Korean J Physiol Pharmacol
Vol 13: 361-365, October, 2009
DOI: 10.4196/kjpp.2009.13.5.361

Possible Involvement of Ca^{2+} Activated K^+ Channels, SK Channel, in the Quercetin-Induced Vasodilatation

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Effects of quercetin, a kind of flavonoids, on the vasodilating actions were investigated. Among the mechanisms for quercetin-induced vasodilatation in rat aorta, the involvement with the Ca^{2+} activated K^+ (K_{Ca}) channel was examined. Pretreatment with NE (5 μM) or KCl (60 mM) was carried out and then, the modulation by quercetin of the constriction was examined using rat aorta ring strips (3 mm) at 36.5°C. Quercetin (0.1 to 100 μM) relaxed the NE-induced vasoconstrictions in a concentration-dependent manner. NO synthesis (NOS) inhibitor, N*G*-monomethyl-L-arginine acetate (L-NMMA), at 100 μM reduced 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-N*G*-nitro arginine methyl ester (L-NAME), at 100 μM also had the similar effect. In the presence of both 100 μM L-NMMA and 10 μM indomethacin, the quercetin-induced vasodilatation was further attenuated by 100 μM tetraethylammonium (TEA, a K_{Ca} channel inhibitor). Also TEA decreased the quercetin-induced vasodilatation in endothelium-denuded rat aorta. Used other K_{Ca} channel inhibitors, the quercetin-induced vasodilatation was attenuated by 0.3 μM apamin (a SK channel inhibitor), but not by 30 nM charybdotoxin (a BK and IK channel inhibitor). Quercetin caused a concentration-dependent vasodilatation, due to the endothelium-dependent and -independent actions. Also quercetin contributes to the vasodilatation selectively with SK channel on smooth muscle.

Key Words: Quercetin, Vasodilatation, K_{Ca} channels, PK-C, Endothelium

INTRODUCTION

Quercetin is a polyphenolic flavonoid existing in a wide variety of plants and foods (Hertog et al., 1993a). It has been reported that flavonoids reduce the incidence of cardiovascular diseases and carcinogenesis (Hertog et al., 1993b). Flavonoid is a vasodilator (Duarte et al., 1993) and a scavenger for free radicals (Murota and Terao, 2003). Thus, flavonoids migtht excert cardiovascular protective actions through their various pharmacological effects. Quercetin has also been reported to possess various pharmacological actions: modulation of epoxyeicosanoic acids synthesis, prevention of platelet aggregation (Formica and Regelson, 1995) as well as vasodilatation (Duarte et al., 1993). Therefore, quercetin may play major role for the pharmacological actions of dietary polyphenols or polyphenol-rich herbal medicine. For example, *Ginkgo biloba* extract, a flavonoid-rich herbal medicine, contains quercetin and ehibits a lot of pharmacological effects (Sticher, 1993; Satoh and Nishida, 2004). In our previous report, *Ginkgo biloba* extract and quercetin cause the vasodilating actions (Nishida and Satoh, 2004). Therefore, quercetin would be a key for the pharmacological effects induced by *Ginkgo biloba* extract.

In some reports for the vasodilating mechanisms, quercetin possesses protein kinase C (PK-C) inhibition (Duarte et al., 1993; Murota and Terao, 2003), tyrosine kinase inhibition (Catalin, 1995), and activation of endothelium-dependent actions (Chen and Pace-Asciak, 1996; Kubota et al., 2001). In addition, flavonoids such as hesperidin, luteoline and 7-hydroxyflavone produce vasodilatation due to the Ca^{2+} activated K^+ (K_{Ca}) channel modulation on vascular smooth muscle cells (Calderone et al., 2004). The K_{Ca} channels hyperpolarize the membrane. They are classified by their conductances as follows: big conductance K_{Ca} (BK) channel (200 pS), intermediate conductance K_{Ca} (IK) channel (37 pS), and small conductance K_{Ca} (SK) channel (32 pS) (Brayden and Nelson, 1992; Neylon et al., 1999). Most recently, quercetin has been demonstrated to activate BK channel in coronary arteries via production of H_2O_2

Received August 4, 2009, Revised August 18, 2009, Accepted October 17, 2009

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ABBREVIATIONS: Akt, phosphatidylinositol 3-kinase (PI_3-kinase)/protein kinase B; BK, big conductance K_{Ca} channel; cGMP-PK, cGMP-dependent protein kinase; EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived releasing factor; EGTA, ethylene glycol-O,O'-bis (2-aminoethyl)-N,N,N',N'-tetraacetic acid; ERK, extracellular signal-regulated kinase; IK, intermediate conductance K_{Ca} channel (37 pS); JNK, c-jun amino-terminal kinase; K_{Ca}, Ca^{2+} activated K^+; L-NMMA, N*G*-monomethyl-L-arginine acetate; L-NAME, L-N*G*-nitro arginine methyl ester; MAPKs, mitogen-activated protein kinases; NE, norepinephrine; NOS, NO synthesis; PK-C, protein kinase C; SK, small conductance K_{Ca} channel; TEA, tetraethylammonium; VASP, vasodilator-stimulated phosphoprotein.

(Congolludo et al., 2007). In other study, however, TEA and glibenclamide (K_{ATP} channel inhibitor) have not been reported to affect the quercetin-induced vasodilatation in rat aorta (Perez-Vizcaino, 2002). Thus, the effects of quercetin on K_{Ca} channels are not clear yet. Aim of this study is to investigate the involvement of K_{Ca} channels in the quercetin-induced vasodilatation in rat aorta.

METHODS

All 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.

Wistar male rats (4~10 weeks old) were anesthetized with ether, and euthanized by exsanguination. The thoracic aorta was quickly removed, and the isolated aorta was cut into 3-mm rings in length. The rings were suspended between two triangular-shaped stainless steel stirrups in a jacketed organ chamber filled with 20 ml modified Krebs-Henseleit solution. The modified Krebs-Henseleit solution was, in mM: 118 NaCl, 4.6 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.1 glucose, 27.2 $NaHCO_3$, 0.03 ethylene glycol- O,O'-bis (2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), and 1.8 $CaCl_2$. The chamber solution was kept at 36.5°C and oxygenated with 95% O_2 and 5% CO_2. The lower stirrup was anchored and the upper stirrup was attached to a force-displacement transducer (TB-652T; Nihon Kohden, Tokyo, Japan) to record the isometric force. All rings were stretched to generate a resting tension of 1.2 g.

After 40 min of resting, addition of 5 μM norepinephrine (NE) or setting the concentration of KCl to 60 mM in the bath was performed to induce vasoconstriction. After the contractile response became steady, quercetin was cumulatively administrated into the bath solution. The effects of quercetin were measured 6~10 min after the responses became steady. The relaxation response was analyzed as a percentage decrease from the maximal contraction induced by NE. Pretreatment with the inhibitors was carried out for 40-min before NE was administrated.

The drugs used were quercetin (Tocris Biosci., Northpoint, UK), N*G*-monomethyl-L-arginine acetate (L-NMMA), L-N*G*-nitro arginine methyl ester (L-NAME), charybdotoxin, apamin (Sigma Chemical Co. St. Louis, MO, U.S.A.), indomethacin and tetraethylammonium (TEA) (Nacalai Tesque Inc., Kyoto Japan). All values are represented as means±S.E.M. The differences of data in mean values were analyzed by Student's t-test and analysis of variance (ANOVA), and a p value of less than 0.05 was considered significant.

RESULTS

The aorta ring strip of rat exhibited a strong contraction induced by initial application of 5 μM NE. Subsequent applications of quercetin (0.1 to 100 μM) were performed. The responses were concentration-dependent. Quercetin caused significant vasodilatation at concentrations higher than 0.3 μM; by 97.8±3.7% (n=10, $p<0.001$) at 100 μM (Table 1).

Prior administration of L-NMMA (100 μM), an NO synthesis (NOS) inhibitor, significantly inhibited the quercetin-induced vasodilatation (Fig. 1). At 100 μM quercetin, the vasodilatation was attenuated 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 (Table 1). This is enforced by our experiments using the aorta removed endothelium. Also,

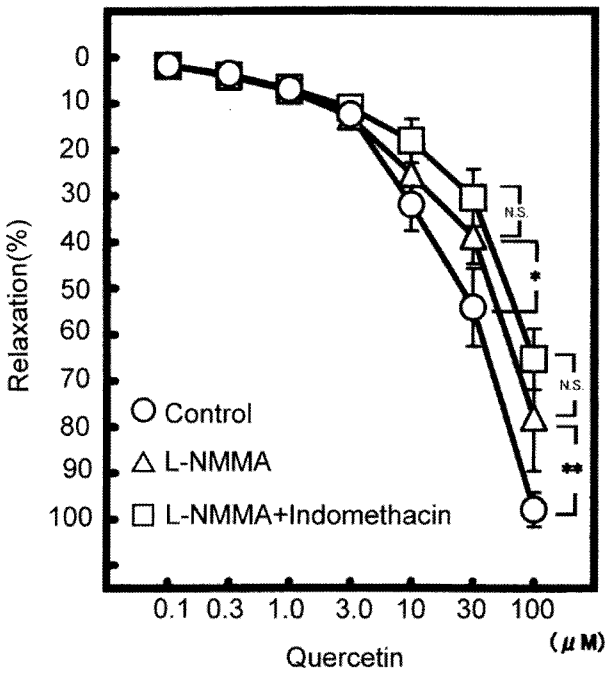

Fig. 1. Concentration-dependent vasodilatation by cumulative administrations of quercetin. Symbols used are control (open circles, n=10), pretreatment with L-NMMA (triangles, n=5), L-NMMA and indomethacin (squares, n=5). Values (%) are represented as mean±S.E.M. *$p<0.05$, **$p<0.01$, with respect to control value.

Table 1. Modulation of the quercetin-induced vasodilatation

		Quercetin (μM)						
	N	0.1	0.3	1	3	10	30	100
Control	10	1.7±0.53	3.6±0.90[a]	6.9±0.91[a]	12.4±1.1[b]	32.0±5.7[c]	54.1±4.4[c]	97.8±3.7[c]
L-NMMA 100 μM	5	1.9±0.55	4.1±1.3	7.3±1.8	13.0±2.4	25.4±4.8	38.7±6.0[x]	78.0±11.6[x]
L-NAME 100 μM	5	1.2±0.89	3.0±1.1	7.0±2.0	13.2±3.7	21.1±4.6[x]	33.0±5.3[y]	69.5±6.1[y]
Endothelium-denuded	5	1.8±0.55	3.8±1.6	8.5±1.9	13.0±2.6	30.8±4.9	44.2±4.2[x]	77.9±2.4[x]

Values (%) represent mean±S.E.M. [a] and [x]: $p<0.05$, [b] and [y]: $p<0.01$, [c]: $p<0.001$. Symbols of [a], [b], and [c] mean significant difference in comparison between effect of quercetin itself at each concentration and the maximal contraction induced by NE. Symbols of [x] and [y] mean significant difference as compared with control (quercetin alone).

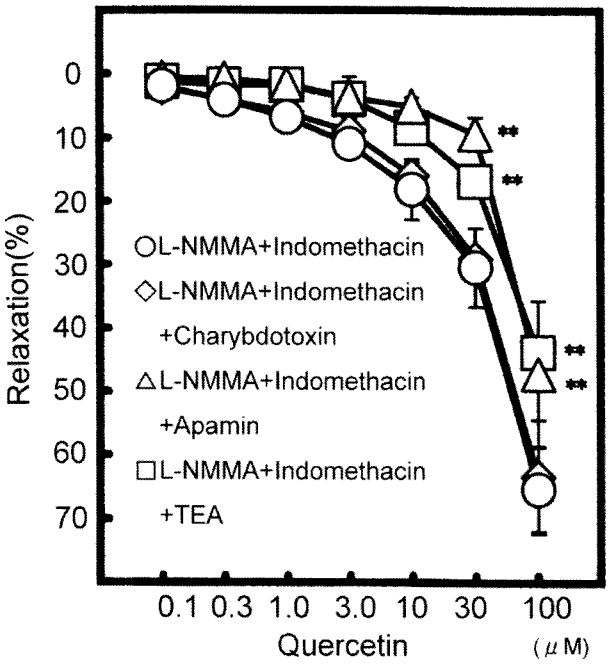

Fig. 2. Modulation of L-NMMA and indomethacin resistant relaxation at 100 μM quercetin. The vasodilatation in the presence of L-NMMA and indomedhacin (n=5) means as control value in this graph. The vasodilatations by L-NMMA and indomethacin plus TEA (n=5), by L-NMMA and indomethacin plus apamin (n=5), and by L-NMMA and indomethacin plus charybdotoxin (n=5) were compared with that of L-NMMA and indomedhacin. Values (%) are represented as mean±S.E.M. **p<0.01, with respect to control value.

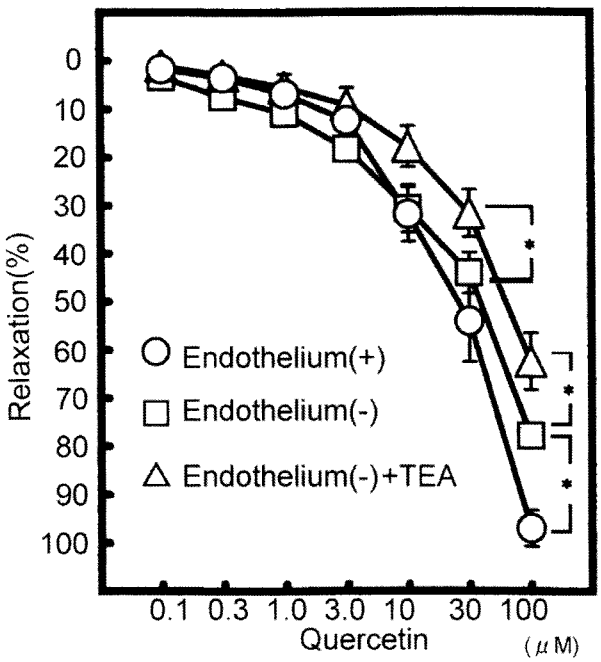

Fig. 3. Modulation of quercetin (100 μM)-induced vasodilatation (n=10) in the removal of endothelium (n=5) and the removal of endothelium plus TEA (n=5). Values (%) are represented as mean± S.E.M. *p<0.05, with respect to control value.

administration of both L-NMMA (100 μM) and indomethacin (10 μM) attenuated the quercetin-induced vasodilatation more than that with L-NMMA alone, but not significantly.

To investigate whether quercetin produces the relaxation involved with Ca^{2+} activated K^+ channel (K_{Ca} channel), the pretreatment with TEA (a K_{Ca} channel inhibitor) was carried out in the presence of indomethacin and L-NMMA. The L-NMMA and indomethacin-resistant relaxation induced by quercetin (100 μM) was significantly reduced by 100 μM TEA to 41.1±11.5% (n=5, $p<0.01$), as shown in Fig. 2. In high K^+ (30 mM) solution, furthermore, TEA also attenuated the L-NMMA and indomethacin-resistant relaxation to 43.8±10.2% (n=5, $p<0.05$). These results indicate that quercetin modulates the K_{Ca} channel.

We examined which type of K_{Ca} channels quercetin affects. Apamin (0.3 μM), a SK channel inhibitor, strongly decreased the L-NMMA and 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 (Fig. 2).

In addition, to clarify which K_{Ca} channel on smooth muscle or endothelium quercetin modulates, the experiments using endothelium-denuded aorta were carried out. Under the conditions, TEA significantly decreased the quercetin-induced relaxation from 77.9±2.4% to 62.5±5.9% (n=6, $p<0.05$) (Fig. 3). Therefore, these results indicate that quercetin affects the K_{Ca} channel on smooth muscle cells.

DISCUSSION

The present experiments in rat aorta strips showed that quercetin caused a concentration-dependent vasodilatation. The vasodilatation was modified (1) by L-NMMA or L-NAME, (2) by removal of endothelium, (3) by both L-NMMA and indomethacin, (4) by both L-NMMA and indomethacin plus TEA, (5) also by apamin but not by charybdotoxin, and (6) by TEA in endothelium-denuded aorta.

A lot of the mechanisms for the vasodilatation induced by quercetin have been shown. However, the mechanisms are still conflicting. In some reports, quercetin has less endothelium-dependent mechanism (Perez-Vizcaino, 2002) or possesses only weak endothelium-dependency (at lower concentrations) (Fusi et al., 2003). In this study, however, quercetin exhibited the remarkable endothelium-dependent actions. NOS inhibitors and removal of endothelium abolished or attenuated the quercetin-induced vasodilatation in rat aorta. Our findings are also supported by other previous reports (Kubota et al., 2001; Ajay et al., 2003). Thus, the vasodilatation by quercetin in rat aorta is considered to be due to NO secretion from endothelium (EDRF). Although the difference is unable to explain now, there might be some conditions to disguise the quercetin's endothelium-dependent mechanisms. In addition, the pretreatment with both indomethacin and L-NMMA reduced the relaxation more strongly than the pretreatment with L-NMMA alone (but not significantly). Thus, it appears possible that the quercetin-induced relaxation is also partly responsible for PGI_2 secretion from endothelium, consistent with a report of Ajay et al. (2003).

Quercetin also dilated the KCl-induced vasoconstriction (Duarte et al., 1993). In our laboratory, quercetin dilated

the KCl-induced vasoconstriction and the quercetin-induced vasodilatation was inhibited by nicardipine in rat aorta (Nishida and Satoh, 2004; Satoh and Nishida, 2004). These results indicate that quercetin also causes the vasodilatation via its Ca^{2+} channel inhibitory action. Satoh (2005) has recently reported that quercetin is an inhibitor of Ca^{2+} channels of cardiomyocytes by means of patch-clamp experiments. On the other hand, quercetin has been shown to be a stimulator of Ca^{2+} channel (Fusi et al., 2003). From our results, however, it is possible that quercetin inhibits the L-type Ca^{2+} channel mediated through second messengers such as PK-A, PK-G and PK-C (Satoh and Sperelakis, 1991; 1995; Satoh, 1996).

PK-C phosphrylates tyrosin kinase and vasodilator-stimulated phosphoprotein (VASP) as a substrate of cGMP-dependent protein kinase (cGMP-PK) (Catalin et al., 1995; Moussazadeh and Haimovich, 1998; Wentworth et al., 2006). But genistein (tyrosine kinase inhibitor) in this study failed to affect the quercetin-induced constriction. The activation of MLCK was abolished by PK-C (Hagiwara et al., 1988; Murthy et al., 2000). 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), leading to protection of proliferation and inflammation (Shin et al., 2004; Granado-Serrano et al., 2008; Lin et al., 2008; Kwon et al., 2009). Thus, quercetin produces the beneficial effects mediated through many cellular signaling pathways.

It has recently been shown that flavonoids-induced vasodilatation is involved with potassium channels (Calderone et al., 2004). Some types of potassium channels are expressed in vascular smooth muscle cells and cause the vasodilating actions by hyperpolarizing the membrane. The K_{Ca} channels are classified by their conductances as follows: BK channel, IK channel, and SK channel (Brayden and Nelson, 1992; Neylon et al., 1999). In the present experiments, three types of K_{Ca} channel inhibitors were used. TEA is sensitive to all K_{Ca} channels (Neylon et al., 1999), apamin to SK channels (Garcia-Pascual et al., 1995; Murphy and Brayden, 1995), and charybdotoxin to BK and IK channels (Carl et al., 1995; Vogalis et al., 1998; Mitamura et al., 2002). Apamin and TEA attenuated the quercetin-induced vasodilatation in the presence of L-NMMA and indomethacin, but charybdotoxin failed to affect it. Thus, quercetin would selectively possess a sensitivity to SK channel (but less to BK and IK channels) among the K_{Ca} channels. In this study, furthermore, TEA attenuated the quercetin-induced vasodilatation of endothelium-denuded rat aorta. Therefore, these results indicate that quercetin regulates the SK channels on smooth muscles.

Most recently, plant polyphenols have been reported to induce endothelium-derived hyperpolarizing factor (EDHF)-type relaxation (Ndiaye et al., 2003). The vasodilatation induced by EDHF has recently been considered to be resistant to both inhibitors of NO synthase and cyclo-oxygenase (Chen and Suzuki, 1990; Fukao et al., 1997; Félétou and Vanhoutte, 2002). Furthermore, the EDHF-induced relaxation is attenuated by high K^+ or TEA (Campbell et al., 1996; Martinez-Orgado et al., 1999). In our laboratory, the quercetin-induced vasodilatation had less or no involvement in EDHF in rat aorta and mesenteric artery (unpublished data). Therefore, it is concluded that quercetin possesses no effect on EDHF.

In conclusion, quercetin caused a concentration-dependent vasodilatation. Quercetin's effects are due to endothelium-dependent actions mediated through the NO (EDRF) and partly PGI_2 syntheses, and also to endothelium-independent actions mediated through the Ca^{2+} channel and PK-C inhibitions (Nishida and Satoh, 2004; Satoh and Nishida, 2004). Moreover, quercetin modulates the K_{Ca} channels on smooth muscles with the selectivity to SK channel. Thus, quercetin possesses multiple mechanisms. Further studies are needed to elucidate the detailed mechanism about the quercetin-induced vasodilatation.

ACKNOWLEDGEMENTS

This work was in part supported by the grants from Tokiwa Phytochemical Co.

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