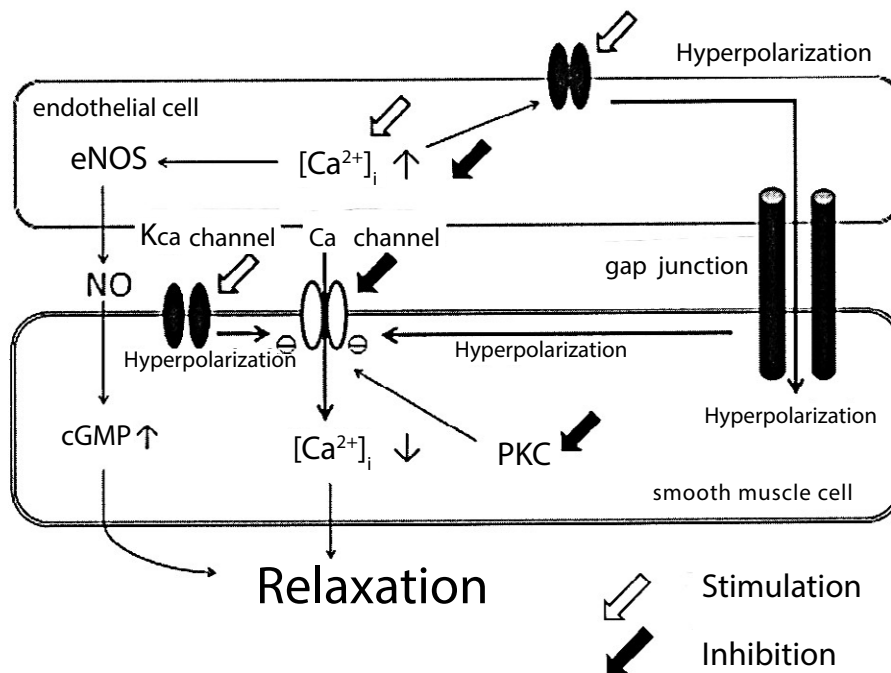


Figure 2: Diagram for the mechanism of quercetin-induced vasorelaxation. Quercetin induces $[Ca^{2+}]_i$ elevation, leading to NO production and K_{Ca} channel activation in endothelium cell. The hyperpolarization induced by the activation of K_{Ca} channel inhibits Ca^{2+} channel in smooth muscle cell.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

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