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Quercetin, a phytoestrogen and dietary flavonoid, activates different membrane-bound guanylate cyclase isoforms in LLC-PK1 and PC12 cells

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Abstract

Accumulated evidence suggests that quercetin, a dietary flavonoid, has beneficial effects in protection against cardiovascular diseases and in the inhibition of tumour growth. We have recently shown that antioxidants such as 17β-estradiol, resveratrol, dithiothreitol and vitamin C activate membrane-bound guanylate cyclase GC-A, a receptor for atrial natriuretic factor (ANF). Since quercetin is a phytoestrogen and potent antioxidant, it is possible that it may activate GC-A or other guanylate cyclase isoforms. We examined whether quercetin activates GC-A or GC-B (the receptor for C-type natriuretic peptide, CNP) in PC12 and porcine kidney proximal tubular LLC-PK1 cells. The results showed that quercetin activated a guanylate cyclase isoform in both cell types. Quercetin inhibited CNP-stimulated GC-B activity, but had little effect on ANF-stimulated GC-A activity in PC12 cells, suggesting that quercetin mainly activates GC-B in PC12 cells. In contrast, CNP had no effect on guanylate cyclase activity in LLC-PK1 cells, indicating that GC-B is not expressed in LLC-PK1 cells. Furthermore, quercetin had a small effect on ANF-stimulated GC-A activity and had no effect on soluble guanylate cyclase activity in LLC-PK1 cells, suggesting that quercetin does not activate GC-A, GC-B or soluble guanylate cyclase in LLC-PK1 cells. However, quercetin did stimulate membrane-bound guanylate cyclase activity in LLC-PK1 cell membranes. These results indicate that quercetin activates the GC-B isoform in PC12 cells, but activates an unknown membrane-bound guanylate cyclase isoform in LLC-PK1 cells.

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Introduction

Hypertension is a risk factor for cardiovascular disease, the leading cause of death in the USA. A diet rich in fruits, vegetables and low-fat dairy foods has been found to substantially lower blood pressure in normal subjects and hypertensive patients (Appel et al 1997; Moore et al 1999). The fruits and vegetables diet also reduces blood pressure, although to a lesser extent. These results suggest that fruits and vegetables contain some compounds that can lower blood pressure. Quercetin, a flavonoid and phytoestrogen, is present in a wide range of fruits and vegetables (Formica & Regelson 1995; Soleas et al 1997; Jovanovic & Simic 2000). Quercetin is also a potent antioxidant in-vivo and in-vitro, and thus quercetin and other flavonoids have been considered as therapeutic agents for a wide range of pathology, including cancer, viral infection, inflammation/allergy, hypertension and atherosclerosis (for review, see Graefe et al 1999; Soleas et al 1997; Wiseman 1999; Jovanovic & Simic 2000; Lamson & Brignall 2000).

The protective action of quercetin and other antioxidants on cancer, hypertension, atherosclerosis and inflammation/allergy may be partially related to their antioxidant function. Besides detoxifying free radicals, quercetin is also known to affect the function or expression of several proteins. For instance, quercetin has been shown to inhibit protein tyrosine kinase (Levy et al 1984) and protein kinase C (Nishino et al 1984), to bind the oestrogen ERβ receptor (Piantelli et al 1995; Caltagirone et al 1997) and to inhibit the expression of ras (Ranelletti et al 2000), mutant p53 protein (Avila et al 1994) and heat shock proteins (Hosokawa et al 1990; Wei et al 1994). Quercetin has also been shown to increase cGMP levels by inhibiting phosphodiesterase activity

(Davis 1984) or by activating the NO/soluble guanylate cyclase pathway (Guerrero et al 2002). However, it has not been documented whether quercetin can affect vasoactive hormone receptors such as membrane-bound GC-A (a receptor for atrial natriuretic factor, ANF) and GC-B (the receptor for C-type natriuretic peptide, CNP).

Membrane-bound guanylate cyclase GC-A and GC-B are activated by ANF and CNP, respectively (for review, see Wedel & Garbers 2001). Recently, we have found that antioxidants such as 17β-estradiol, resveratrol (a phytoestrogen), dithiothreitol and vitamin C activate GC-A in PC12 cells (Chen et al 1998, 2000, 2001a). Since quercetin is a potent antioxidant (Soleas et al 1997, Wiseman 1999, Jovanovic & Simic 2000, Lamson & Brignall 2000), and expresses oestrogen-like (Piantelli et al 1995; Caltagirone et al 1997) or anti-oestrogen properties (Miodini et al 1999), we examined whether quercetin affects the activation of GC-A or GC-B. In this report, we measured the effect of quercetin on basal, ANF-stimulated GC-A and CNP-stimulated GC-B activity in LLC-PK1 and PC12 cells. Quercetin increased basal cGMP formation in a dose-dependent manner in both cell types, indicating that it activates a guanylate cyclase isoform. Interestingly, quercetin inhibited CNP-stimulated GC-B activity, but not ANF-stimulated GC-A activity in PC12 cells, indicating that quercetin activates GC-B in PC12 cells. However, quercetin did not affect ANF-stimulated GC-A activity or CNP-stimulated GC-B activity in LLC-PK1 cells. Quercetin also did not affect soluble guanylate cyclase activity in LLC-PK1 cells, but activated membrane-bound guanylate cyclase in LLC-PK1 cell membranes. These results indicate that quercetin activates an unknown membrane-bound guanylate cyclase isoform in LLC-PK1 cells. Thus, quercetin activates different membrane-bound guanylate cyclase isoforms in PC12 and LLC-PK1 cells.

Materials and Methods

Materials

Synthetic atrial natriuretic factor (rat ANF, 8–33) and C natriuretic peptide were purchased from Peninsula Laboratories, Inc. (Belmont, CA). Quercetin and other common chemicals were purchased from Sigma (St Louis, MO).

cGMP Determination

PC12 and LLC-PK1 cells were cultured until confluence in 6-well plates (35 mm) with RPMI medium containing 5% bovine fetal serum and 10% horse serum. The cells were washed with 2 mL of serum-free medium and pre-incubated at 37 °C for 10 min with 1 mL of medium containing 0.5 mM isobutylmethylxanthine. Various concentrations of quercetin, ANF or CNP were added to the cells and incubated for 10 min at 37 °C. After incubation, the medium was aspirated and 1 mL cold 10% trichloroacetic acid was added to the plates. The cell extracts were scraped and centrifuged for 15 min at 2000 *g*, and the supernatant fractions were extracted with water-saturated ether to remove trichloroacetic acid. The cGMP levels in the supernatants were determined by radioimmunoassay (Chang & Song 1993; Chen et al 1998, 2000, 2001a, b).

Preparation of LLC-PK1 cell membranes

LLC-PK1 cells were grown to near confluence in 75-cm^2 flasks with RPMI medium containing 10% bovine fetal serum and 5% horse serum. Cultured cells were washed with cold PBS and then resuspended in 25 mM Tris-HCl (pH 7.6) buffer containing 250 mM sucrose. The cells were frozen at −80 °C and thawed on ice to break down the cell membranes. The broken cells were then centrifuged (12 000 *g*, 4 °C) for 15 min. The membrane pellets were resuspended with 50 mM Tris buffer, pH 7.6, containing 250 mM sucrose. Both the supernatant (soluble proteins) and the membrane fractions were then assayed for guanylate cyclase activity.

Guanylate cyclase assay

Guanylate cyclase was assayed at 37 °C in the presence of 50 mM Tris, pH 7.6, 0.5 mM isobutylmethylxanthine, 1 mM GTP, 4 mM $MgCl_2$, 0.1% (w/v) bovine serum albumin, 25 mM creatine phosphate, 55 U mL^{-1} creatine kinase (135 U (mg protein)$^{-1}$) and about 8 μg of cytosolic or membrane proteins with or without various doses of quercetin in a final volume of 0.1 mL. Reactions were initiated by the addition of LLC-PK1 cell membranes, incubated for 10 min and terminated by the addition of 0.5 mL 50 mM chilled sodium acetate, pH 4.0. Generated cGMP was quantified by the radioimmunoassay as previously described (Chang & Song 1993; Chen et al 1998, 2000, 2001a, b).

Results

Effects of quercetin on cGMP formation in PC12 cells

We examined whether quercetin, an antioxidant and dietary flavonoid, can affect guanylate cyclase activity. We measured its effects on cGMP formation in PC12 cells. Quercetin increased cGMP levels in PC12 cells in a dose-dependent manner (Figure 1). The maximal activation for quercetin was about 7 fold. The EC50 (concentration for the half-maximal activation) for the activation of guanylate cyclase by quercetin was about 150 μM in PC12 cells.

Effects of quercetin on ANF- and CNP-stimulated guanylate cyclase activity in PC12 cells

Membrane-bound GC-A and GC-B are the receptors for ANF and CNP, respectively (Wedel & Garbers 2001). PC12 cells are known to express GC-A (Chen et al 1998, 2000, 2001a, b) and GC-B (Suga et al 1992), and thus serve as a good cell model system for studying the effects of quercetin on the activity of GC-A and GC-B. To

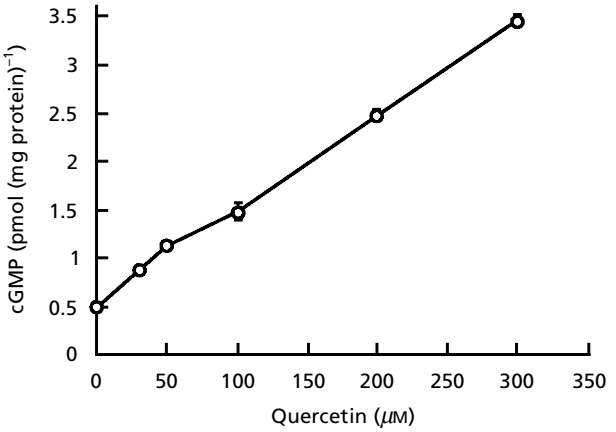

Figure 1 Effect of quercetin on cGMP formation in PC12 cells. Confluent cells were exposed to 0.5 mM isobutylmethylxanthine at 37 °C for 10 min and then stimulated with various concentrations of quercetin for another 10 min. The reaction was stopped with 10% trichloroacetic acid. Generated cGMP was measured by radioimmunoassay. Quercetin stimulated cGMP formation in a dose-dependent manner with an EC50 at around 150 μM. The error bar represents the deviation from the mean of the four replicates.

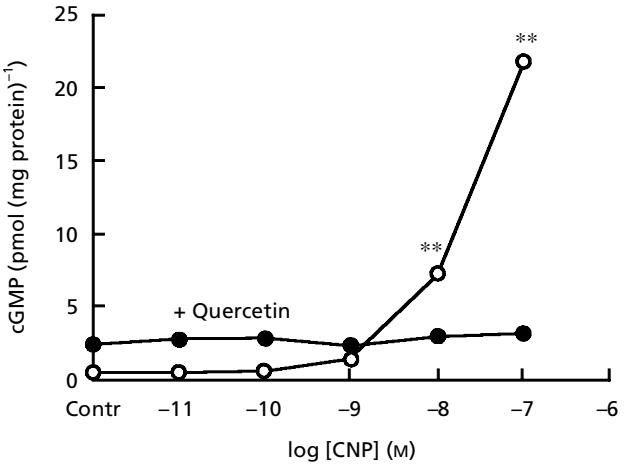

Figure 3 Effect of quercetin on CNP-stimulated GC-B activity in PC12 cells. PC12 cells were exposed to 0.5 mM isobutylmethylxanthine at 37 °C for 10 min, incubated without (○) or with (●) 200 μM quercetin for 10 min and then challenged with various concentrations of CNP for an additional 10 min. The reaction was stopped with 10% trichloroacetic acid. The generated cGMP was measured with the radioimmunoassay. Quercetin abolished CNP-stimulated GC-B activity. The error bar represents the deviation from the mean of the four replicates. $^{**}P < 0.0001$, with quercetin vs without quercetin.

examine whether quercetin activates GC-A or GC-B, we measured the effects of quercetin on ANF-stimulated GC-A and CNP-stimulated GC-B activity in PC12 cells. Quercetin 200 μM slightly decreased ANF-stimulated GC-A activity (Figure 2). However, 200 μM quercetin inhibited CNP-stimulated GC-B activity (Figure 3). These results suggest that quercetin increases cGMP levels mainly through the activation of GC-B, but not GC-A.

Effects of quercetin on cGMP formation in porcine kidney proximal tubular LLC-PK1 cells

To examine whether quercetin also activates GC-B in other cell types, we measured the effect of ANF, CNP and quercetin on cGMP levels in LLC-PK1 cells. LLC-PK1 cells are known to express GC-A (Inui et al 1985).

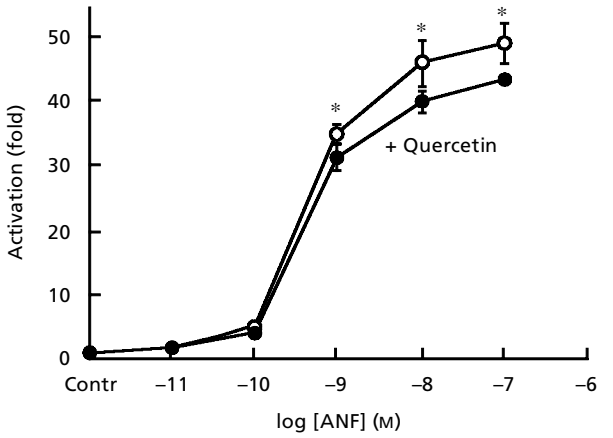

Figure 2 Effect of quercetin on ANF-stimulated GC-A activity in PC12 cells. Confluent cells were exposed to 0.5 mM isobutylmethylxanthine at 37 °C for 10 min, incubated without (○) or with (●) 200 μM quercetin for 10 min and then stimulated with various concentrations of ANF for another 10 min. The reaction was stopped with 10% trichloroacetic acid. Generated cGMP was measured by radioimmunoassay. Quercetin slightly decreased ANF-stimulated GC-A activity. The error bar represents the deviation from the mean of the four replicates. $^{*}P < 0.05$, with quercetin vs without quercetin.

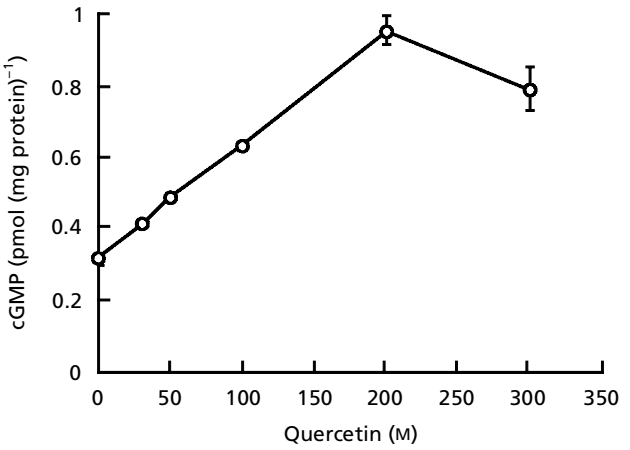

Figure 4 Effect of quercetin on cGMP formation in LLC-PK1 cells. Confluent cells were exposed to 0.5 mM isobutylmethylxanthine at 37 °C for 10 min and then stimulated with various concentrations of quercetin for another 10 min. The reaction was stopped with 10% trichloroacetic acid. Generated cGMP was measured by radioimmunoassay. Quercetin increased cGMP formation in a dose-dependent manner with an EC50 at around 100 μM. The error bar represents the deviation from the mean of the four replicates.

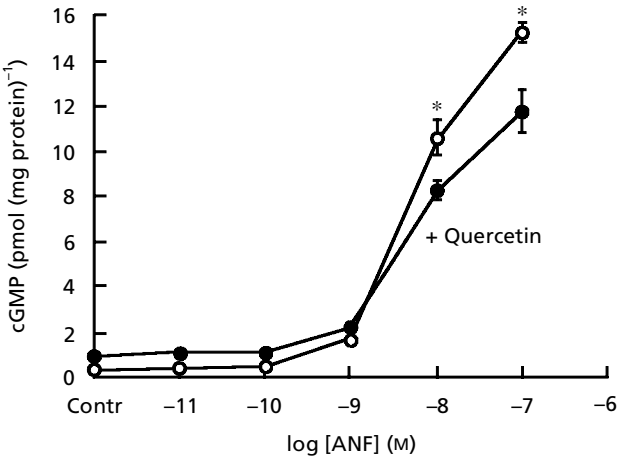

Figure 5 Effect of quercetin on ANF-stimulated GC-A activity in LLC-PK1 cells. Confluent cells were exposed to 0.5 mM isobutylmethylxanthine at 37 °C for 10 min, incubated without (○) or with (●) 200 μM quercetin for 10 min and then various concentrations of ANF for another 10 min. The reaction was stopped with 10% trichloroacetic acid. Generated cGMP was measured by radioimmunoassay. Quercetin slightly inhibited ANF-stimulated GC-A in LLC-PK1 cells. The error bar represents the deviation from the mean of the four replicates. *$P < 0.005$, with quercetin vs without quercetin.

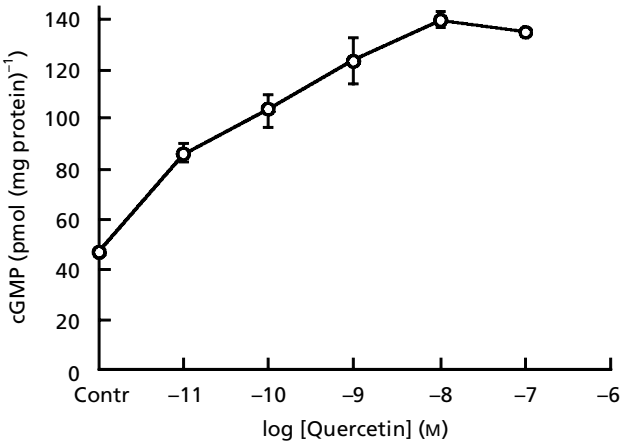

Figure 6 Effects of quercetin on guanylate cyclase activity in membrane proteins from LLC-PK1 cells. Guanylate cyclase assay was performed at 37 °C for 15 min in the presence and absence of various concentrations of quercetin using membrane proteins from LLC-PK1 cells. Generated cGMP was measured by radioimmunoassay. The error bar represents the deviation from the mean of four replicates. Quercetin activated membrane-bound guanylate cyclase in LLC-PK1 cells. The error bar represents the deviation from the mean of the four replicates.

As in PC12 cells, quercetin increased cGMP levels in a dose-dependent manner in LLC-PK1 cells (Figure 4). Figure 5 shows that ANF increased cGMP levels, confirming that GC-A is present in LLC-PK1 cells. In the presence of 200 μM quercetin, ANF was still able to activate GC-A although with a slightly lower potency in LLC-PK1 cells, suggesting that, like in PC12 cells, GC-A is not the major guanylate cyclase isoform activated by quercetin. However, unlike in PC12 cells, 0.1 μM CNP had no effect on cGMP formation in LLC-PK1 cells (data not shown), indicating that GC-B is not expressed in LLC-PK1 cells. These results indicate that quercetin does not increase cGMP levels by activating GC-A and GC-B in LLC-PK1 cells.

To examine whether quercetin activates soluble or membrane-bound guanylate cyclase in LLC-PK1 cells, we measured the effect of quercetin on guanylate cyclase activity in soluble proteins and membranes prepared from LLC-PK1 cells. Figure 6 shows that quercetin stimulated guanylate cyclase activity in LLC-PK1 cell membranes in a dose-dependent manner with an EC50 at around 10^{-11} M. However, quercetin did not significantly affect the activity of soluble guanylate cyclase in soluble proteins from LLC-PK1 cells (data not shown).

Discussion

Recently, we have shown that 17β-estradiol and resveratrol, a phytoestrogen, activate GC-A in PC12 cells (Chen et al 1998, 2000). Other antioxidants, such as dithiothreitol and vitamin C, also activate GC-A in PC12 cells (Chen et al 2001a). Since 17β-estradiol (Chen et al 1998), resveratrol (Chen et al 2000) and quercetin (Piantelli et al 1995, Caltagirone et al 1997, Miodini et al 1999) exert both antioxidant and oestrogenic actions, we examined whether quercetin can affect the activity of guanylate cyclase isoforms in PC12 and LLC-PK1 cells. The results showed that quercetin increases cGMP formation in both cell types, indicating that quercetin stimulates the activity of a guanylate cyclase isoform.

Accumulated evidence indicates that quercetin increases cGMP formation mainly through the activation of GC-B, but not GC-A, in PC12 cells. Firstly, quercetin has a small effect on ANF-stimulated GC-A activity in PC12 cells, suggesting that GC-A is not the main guanylate cyclase isoform activated by quercetin; and secondly, quercetin abolishes CNP-stimulated GC-B activity in PC12 cells. As compared with CNP, quercetin is less potent in stimulating GC-B activity. Therefore, when quercetin is added together with CNP, it competes with CNP for the activation of GC-B and thus lowers the CNP-stimulated GC-B activity in PC12 cells.

In contrast to PC12 cells, the guanylate cyclase isoform activated by quercetin in LLC-PK1 cells remains unknown. Unlike PC12 cells, CNP fails to increase cGMP levels in LLC-PK1 cells, indicating that GC-B is not expressed in LLC-PK1 cells. Therefore, quercetin cannot activate GC-B in LLC-PK1 cells. Although ANF activates GC-A effectively in LLC-PK1 cells, quercetin only slightly decreases ANF-stimulated GC-A activity, indicating that GC-A is not the main target of quercetin in LLC-PK1 cells. Guanylate cyclase assay reveals that quercetin activates a membrane-bound, but not soluble,

guanylate cyclase in LLC-PK1 cells. Besides GC-A and GC-B, GC-C is another membrane-bound guanylate cyclase with a known ligand (guanylin or *E. coli* heat-stable toxin STa) (Wedel & Garbers 2001). However, we have found that guanylin or STa increased cGMP levels only about 70–80% (unpublished results). Since quercetin increases guanylate cyclase activity about 3-fold, GC-C is unlikely to be the target of quercetin in LLC-PK1 cells. Thus, the major membrane-bound guanylate cyclase isoform activated by quercetin remains unknown in LLC-PK1 cells. Several membrane-bound guanylate cyclase isoforms have been cloned (Wedel & Garbers 2001). However, their ligands remain unknown. We are in the process of examining whether these orphan guanylate cyclase receptors are involved in the stimulation of guanylate cyclase activity by quercetin in LLC-PK1 cells.

It is interesting to note that the EC50 of quercetin for the activation of membrane-bound guanylate cyclase in whole cells and membrane suspension differs substantially. The reason for this discrepancy remains unknown. Similar phenomena have been observed in the activation of guanylate cyclase by oestrogen (Chen et al 1998) and resveratrol (Chen et al 2000). It is likely that 17β-estradiol, resveratrol and quercetin may have better accessibility to guanylate cyclase in the membrane suspension. Additionally, we have recently found that 17β-estradiol also inhibits the activity of soluble guanylate cyclase besides activating membrane-bound guanylate cyclase (Chen et al 2001b). Therefore, the EC50 of 17β-estradiol in the intact cells is shifted to the higher concentration. Similarly, the higher EC50 of resveratrol and quercetin in intact cells may also be due to the inhibition of soluble guanylate cyclase activity. Another possible reason for the high EC50 of 17β-estradiol, resveratrol and quercetin in the whole cells may be that their binding sites are located within cells rather than on the extracellular cell surface. Further experiments are required to distinguish these possibilities.

Transgenic and knock-out studies have clearly demonstrated the involvement of the ANF/GC-A system in the regulation of blood pressure (for review, see Wedel & Garbers 2001). Compared with GC-A, the function of GC-B, the receptor for CNP, is not well understood. GC-B is expressed in many tissues (Wedel & Garbers 2001). It is more abundant than GC-A in the brain, lung, kidney and aortic smooth cells. On the other hand, CNP is expressed in the brain, kidney, heart and endothelial cells. This tissue and cell distribution suggests that the CNP/GC-B system may play a role in the regulation of blood pressure or other cardiovascular functions. Indeed, CNP has been shown to lower blood pressure, although not as potently as ANF (Lopez et al 1997). Besides its involvement in blood pressure regulation, recent studies show that the CNP/GC-B system is also involved in endochondral ossification (Yasoda et al 1998). Since PC12 cells are neuron precursor cells, the fact that quercetin activates GC-B in PC12 cells suggests that the CNP/GC-B system may also play a role in neuronal functions.

Quercetin has been shown to exert cardiovascular protective effects and has been considered as a therapeutic agent for hypertension (Soleas et al 1997; Graefe et al 1999; Wiseman 1999; Jovanovic & Simic 2000; Lamson & Brignall 2000). Some of the cardioprotective effects of quercetin may be attributable to decreases in the serum low-density lipoprotein concentrations, to its inhibition of platelet aggregation, to its inhibition of cyclooxygenase and to its promotion of vasodilatory effects (Soleas et al 1997; Graefe et al 1999; Wiseman 1999; Jovanovic & Simic 2000; Lamson & Brignall 2000). Interestingly, similar mechanisms have also been suggested to account for the cardioprotective effects of resveratrol (Pace-Asciak et al 1995; Ashby et al 1999). It is well known that cGMP can relax smooth muscle and inhibit platelet aggregation (Ulker et al 1995, Riddell & Owens 1999). Although quercetin and resveratrol may activate different guanylate cyclase isoforms in different cell types, the end effect of quercetin and resveratrol is an increase in the cGMP levels. Therefore, some of the cardioprotective effects of quercetin may be due to the activation of GC-B and an unknown guanylate cyclase isoform.

In conclusion, our results demonstrate for the first time that quercetin, a major flavonoid in fruits and vegetables, activates membrane-bound guanylate cyclase GC-B in PC12 cells and an unknown membrane-bound guanylate cyclase isoform in LLC-PK1 cells. Our previous studies have shown that antioxidants 17β-estradiol, resveratrol, dithiothreitol and vitamin C activate GC-A in PC12 cells. These results indicate that antioxidants can also exert their effects by interacting with hormone receptors such as GC-A and GC-B besides eliminating oxidizing free radicals. Since oestrogenic compounds (17β-estradiol, resveratrol and quercetin), dithiothreitol and vitamin C are not structurally related antioxidants, these results suggest that cGMP, the product of GC-B and other guanylate cyclase isoforms, may be involved in the antioxidant defence against hypertension and other age-related diseases.

References

Appel, L. J., Moore, T. J., Obarzanek, E., Vollmer, W. M., Svetkey, L. P., Sacks, F. M., Bray, G. A., Vogt, T. M., Cutler, J. A., Windhauser, M. M., Lin, P., Karanja, N., Simons-Morton, D., McCullough, M., Swain, J., Steele, P., Evans, M. A., Miler, E. R., Harsha, D. W. (1997) Clinical trial of the effects of dietary patterns on blood pressure. *N. Engl. J. Med.* **336**: 1117–1124

Ashby, J., Tinwell, H., Pennie, W., Brooks, A. N., Lefevre, P. A., Beresford, N., Sumpter, J. P. (1999) Partial and weak oestrogenicity of the red wine constituent resveratrol: consideration of its superagonist activity in MCF-7 cells and its suggested cardiovascular protective effects. *J. Appl. Toxicol.* **19**: 39–45

Avila, M. A., Velasco, J. A., Cansado, J., Notario, V. (1994) Quercetin mediates the down-regulation of mutant p53 in the human breast cancer cell line MDA-MB468. *Cancer Res.* **54**: 2424–2428

Caltagirone, S., Ranelletti, F. O., Rinelli, A., Maggiano, N., Colasante, A., Musiani, P., Aiello, F. B., Piaantelli, M. (1997) Interaction with type II estrogen binding sites and antiproliferative activity of tamoxifen and quercetin in

human non-small-cell lung cancer. *Am. J. Respir. Cell Mol. Biol.* **17**: 51–59

Chang, C.-H., Song, D.-L. (1993) Melittin potentiates guanylate cyclase activation stimulated by atrial natriuretic factor and ATP. *J. Biol. Chem.* **268**: 4908–4911

Chen, Z.-J., Yu, L., Chang, C.-H. (1998) Stimulation of membrane-bound guanylate cyclase activity by 17-β estradiol. *Biochem. Biophys. Res. Commun.* **252**: 639–642

Chen, Z.-J., Che, D., Chang, C.-H. (2000) Resveratrol activates membrane-bound guanylate cyclase in PC12 cells. *Pharm. Pharmacol. Commun.* **6**: 211–215

Chen, Z.-J., Che, D., Chang, C.-H. (2001a) Antioxidants, vitamin C and dithiothreitol, activate membrane-bound guanylate cyclase in PC12 cells. *J. Pharm. Pharmacol.* **53**: 243–247

Chen, Z.-J., Che, D., Vetter, M., Liu, S., Chang, C.-H. (2001b) 17β-estradiol inhibits soluble guanylate cyclase activity through a protein tyrosine phosphatase in PC12 cells. *J. Steroid Biochem. Mol. Biol.* **78**: 451–458

Davis, C. W. (1984) Assessment of selective inhibition of rat cerebral cortical calcium-independent and calcium-dependent phosphodiesterases in crude extracts using deoxycyclic AMP and potassium ions. *Biochim. Biophys. Acta* **797**: 354–362

Formica, J. V., Regelson, W. (1995) Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol.* **33**: 1061–1080

Graefe, E. U., Derendorf, H., Veit, M. (1999) Pharmacokinetics and bioavailability of the flavonol quercetin in humans. *Int. J. Clin. Pharmacol. Ther.* **37**: 219–233

Guerrero, M. F., Puebla, P., Carron, R., Martin, M. L., San roman, L. (2002) Quercetin 3,7-dimethyl ether: a vasorelaxant flavonoid isolated from Croton schiedeanus Schlecht. *J. Pharm. Pharmacol.* **54**: 1373–1378

Hosokawa, N., Hirayoshino, K., Nakai, A., Hosokawa, Y., Marui, N., Yoshida, M., Sakai, T., Tinoshino, H., Aoike, A., Kawai, K., Nagata, K. (1990) Flavonoids inhibit the expression of heat shock proteins. *Cell Struct. Funct.* **15**: 393–401

Inui, K., Matsukawa, Y., Nakao, K., Moril, N., Imura, H., Shimokura, M., Kiso, Y., Hori, R. (1985) Specific binding activities and cyclic GMP response by atrial natriuretic peptide in kidney epithelial cell line (LLC-PK1). *Biochem. Biophys. Res. Commun.* **132**: 253–260

Jovanovic, S. V., Simic, M. G. (2000) Antioxidants in nutrition. *Ann. N. Y. Acad. Sci.* **899**: 326–334

Lamson, D. W., Brignall, M. S. (2000) Antioxidants and cancer III: Quercetin. *Altern. Med. Rev.* **5**: 196–208

Levy, J., Teuerstein, I., Marbach, M., Radian, S., Sharoni, Y. (1984) Tyrosine protein kinase activity in the DMBA-induced rat mammary tumor: inhibition by quercetin. *Biochem. Biophys. Res. Commun.* **123**: 1227–1233

Lopez, M. J., Garbers, D. L., Kuhn, M. (1997) The guanylyl cyclase-deficient mouse defines differential pathways of natriuretic peptide signaling. *J. Biol. Chem.* **272**: 23064–23068

Miodini, P., Fioravanti, L., Di Fronzo, G., Cappelletti, V. (1999) The two phyto-oestrogens genistein and quercetin exert different effects on oestrogen receptor function. *Br. J. Cancer* **80**: 1150–1155

Moore, T. J., Vollmer, W. M., Appel, L. J., Sacks, F. M., Svetkey, L. P., Vogt, T. M., Conlin, P. R., Simons-Morton, D. G., Carter-Edwards, L., Harsha, D. W. (1999) Effect of dietary patterns on ambulatory blood pressure. Results from the dietary approaches to stop hypertension (DASH) trial. *Hypertension* **34**: 472–477

Nishino, H., Nishino, A., Jwasima, A. (1984) Quercetin inhibits the action of 12-O-tetradeconylphorbol-13-acetate, a tumor promoter. *Oncology* **41**: 120–123

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

Piantelli, M., Maggiano, N., Ricci, R., Larocca, L. M., Capelli, A., Scambia, G., Isda, G., Natali, P. G., Ranelletti, F. O. (1995) Tamoxifen and quercetin interact with type II estrogen binding sites and inhibit the growth of human melanoma cells. *J. Invest. Dermatol.* **105**: 248–253

Ranelletti, F. O., Maggiano, N., Serra, F. G., Ricci, R., Larocca, L. M., Lanza, P., Scambia, G., Fattorossi, A., Capelli, A., Piantelli, M. (2000) Quercetin inhibits p21-ras expression in human colon cancer cell lines and in primary colorectal tumors. *Int. J. Cancer* **85**: 438–445

Riddell, D. R., Owens, J. S. (1999) Nitric oxide and platelet aggregation. *Vitam. Horm.* **57**: 25–48

Soleas, G. J., Diamandis, E. P., Goldberg, D. M. (1997) Wine as a biological fluid: history, production, and role in disease prevention. *J. Clin. Lab. Anal.* **11**: 287–313

Suga, S., Nakao, K., Hosoda, K., Mukoyama, M., Ogawa, Y., Shirakami, G., Arai, H., Saito, Y., Kambayashi, Y., Inouye, K., Imura, H. (1992) Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinology* **130**: 229–239

Ulker, S., Akgur, S., Evine, A., Soykan, N., Kosay, S. (1995) Platelet aggregation and atrial natriuretic peptide. *Gen. Pharmacol.* **26**: 1409–1412

Wedel, B. J., Garbers, D. L. (2001) The guanylyl cyclase family at Y2K. *Annu. Rev. Physiol.* **63**: 215–233

Wei, Y. Q., Zhao, X., Kariya, Y., Fukata, H., Teshigawara, K., Uchida, A. (1994) Induction of apoptosis by quercetin: Involvement of heat shock protein. *Cancer Res.* **54**: 4952–4957

Wiseman, H. (1999) The bioavailability of non-nutrient plant factors: dietary flavonoids and phyto-oestrogens. *Proc. Nutr. Soc.* **58**: 139–146

Yasoda, A., Ogawa, Y., Suda, M., Tamura, N., Mori, K., Sakuma, Y., Chusho, H., Shiota, K., Tanaka, K., Nakao, K. (1998) Natriuretic peptide regulation of endochondral ossification. Evidence for possible roles of the C-type natriuretic peptide/guanylyl cyclase-B. *J. Biol. Chem.* **273**: 11695–11700