Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 503 (2004) 31–34

www.elsevier.com/locate/ejphar

Short communication

Effect of the flavonol quercetin on membrane conductances in rat colonic crypt cells

Rainer Cermak[a,*], Siegfried Wolffram[a], Gisela Kuhn[b]

[a]*Institute of Animal Nutrition and Physiology, Christian-Albrechts-University of Kiel, Olshausenstr. 40, D-24098 Kiel, Germany*
[b]*Institute of Veterinary Physiology, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland*

Received 10 May 2004; received in revised form 2 September 2004; accepted 7 September 2004
Available online 5 October 2004

Abstract

The plant polyphenol quercetin was shown to induce Cl^- secretion in rat colon. This study was performed to investigate the alterations of membrane conductances in isolated epithelial cells induced by quercetin. Whole-cell patch-clamp recordings were performed in isolated crypts from rat distal colon. In cells of the crypt basis, quercetin significantly hyperpolarized the membrane potential at concentrations ≥3 μM and increased the K^+ conductance without visibly altering the Cl^- conductance. Thus, quercetin induces Cl^- secretion merely by activation of K^+ channels in the colon epithelium.

Keywords: Quercetin; Flavonoid; K^+ conductance; Cl^- secretion; Colon

1. Introduction

The flavonol quercetin is widely distributed in plants and, therefore, the most abundant flavonoid ingested by man and herbivorous animals (Herrmann, 1988; Hertog et al., 1993). We have recently demonstrated that this flavonol induces a Cl^- and HCO_3^- secretion in intestinal epithelium of rats (Cermak et al., 1998). Intestinal Cl^- secretion depends on the combined activity of several transport mechanisms. Basolateral localized Na^+/K^+ ATPases generate a Na^+ gradient, which is responsible for the combined influx of Na^+, K^+, and Cl^- via basolateral $Na^+/K^+/2\ Cl^-$ cotransport. The Cl^- ions are secreted via the cystic fibrosis transmembrane conductance regulator (CFTR), which represents the Cl^- channel in the apical membrane of the colon mucosa. The K^+ ions can recycle via basolateral K^+ channels (Greger et al., 1997). Changes in any of these mechanisms will alter Cl^- secretion.

Two major second messenger pathways are usually involved in intestinal Cl^- secretion. Secretagogues increasing intracellular cyclic adenosine 3′,5′-monophosphate (cAMP), like vasoactive intestinal peptide or prostaglandin E_2, induce protein kinase A-dependent phosphorylation of CFTR. This results in a higher Cl^- conductance of the apical enterocyte membrane leading to Cl^- secretion (Greger, 2000). In contrast, secretagogues increasing intracellular Ca^{2+}, like carbachol or histamin, do not directly influence CFTR, but activate Ca^{2+}-dependent K^+ channels on the apical and basolateral membrane. This hyperpolarizes the membrane potential, thereby increasing the electrochemical driving force for the apical exit of Cl^- ions (Greger et al., 1997).

In a previous study, we could demonstrate that the Cl^- secretion evoked by quercetin was dependent on Ca^{2+} (Cermak et al., 2000). In agreement with this finding, the flavonol was able to activate basolateral K^+ channels in apically permeabilized epithelium of the distal colon (Cermak et al., 2002). However, the question remains open if quercetin is able to directly activate CFTR in this tissue, as such a direct activation was demonstrated for the related isoflavonoid genistein (French et al., 1997; Wang et al.,

* Corresponding author. Tel.: +49 431 880 2078; fax: +49 431 880 1528.
E-mail address: cermak@aninut.uni-kiel.de (R. Cermak).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.09.021

Table 1
Concentration dependence of the effect of quercetin on membrane potential in crypt base cells

Quercetin concentration (μM)	Change in membrane potential (mV)	*n*
1	−1.0±0.5	8
3	−4.2±0.7**	6
10	−5.8±0.7**	18
30	−9.6±1.2*	6
100	−3.7±0.9*	10

Values are means±S.E.M.

* $P<0.01$ versus mean of the control values before quercetin addition and after quercetin washout.

** $P<0.001$ versus mean of the control values before quercetin addition and after quercetin washout.

1998; Weinreich et al., 1997). In our previous studies, we used tissue preparations that also contained subepithelial cell layers. Therefore, we could not exclude that the effect of quercetin in colon epithelium was dependent on neural transmitters or mediators released by subepithelial cells (Cermak et al., 2000). Thus, we performed experiments with isolated colon crypts in order to check if quercetin acts directly on epithelial cells and if it activates a Cl^- conductance in addition to the K^+ conductance already described.

2. Materials and methods

2.1. Crypt isolation

Male Wistar rats (RCC) with a body weight of 200–250 g were used. The animals were kept and treated according to institutional guidelines approved by the Kantonales Veterinäramt Zürich. The animals had free access to tap water and a standard diet (Eberle Nafag AG) until the day of the experiment. Animals were stunned by a blow on the head and killed by exsanguination. Crypts of the distal colon were isolated according to a previously published method (Ecke et al., 1996b).

2.2. Whole-cell recordings

Conventional whole-cell patch-clamp recordings were performed as already described (Cermak and Scharrer, 1999). Isolated crypts were fixed to the bottom of a perfusion chamber with the aid of poly-L-lysine (0.01 w/v) and additionally held with a suction pipette. The perfusion rate of the extracellular buffer solution was around 10 ml min^{-1}, the volume of the perfusion chamber was about 0.5 ml. All experiments were performed at 37 °C. Membrane voltage was recorded in the zero current clamp mode. Current voltage curves were obtained by clamping the cell to a holding potential of −80 mV and stepwise clamping the cell to potentials from −100 to +60 mV for 30 ms in 10-mV increments. For statistical comparison of membrane currents, the last 5 ms of each current pulse were evaluated.

2.3. Chemicals and solutions

All standard chemicals and quercetin were obtained from Fluka, Buchs, Switzerland. The perfusion buffer contained (in mM): NaCl 145; KH_2PO_4 0.4, K_2HPO_4 1.6, Ca^{2+} gluconate 1.3, $MgCl_2$ 1, D-glucose 5; pH was adjusted to 7.4. The solution for the patch pipettes contained (in mM): KCl 30, K^+ gluconate 95, NaH_2PO_4 1.2, Na_2HPO_4 4.8, Ca^{2+} gluconate 0.726, $MgCl_2$ 1.03, D-glucose 5, ethylene glycol-bis (β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) 1, Na_2ATP 1; pH was adjusted to 7.2.

2.4. Statistics

Data are presented as mean values±standard error of the mean (S.E.M.). Statistical significance of the effects was tested by comparing the value under the drug with the mean of the control values before drug addition and after drug removal (paired two-sided Student's *t*-test). A *P* value of less than 0.05 was considered significant.

3. Results

All recordings were made from cells of the crypt basis since these cells have the highest expression of the apical chloride channel CFTR (Trezise and Buchwald, 1991; Umar et al., 2000). Basal membrane potential in these cells was −79.4±1.1 mV (n=59), indicating a high fractional K^+ conductance. This was confirmed by administration of the K^+ channel blocker Ba^{2+} (1 mM), which depolarized the membrane potential reversibly by 24.3±1.1 mV (n=59).

Superfusion with various concentrations of quercetin induced a hyperpolarization in cells of the crypt basis, which was reversible after washout of the flavonol. This effect was significant at a concentration of 3 μM and maximal at a concentration of 30 μM (Table 1). To identify the underlying

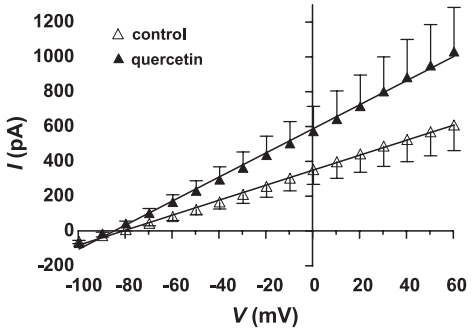

Fig. 1. Effect of quercetin on current–voltage relation in crypt base cells. Filled triangles are values in the presence of quercetin (10 μM), open triangles are values of the means of the control values before quercetin addition and after quercetin washout (means±S.E.M., n=10).

currents, current voltage curves were obtained at a concentration of 10 μM quercetin. In these experiments, the flavonol significantly hyperpolarized crypt cells from −82.0±1.7 to -87.9±1.4 mV (n=10) and increased the outward currents at all clamp potentials above K^+ equilibrium potential (Fig. 1). The membrane conductance was significantly increased from 3.6±0.8 to 6.0±1.5 nS in the presence of the flavonol. The quercetin-induced current had a reversal potential of −98.0±5.8 mV. This was not significantly different from the K^+ equilibrium potential under our experimental conditions, which was −94 mV (calculated with the Nernst equation; $[K^+]_{IN}$=125 mM and $[K^+]_{EX}$=3.6 mM).

4. Discussion

In previous studies that investigated the effect of quercetin on colonic Cl^- secretion, we could exclude a participation of submucosal neurons and also of prostaglandins, which are mainly released by subepithelial cells, in the activation pathway of the flavonol (Cermak et al., 1998, 2000). However, because we used tissue preparations containing a variety of submucosal cell types, we could not definitely exclude the necessity for other local mediators released by subepithelial cells. The demonstration of a quercetin effect in an isolated crypt cell preparation devoid of nonepithelial cells finally proves that the flavonol is able to act directly on colonocytes.

Quercetin increased the cellular K^+ conductance of cells in the crypt base, which show the strongest expression of CFTR along the crypt axis (Trezise and Buchwald, 1991; Umar et al., 2000) and exert a pronounced Cl^- secretion when activated by secretagogues (Böhme et al., 1991; Ecke et al., 1996a,b). This observation is in accordance with a previous study which demonstrated the activation of basolateral K^+ channels in colon epithelium by quercetin (Cermak et al., 2002). Due to the blocker specificity of these channels and due to the Ca^{2+} dependence of the quercetin effect (Cermak et al., 2000), we concluded that the flavonol uses the Ca^{2+} pathway for its secretory activity. This is supported by the observation that quercetin seems to increase the intracellular Ca^{2+} concentration in isolated colon crypt cells (R. Cermak, unpublished observations).

The related isoflavone genistein was shown to directly activate CFTR (French et al., 1997; Wang et al., 1998; Weinreich et al., 1997). An increase in Cl^- conductance by genistein was also demonstrated in rat colon crypt cells, at least when it was administered together with the cAMP-agonist forskolin (Diener and Hug, 1996). In the same cells, those authors observed an inhibition of a K^+ conductance by genistein (Diener and Hug, 1996). Our present experiments demonstrate a different mode of action for quercetin. Whereas the K^+ conductance was clearly increased, the current voltage curves did not indicate any change of the cellular Cl^- conductance. The activation of a distinct Cl^- conductance should have increased inward currents at clamp potentials below the Cl^- equilibrium potential, which was −40 mV under our experimental conditions ($[Cl^-]_{IN}$=32 mM and $[Cl^-]_{EX}$=147 mM). However, this was not the case. Although we cannot totally exclude any activation of Cl^- channels by quercetin, such an effect could have only been minor and negligible for the whole cell conductance in crypt cells.

Thus, we conclude that quercetin had no visible effect on secretory Cl^- channels, that is, CFTR. The different effects on K^+ conductance by quercetin and genistein can explain the stronger secretory effect of quercetin observed in a previous study where we already suggested different secretory mechanisms for these two flavonoids (Cermak et al., 1998).

In contrast to our findings, other authors suggested a similar secretory mode of action for both flavonoids. They showed that several flavonoids, among them the isoflavone genistein, the flavone apigenin, and the flavonols kaempferol and quercetin, activated CFTR-mediated Cl^- currents in Calu-3 airway epithelial cells (Illek and Fischer, 1998). However, studies with renal epithelial A6 cells support our findings of different effects on CFTR between genistein and quercetin. Whereas genistein increased a CFTR-mediated Cl^- conductance in A6 cells, this was neither the case for the flavones apigenin and luteolin nor for the flavonols kaempferol and quercetin (Niisato et al., 1999, 2004).

In summary, we have shown that the flavonol quercetin increases a K^+ conductance in isolated crypt cells of rat colon. The concomitant hyperpolarization increases the electrochemical driving force for Cl^- secretion. No direct effect on Cl^- currents, that is, CFTR, could be demonstrated. Thus, the flavonol quercetin acts differently than the related isoflavone genistein in rat colon.

References

Böhme, M., Diener, M., Rummel, W., 1991. Calcium- and cyclic-AMP-mediated secretory responses in isolated colonic crypts. Pflügers Arch. 419, 144–151.

Cermak, R., Scharrer, E., 1999. Effect of 2,5-anhydro-D-mannitol on membrane potential in rat hepatocyte couplets and hepatocyte monolayer cultures. Biochim. Biophys. Acta 1421, 116–124.

Cermak, R., Föllmer, U., Wolffram, S., 1998. Dietary flavonol quercetin induces chloride secretion in rat colon. Am. J. Physiol. 275, G1166–G1172.

Cermak, R., Vujicic, Z., Kuhn, G., Wolffram, S., 2000. The secretory response of the rat colon to the flavonol quercetin is dependent on Ca^{2+}-calmodulin. Exp. Physiol. 85, 255–261.

Cermak, R., Kuhn, G., Wolffram, S., 2002. The flavonol quercetin activates basolateral K^+ channels in rat distal colon epithelium. Br. J. Pharmacol. 135, 1183–1190.

Diener, M., Hug, F., 1996. Modulation of Cl^- secretion in rat distal colon by genistein, a protein tyrosine kinase inhibitor. Eur. J. Pharmacol. 299, 161–170.

Ecke, D., Bleich, M., Greger, R., 1996a. Crypt base cells show forskolin-induced Cl^- secretion but no cation inward conductance. Pflügers Arch. 431, 427–434.

Ecke, D., Bleich, M., Schwartz, B., Fraser, G., Greger, R., 1996b. The ion conductances of colonic crypts from dexamethasone-treated rats. Pflügers Arch. 431, 419–426.

French, P.J., Bijman, J., Bot, A.G., Boomaars, W.E.M., Scholte, B.J., de Jonge, H.R., 1997. Genistein activates CFTR Cl^- channels via a tyrosine kinase- and protein phosphatase-independent mechanism. Am. J. Physiol. 273, C747–C753.

Greger, R., 2000. Role of CFTR in the colon. Annu. Rev. Physiol. 62, 467–491.

Greger, R., Bleich, M., Leipziger, J., Ecke, D., Mall, M., Kunzelmann, K., 1997. Regulation of ion transport in colonic crypts. News Physiol. Sci. 12, 62–66.

Herrmann, K., 1988. On the occurrence of flavonol and flavone glycosides in vegetables. Z. Lebensm.-Unters. Forsch. 186, 1–5.

Hertog, M.G.L., Hollman, P.C.H., van de Putte, B., 1993. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. J. Agric. Food Chem. 41, 1242–1246.

Illek, B., Fischer, H., 1998. Flavonoids stimulate Cl conductance of human airway epithelium in vitro and in vivo. Am. J. Physiol. 275, L902–L910.

Niisato, N., Ito, Y., Marunaka, Y., 1999. Activation of Cl^- channel and $Na^+/K^+/2Cl^-$ cotransporter in renal epithelial A6 cells by flavonoids: genistein, daidzein, and apigenin. Biochem. Biophys. Res. Commun. 254, 368–371.

Niisato, N., Nishino, H., Nishio, K., Marunaka, Y., 2004. Cross talk of cAMP and flavone in regulation of cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel and $Na^+/K^+/2Cl^-$ cotransporter in renal epithelial A6 cells. Biochem. Pharmacol. 67, 795–801.

Trezise, A.E.O., Buchwald, M., 1991. In vivo cell-specific expression of the cystic fibrosis transmembrane conductance regulator. Nature 353, 434–437.

Umar, S., Scott, J., Sellin, J.H., Dubinsky, W.P., Morris, A.P., 2000. Murine colonic mucosa hyperproliferation. I. Elevated CFTR expression and enhanced cAMP-dependent Cl^- secretion. Am. J. Physiol. 278, G753–G764.

Wang, F., Zeltwanger, S., Yang, I.C.H., Nairn, A.C., Hwang, T.C., 1998. Actions of genistein on cystic fibrosis transmembrane conductance regulator channel gating—evidence for two binding-sites with opposite effects. J. Gen. Physiol. 111, 477–490.

Weinreich, F., Wood, P.G., Riordan, J.R., Nagel, G., 1997. Direct action of genistein on CFTR. Pflügers Arch. 434, 484–491.