The heart was dissected out and perfused with normal Tyrode solution in situ (Krebs-Henseleit 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, 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 in DMSO. The final concentration of DMSO was diluted to 100 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% (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_{K1}). Thus, the APD prolongation increases the refractory period.

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

**Introduction**

Quercetin is a flavonoid, 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_{Ca,L}, I_{K1a}, I_{Kr}, 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 concentration-dependent manner. NG-nitro-L-arginineacetate (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 tetrodiallonmonium (TEA). Among K_{Ca} channel inhibitors, the quercetin-induced vasodilatation was attenuated by 0.3 μM amapin (sensitive to SK), but not by 30 mM charybotoxin (sensitive to BK and IK). Under KCl-induced vasoconstriction, the quercetin-induced vasorelaxation was attenuated by PK-C inhibitors; Gö6983 (α-, β-, γ- and ε-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-NG-nitro arginine methyl ester (L-NNAME) and 100 μM indomethacin. The LNAME/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-glycyrhretinic acids (gap junction inhibitors). Therefore, quercetin dilates the vascular smooth muscle mediated by endothelium-dependent and -independent mechanisms.
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Citation:

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 IKrec 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 IKrec enhancement may protect a cell due to an APD shortening and a decline of [Ca²⁺], 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_{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⁺], 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

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Quercetin 0.1 µM</th>
<th>Quercetin 0.3 µM</th>
<th>Quercetin 1 µM</th>
<th>Quercetin 3 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>APA (mV)</td>
<td>117.4 ± 1.2</td>
<td>117.6 ± 1.2</td>
<td>117.8 ± 1.1</td>
<td>114.6 ± 1.4</td>
<td>113.7 ± 1.2</td>
</tr>
<tr>
<td>RP (mV)</td>
<td>-80.9 ± 0.5</td>
<td>-80.6 ± 0.6</td>
<td>-80.7 ± 1.0</td>
<td>-80.4 ± 2.3</td>
<td>-79.7 ± 2.1</td>
</tr>
<tr>
<td>APD₃₀ (ms)</td>
<td>77.0 ± 2.2</td>
<td>78.6 ± 2.4</td>
<td>79.8 ± 3.8</td>
<td>82.9 ± 2.3</td>
<td>86.2 ± 2.0</td>
</tr>
<tr>
<td>APD₉₀ (ms)</td>
<td>94.1 ± 2.0</td>
<td>95.0 ± 2.1</td>
<td>97.3 ± 2.0</td>
<td>100.3 ± 2.6</td>
<td>110.9 ± 2.3</td>
</tr>
<tr>
<td>V_{max} (V/s)</td>
<td>182.8 ± 2.5</td>
<td>182.1 ± 1.2</td>
<td>182.5 ± 2.3</td>
<td>173.8 ± 2.2</td>
<td>161.7 ± 2.4</td>
</tr>
<tr>
<td>Washout</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>APA (mV)</td>
<td>110.7 ± 1.0</td>
<td>-80.0 ± 0.6</td>
<td>79.4 ± 2.2</td>
<td>96.8 ± 2.4</td>
<td>172.3 ± 2.2</td>
</tr>
</tbody>
</table>

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

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 [Ca2+], and eNOS phosphorylation [21,22]. The [Ca2+]i elevation in endothelium facilitates other actions such as a production of hydroxyl hydrogen [23], endothelial hyperpolarization [24,25] induced by activation of K+ channels [26], and production of endothelium-derived hyperpolarizing factor (EDHF) [12,27].

On endothelium-derived hyperpolarizing factor (EDHF): Quercetin increases [Ca2+]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 be resistant to both inhibitors of NOS and cyclooxygenase [29]. So, we examined using rat mesenteric artery as mentioned.

On Ca2+-activated K+ (KCa) channel: The elevation of [Ca2+]i induced by quercetin may stimulate the KCa channel. The activation of KCa 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 KCa 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 KCa channels [32,33], apamine to SK channels [34], and carybtdotoxin to BK and IK channels [35]. Apamin (0.3 µM), a BK 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 carybtdotoxin (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) I2: 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 PGI2 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 PGI2.

Effects on vascular smooth muscle

On L-type Ca2+ channel: L-type Ca2+ 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 IcaL in A7R5 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 Ca2+-free solution (p<0.01) [7,18]. The quercetin-induced vasorelaxation was also attenuated by nicardipine, and by a switch from normal Krebs' solution (Ca2+=1.8 mM) to Ca2+-free solution. Also, quercetin dilated the KCl-induced vasoconstriction [3,19]. These findings demonstrate the vasorelaxation due to its Ca2+ channel inhibitory action. Satoh [17] has already reported that quercetin is an inhibitor of IcaL channel in cardiomyocytes. These are summarized in Table 3.

On protein kinase C: The Ca2+ 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, stauroporine (100 nM), a PK-C inhibitor, decreased the relaxation induced by 100 µM quercetin by approximately 40%. It has been shown that PK-C and/or 6 are necessary for phorbol ester-mediated constriction of aortic smooth muscle, but are not essential for NE, vasopressin-, or K+ induced

<table>
<thead>
<tr>
<th>Quercetin</th>
<th>0.1</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
<th>10</th>
<th>30</th>
<th>100 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>1.7 ± 0.5</td>
<td>3.6 ± 0.9</td>
<td>6.9 ± 0.9</td>
<td>12.4 ± 1.1</td>
<td>32.0 ± 5.7</td>
<td>54.1 ± 8.4</td>
</tr>
<tr>
<td>Endothelium-denuded</td>
<td>5</td>
<td>1.5 ± 1.3</td>
<td>3.1 ± 1.6</td>
<td>6.9 ± 1.8</td>
<td>11.2 ± 2.4</td>
<td>30.8 ± 4.9</td>
<td>44.1 ± 4.2</td>
</tr>
<tr>
<td>L-NAME 100 µM</td>
<td>5</td>
<td>1.9 ± 0.9</td>
<td>3.6 ± 1.1</td>
<td>7.1 ± 2.1</td>
<td>13.2 ± 3.7</td>
<td>21.1 ± 4.6</td>
<td>33.0 ± 5.3</td>
</tr>
<tr>
<td>L-NMMA 100 µM</td>
<td>5</td>
<td>1.9 ± 0.6</td>
<td>4.1 ± 1.3</td>
<td>7.3 ± 1.8</td>
<td>12.0 ± 2.4</td>
<td>25.4 ± 4.8</td>
<td>38.7 ± 6.0</td>
</tr>
<tr>
<td>L-NMMA 100 µM + Indomethacin 10 µM</td>
<td>5</td>
<td>1.9 ± 0.8</td>
<td>4.1 ± 1.3</td>
<td>6.8 ± 1.3</td>
<td>11.0 ± 2.0</td>
<td>18.2 ± 4.7</td>
<td>30.4 ± 6.2</td>
</tr>
</tbody>
</table>

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.
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; G69983 sensitive to α, β, γ, and δ isozymes, and Ro-31-8425 to α, β, γ, and ε isozymes 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) chosen; Gö6983 sensitive to α, β, γ, δ and ζ isozymes, and Ro-31-8425 (0.1 µM) do not affect the quercetin-induced constriction (by just 0.7% decrease).

Flavonoids have been shown to produce vasodilatation due to the K Ca channel to hyperpolarize the membrane of vascular smooth muscle [30]. The Ca2+-activated K+ (Kv) channels: Flavonoids have been shown to produce vasodilatation due to the Kv channel to hyperpolarize the membrane of vascular smooth muscle [30]. The Ca2+-activated K+ channel and phosphatidylinositol 3-kinase (PI3-kinase)/protein kinase B (Akt) pathways [43].

On Ca2+-activated K+ (Kv) channels: Flavonoids have been shown to produce vasodilatation due to the Kv channel to hyperpolarize the membrane of vascular smooth muscle [30]. The Ca2+-activated K+ channel and phosphatidylinositol 3-kinase (PI3-kinase)/protein kinase B (Akt) pathways [43].

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

<table>
<thead>
<tr>
<th>Quercetin</th>
<th>n</th>
<th>0.1</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
<th>10</th>
<th>30</th>
<th>100 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Constriction induced by Norepinephrine A)</td>
<td>10</td>
<td>1.7±0.5</td>
<td>3.6±0.9</td>
<td>6.9±0.9</td>
<td>12.4±1.2</td>
<td>32.0±5.7</td>
<td>54.1±8.4</td>
<td>97.8±3.7</td>
</tr>
<tr>
<td>Ca2+-free</td>
<td>5</td>
<td>2.1±1.5</td>
<td>3.1±1.4</td>
<td>9.0±2.0</td>
<td>12.8±3.3</td>
<td>22.8±2.5</td>
<td>45.0±3.3</td>
<td>67.5±7.4</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>5</td>
<td>0.0±0.0</td>
<td>1.4±1.4</td>
<td>1.4±1.4</td>
<td>1.4±1.4</td>
<td>6.8±3.6</td>
<td>15.0±7.2</td>
<td>61.2±16.6</td>
</tr>
<tr>
<td>Ro-31-8425 (0.1µM)</td>
<td>5</td>
<td>1.5±1.0</td>
<td>3.0±1.9</td>
<td>6.6±2.5</td>
<td>12.8±4.0</td>
<td>23.0±4.9</td>
<td>42.8±4.2</td>
<td>80.8±2.6</td>
</tr>
<tr>
<td>G69983 (0.1µM)</td>
<td>5</td>
<td>1.0±0.6</td>
<td>2.0±1.2</td>
<td>2.8±1.7</td>
<td>8.1±2.5</td>
<td>18.8±3.5</td>
<td>40.0±6.2</td>
<td>84.6±7.7</td>
</tr>
<tr>
<td>B)</td>
<td>1.5±1.3</td>
<td>3.1±1.6</td>
<td>6.9±1.8</td>
<td>11.2±2.4</td>
<td>30.8±4.9</td>
<td>44.1±4.2</td>
<td>77.9±2.3</td>
<td></td>
</tr>
<tr>
<td>+TEA (100µM)</td>
<td>5</td>
<td>1.0±0.75</td>
<td>3.0±1.3</td>
<td>5.5±2.6</td>
<td>9.2±3.6</td>
<td>17.9±4.2</td>
<td>31.7±4.9</td>
<td>62.5±4.9</td>
</tr>
<tr>
<td>C) L-NMMA (100µM) +Indomethacin (10µM)</td>
<td>5</td>
<td>1.9±0.8</td>
<td>4.1±1.3</td>
<td>8.8±1.3</td>
<td>11.0±2.0</td>
<td>18.2±4.7</td>
<td>30.4±6.2</td>
<td>65.2±6.6</td>
</tr>
<tr>
<td>+high K+ (30mM)</td>
<td>5</td>
<td>0.5±0.3</td>
<td>2.6±1.5</td>
<td>5.1±1.6</td>
<td>9.4±2.3</td>
<td>13.6±1.9</td>
<td>24.4±3.9</td>
<td>41.0±5.7</td>
</tr>
<tr>
<td>+TEA (100µM)</td>
<td>5</td>
<td>1.2±0.5</td>
<td>1.8±1.5</td>
<td>1.8±1.5</td>
<td>4.0±4.0</td>
<td>8.5±5.5</td>
<td>16.8±7.0</td>
<td>43.8±9.5</td>
</tr>
<tr>
<td>+Charybdotoxin</td>
<td>5</td>
<td>2.1±1.3</td>
<td>3.8±2.6</td>
<td>4.2±2.0</td>
<td>4.5±2.3</td>
<td>16.8±3.6</td>
<td>28.2±2.7</td>
<td>63.4±11.8</td>
</tr>
<tr>
<td>+Amarin</td>
<td>5</td>
<td>0.6±0.6</td>
<td>0.8±0.8</td>
<td>1.6±1.6</td>
<td>3.7±2.7</td>
<td>5.1±1.6</td>
<td>9.4±2.4</td>
<td>47.1±11.1</td>
</tr>
</tbody>
</table>

**Summary:** The vasodilating 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 L-NMMA and removal of endothelium, (3) NOS by L-NMMA or L-NAME and removal of endothelium, (4) Kv channel by amarin but not by charybdotoxin. Furthermore, the vasodilation was produced by (4) Kv channel by amarin but not by charybdotoxin. As well it is due to endothelium-independent actions mediated through the Ca2+ channel, the Kv 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 100 μM, 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<sub>Ca</sub> 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 vasodilatation 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-NAME 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<sup>2+</sup>]<sub>i</sub>, and then facilitates EDHF synthesis as well as closely involved with EDHF. Therefore, it is possible that quercetin is responsible for EDHF-type relaxation, which which may play a role for the quercetin-induced vasodilatation in mesenteric artery, different from aorta.

<table>
<thead>
<tr>
<th>Quercetin</th>
<th>Control</th>
<th>A</th>
<th>Endothelium-denuded</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>0.1</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>Control A</td>
<td>10</td>
<td>1.6 ± 0.94</td>
<td>8.0 ± 1.7&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>23.4 ± 3.2&lt;sup&gt;2)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Endothelium-denuded</td>
<td>6</td>
<td>1.8 ± 0.55</td>
<td>3.8 ± 1.8&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>8.5 ± 1.9&lt;sup&gt;1)&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-NAME 100 μM</td>
<td>8</td>
<td>1.3 ± 0.9</td>
<td>3.2 ± 1.8</td>
<td>19.2 ± 3.4</td>
</tr>
<tr>
<td>L-NAME+Indomethacin (100 μM)</td>
<td>8</td>
<td>1.9 ± 0.75</td>
<td>4.1 ± 1.3</td>
<td>19.0 ± 7.3</td>
</tr>
<tr>
<td>+TEA(100μM)</td>
<td>6</td>
<td>1.3 ± 0.80</td>
<td>7.5 ± 2.9</td>
<td>20.2 ± 5.7</td>
</tr>
</tbody>
</table>

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

Symbols of a) and b) indicate 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.
involved with EDHF. The vasodilatation induced by the activation of K_{ca} channel is closely dependent. Quercetin produces NO in endothelium, but plays minor role in the quercetin-induced vasodilatation. The vasodilatation to the same significant extent as the endothelium 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 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


Figure 2: Diagram for the mechanism of quercetin-induced vasorelaxation. Quercetin induces Ca^{2+} elevation, leading to NO production and K\textsubscript{ca} channel activation in endothelium cell. The hyperpolarization induced by the activation of K\textsubscript{ca} channel in smooth muscle cell. Moreover, modulation of gap junction aggravates the vasodilatation.


