EFFECTS OF QUERCETIN ON EPITHELIAL CHLORIDE SECRETION

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(Received in final form August 12, 1997)

Summary

The effect of the flavonoid quercetin on epithelial chloride secretion has been studied in vitro. These studies were performed using monolayers of the human colonic epithelial cell line T84, mounted in modified Ussing chambers. Chloride secretion was assessed as changes in short circuit current (Isc) both in basal conditions as well as in response to different secretagogues: carbachol (100 μM), vasoactive intestinal polypeptide (VIP) (10 nM) and prostaglandin E2 (1 μM). Secretion was also induced via protein kinase C by adding phorbol myristate acetate (PMA, 100 nM) in the presence of the calmodulin inhibitor W13 (50 μM). Quercetin (100 μM) was able to promote secretion when the flavonoid was added to the mucosal side of the monolayer. On the contrary, serosal addition of quercetin was devoid of secretory activity and at concentration of 10 μM it was able to inhibit chloride secretion in response to carbachol, prostaglandin E2 and PMA/W13, but not that induced by VIP. We conclude that the effect of quercetin on epithelial chloride secretion is dual, secretory and antisecretory, depending on both the concentration and the side of the monolayer where the addition of the flavonoid is made.

Key Words: quercetin, protein kinase C, T84 cell line, chloride secretion, flavonoid

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a flavonoid which exhibits a broad spectrum of biological activities (1,2). We and other researchers have investigated the pharmacological effects of quercetin on the gastrointestinal tract. Quercetin has been shown to have antiulcer and gastroprotective effects (3). Quercetin also displays acute antiarrheal activity either directly (4) or upon release by hydrolysis of the quercetin glycoside quercitrin (3-rhamnosylquercetin) (5). The antiarrheal activity of quercetin and/or quercitrin has been ascribed to inhibition of intestinal motility (6,7) and/or the antisecretory properties of the flavonoid (4,5). Both anti-kinetic and antisecretory effects have been proposed to be related to calcium antagonism or stimulation of...
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Vol. 61, No. 20, 1997

\( \alpha \)-adrenoceptors (4,8). However, there may be other mechanisms involved. For example, quercetin is a known inhibitor of protein kinase C, as demonstrated in rat brain (9) and vascular smooth muscle (10). Protein kinase C activity has certainly been implicated in the regulation of intestinal motility and water and electrolyte transport. On the other hand, quercetin inhibits prostaglandin synthesis (11) and the production of reactive oxygen radicals (12,13), mediators that can also significantly affect gastrointestinal function (14).

In a previous study, Nguyen et al. (15) described that quercetin exerted a direct secretory effect on intestinal epithelial cells. This contrasts with the antisecretory activity showed by quercetin \textit{in vivo} in the rat using the tied-off colon technique (5). Thus, quercetin (100 \( \mu \)M, luminal) was able to inhibit colonic fluid secretion induced by sodium picosulphate or prostaglandin \( \text{E}_2 \), without affecting the normal absorptive tone. The present study was undertaken to test the effects of quercetin on intestinal chloride secretion both in basal conditions as well as when it is induced by several secretagogues like carbachol, prostaglandin \( \text{E}_2 \) and vasoactive intestinal peptide. For this purpose, monolayers of the human epithelial cell line T\textsubscript{84} mounted in modified \textit{Ussing} chambers were used.

Materials and methods

\textit{Growth and maintenance of T\textsubscript{84} cells.} T\textsubscript{84} cells were grown and maintained in culture as previously described (16,17). Briefly, the cells were cultured in 5\% CO\textsubscript{2} and at 37°C in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium supplemented with 5\% (v/v) newborn calf serum. Cells were seeded onto rat tail collagen-coated Nuclepore filters glued to Lexan rings (surface area: 2.0 cm\textsuperscript{2}, approximately 10\textsuperscript{6} cells/filter). Methods for the preparation of these filter supports have been described previously (16). The filters were then set on glass beads to allow medium (supplemented with 5000 units/l penicillin and 5000 \( \mu \)g/l streptomycin sulfate) to bathe both sides of the cell monolayer. Cells were maintained as confluent monolayers for 7 days after the filters were seeded for use in secretory studies.

\textit{Secretory studies.} The cell monolayers on the filter/ring units were mounted in modified \textit{Ussing} chambers as previously described (16), and both sides of the monolayer were bathed with Ringer's solution, with the following composition (in mM): NaCl 115; CaCl\textsubscript{2} 1.2; MgCl\textsubscript{2} 1.2; KH\textsubscript{2}PO\textsubscript{4} 0.4; K\textsubscript{2}HPO\textsubscript{4} 2.5; NaHCO\textsubscript{3} 25 and glucose 10. The medium was warmed to 37°C with a circulating water jacket and gently mixed and oxygenated with a constant inflow of 95\% O\textsubscript{2}/5\% CO\textsubscript{2}. Spontaneous tissue potential difference was short-circuited continuously via an automatic voltage clamp (W.P. Instruments, New Haven, CT) and Ag-AgCl\textsubscript{2} electrodes, except for brief (2-5 s) intervals at each time point when the open circuit potential difference was recorded. The current necessary to maintain the short circuit \( (I_s) \) was recorded at 5-min intervals. Instrument calibration was performed prior to each experiment using a filter/ring unit without cells. All comparative studies used matched pairs of monolayers seeded at the same time and studied concurrently. Previous studies have shown that \( I_s \) values in this system are wholly reflective of net chloride transport.

Quercetin was dissolved in ethanol and added to the Ringer's solution at a 1:100 dilution (final concentrations of ethanol 1\%) in the serosal (basolateral) or in the mucosal (apical) side of the cell monolayer at concentrations of 10 and 100 \( \mu \)M. Secretagogues (carbachol 100 \( \mu \)M, prostaglandin \( \text{E}_2 \) 1 \( \mu \)M or VIP 10 nM), were added to the serosal side 15 min after quercetin administration. Secretion was also induced in a protein kinase C-dependent fashion by adding phorbol 12-myristate 13-acetate (PMA, 100 nM) to both sides of the monolayer in the presence of the calmodulin kinase inhibitor W13 (50 \( \mu \)M). Previous studies have shown that this treatment induces chloride secretion.
that can be inhibited by protein kinase C inhibitors (18). Control experiments were performed by
adding ethanol (final concentration 1%) at the time point in which quercetin was added.

**Chemicals.** All reagents, including quercetin, were obtained from Sigma (St. Louis, MO, USA). Cell
culture media were purchased from JRH (Lenexa, KS).

**Statistics.** All results are expressed as mean ± S.E.M. Differences between means were tested for
statistical significance using Student's unpaired t-test. Probability values of P<0.05 were considered
significant.

**Results**

**Quercetin-induced secretion.** Quercetin elicited a significant secretory response when added to the
mucosal side of T₈₄ cells at a concentration of 100 μM (Iₐ ~4 μA/cm²), but not at the lowest
concentration (10 μM). No significant secretory effect was observed when quercetin was added to
the serosal side at any concentration. Ethanol, which was used as a solvent for quercetin, was
occasionally observed to increase Iₑ above baseline in these experiments (Table I).

**TABLE I**

<table>
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<th></th>
<th>Basal</th>
<th>Carbachol</th>
<th>PMA/W13</th>
<th>Prostaglandin E₂</th>
<th>VIP</th>
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<tr>
<td>Control</td>
<td>0.9±0.2</td>
<td>35.1±3.1</td>
<td>8.7±0.7</td>
<td>26.0±2.0</td>
<td>20.7±1.3</td>
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<tr>
<td>Quercetin</td>
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<td>46.2±6.7</td>
<td>8.3±0.9</td>
<td>24.6±1.8</td>
<td>25.9±0.8*</td>
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<tr>
<td>(mucosal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.8±0.1</td>
<td>26.5±3.0*</td>
<td>5.8±0.7*</td>
<td>19.4±1.4*</td>
<td>21.0±1.6</td>
</tr>
<tr>
<td>(serosal)</td>
<td></td>
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Quercetin (10 μM) was added to the mucosal or serosal side of the cell monolayer 15 min prior to
administration of secretagogues. Peak Iₑ values (μA/cm²) above baseline, representing maximal
chloride secretion, are shown. Data are expressed as mean ± S.E.M. (n=6 per group). *P<0.05
compared to the control by Student's t-test.

**Effect of quercetin on secretagogue-induced chloride secretion.** A quercetin concentration of 10 μM
was selected for these studies, since this was without a direct secretory effect by itself on either side
of the monolayer. Pretreatment of T₈₄ cells for 15 min with quercetin (10 μM, mucosal) had no
significant effect on carbachol-induced chloride secretion (Table I). In contrast, when the flavonoid
was added to the serosal side, the peak secretory response to carbachol was significantly inhibited,
by approximately 25% (P<0.05) (Fig. 1A). On the other hand, quercetin (10 μM, serosal) inhibited
the maximal secretory response elicited by prostaglandin E₂ by 25% (P<0.05) (Table I, Fig. 1B) but
had no effect on VIP-induced secretion (Table I). However, when added to the mucosal rather than
the serosal side of the monolayer, quercetin significantly enhanced VIP-induced secretion (P<0.05).
In another set of experiments, chloride secretion was induced by PMA in the presence of the
calmodulin kinase inhibitor W13, since the phorbol ester alone has no effect on chloride secretion
by T₈₄ cells (18). Pretreatment of the cells with quercetin inhibited protein kinase C-dependent
chloride secretion by 33%, but only when the flavonoid was added to the serosal side of the
monolayer (Fig. 1C). Mucosal addition was without an effect on protein kinase C-stimulated secretion (Table I).

Fig. 1

Effect of quercetin (Q, 10 μM) on chloride secretion in T₄₄ cells induced by carbachol (C, 100 μM) (panel A), prostaglandin E₂ (PGE₂, 1 μM) (panel B) and PMA (100 nM) in the presence of the calmodulin kinase inhibitor W13 (50 μM) (panel C). Both quercetin and secretagogue compound were added to the serosal side of the monolayer. Data are expressed as mean ± S.E.M. (n=6). *P<0.05, **P<0.01 compared with cells treated with the secretagogue compound alone by Student's t-test. × Secretagogue compound, ▲ Quercetin+secretagogue compound.
Discussion

In the present paper we have studied the effect of quercetin, a flavonoid which showed colonic antisecretory activity in vivo (5), on chloride secretion in T₈₂₂ cell monolayers mounted in Üssing chambers. This was performed first in basal conditions and second in response to several secretagogue agents.

Although in previous studies we showed that quercetin had no effect on basal levels of colonic absorption in vivo (5), quercetin exerted a significant secretory effect in vitro at the highest mucosal concentration assayed. A similar effect was observed by Nguyen et al. (15), which the authors attributed to a small increase in cAMP levels. This could be explained on the basis of the known ability of the flavonoid to inhibit cyclic AMP phosphodiesterase (19). Therefore, a lower concentration (10 μM) was selected for the subsequent experiments.

On the other hand, quercetin exerted an inhibitory effect on secretory responses to carbachol, although the inhibition observed was only about 25%. This was likely not due to toxicity of the flavonoid, since Nguyen et al. (15) previously reported that T₈₂₂ cell monolayers exposed to 100 μM quercetin for >1 h could still exclude trypan blue. It is well known that carbachol induces chloride secretion in T₈₂₂ cells through activation of basolateral muscarinic receptors (20). This suggests that quercetin exerts a direct inhibitory effect on the enterocyte, so that the opening of the apical channels would be somehow compromised.

In contrast to its effect on carbachol-induced secretion, quercetin was ineffective in blocking responses to VIP. In fact, quercetin actually enhanced VIP-induced secretion when it was added to the mucosal side of the preparation. This could be also explained on the basis of the ability of quercetin to increase cytosolic cAMP levels (15,19). Conversely, serosal quercetin was able to inhibit the secretory response induced by prostaglandin E₂. In previous studies we found that quercetin inhibited prostaglandin E₂-induced hydroelectrolytic secretion in vivo (5). Together, the findings may imply that the flavonoid has a direct inhibitory effect on epithelial secretory responses to prostaglandin E₂. Superficially, VIP and prostaglandin E₂ are thought to activate broadly similar intracellular mechanisms leading to chloride secretion. Thus, both agents are thought to activate protein kinase A and take advantage of the same secretory machinery within the enterocyte. However, in a number of epithelial transport models, the effects of prostaglandin E₂ cannot be accounted for by changes in cAMP. Likewise, studies in T₈₂₂ cells have revealed that prostaglandin E₂ has a much greater effect on chloride secretion than would be predicted by its ability to increase cAMP, relative to changes induced by other agents (Barrett, unpublished observations).

One of the enzymes involved in the intracellular regulation of transepithelial hydroelectrolytic transport is protein kinase C, although its role remains controversial. Protein kinase C is activated by calcium-mobilizing agonists such as carbachol via release of diacylglycerol and Ca²⁺. Protein kinase C can also be activated in an experimental setting by phorbol esters. A number of laxatives (bisacodyl, ricinoleic acid) seem to activate protein kinase C as well, resulting in upregulation of phospholipase A₂ and increased production of prostaglandins which contribute to their secretory effect (21). In T₈₂₂ cells, however, protein kinase C activation seems predominantly to exert an inhibitory rather than an activating effect on Ca²⁺ dependent secretion. This may be the result of various consequences of the activation of specific protein kinase C isoforms. Nevertheless, if calmodulin-dependent protein kinase activity is blocked in T₈₂₂ cells, then activation of protein kinase C increases, rather than decreases, chloride secretion. In this setting, pretreatment of T₈₂₂ cells with serosal, but not mucosal, quercetin significantly inhibited PMA-induced chloride secretion by 33%. This effect may be related to a direct inhibitory action of quercetin on protein kinase C, in view of
the known ability of the flavonoid to downregulate this enzyme in other tissues, such as the rat brain (9) or aorta (10).

In conclusion, quercetin is able to promote secretion at 100 μM in Tm cell line when is administered luminally, but it shows no effect when is added at lower concentrations or in the serosal side of the preparation. On the other hand, quercetin inhibits carbachol- and prostaglandin E2-induced secretion in this cell line, and shows no inhibitory effect when VIP is used as secretagogue. The mechanism of action of the flavonoid is still not fully elucidated, but it involves a direct effect on the enterocyte. The effects are not specific for a particular signal transduction mechanism, and may reflect an action distal in the secretory pathway. Other mechanisms may also be involved in the antisecretory effect. Thus, quercetin is a chelator of ions such as iron, and may interfere with Ca²⁺ availability within the enterocyte. It is interesting to note that quercetin is able to exert a dual effect on this epithelial cell line, secretory or antisecretory, depending on its concentration as well as on the side of the monolayer where the flavonoid is added: serosal or mucosal. This dual effect would allow us to think that the flavonoid may exert a regulatory role in the digestive tract. Thus, quercetin is able to facilitate intestinal transit in normal conditions and to delay it when this transit is increased in situations in which the cholinergic system is activated or when there is an increased synthesis or release of prostaglandins, as it happens in different types of diarrhoea.

Acknowledgements

The authors want to thank Dr. Jorge Uribe for his excellent technical assistance. These studies were supported by the Spanish Ministry of Education and Science (CICYT SAF94/0528) and by grants to Dr. Barrett from the National Institutes of Health (USA) (DK28305 and DK47756).

References


