ELSEVIER

Biochemical Pharmacology 62 (2001) 1145–1151

Biochemical Pharmacology

# The impact of different flavonoid classes on colonic $Cl^-$ secretion in rats

Rainer Cermak[a,*], Zoran Vujicic[a], Erwin Scharrer[a], Siegfried Wolfram[b]

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

Received 26 March 2001; accepted 14 June 2001

**Abstract**

The plant polyphenol quercetin was shown to induce a significant $Cl^-$ secretion in intestinal epithelium. In order to elucidate the structural requirements of quercetin and related flavonoids for this activity, we tested the ability of further flavonols and other flavonoids found in edible plants to induce $Cl^-$ secretion which was measured as an increase in short-circuit current ($I_{sc}$) in rat colon. Whereas several flavonols and the flavon luteolin increased $I_{sc}$, other flavonoids such as flavanones, flavans, flavanols, and anthocyanidins failed to do so. Two glycosides of quercetin, spiraeosid, and isoquercitrin, as well as two methoxylated quercetin metabolites, isorhamnetin and tamarixetin, were also able to increase $I_{sc}$. We conclude that a 2,3-double bond in conjunction with the 4-oxo group in the C ring and a hydroxylated B ring are necessary for the secretory activity of flavonoids. This activity requires different structural features than those mandatory for the antioxidative properties of flavonoids. Glucosidation and methoxylation of several hydroxyl groups does not necessarily abolish the secretory potential. 

*Keywords:* Flavonoids; Flavonols; $Cl^-$ secretion; Colon; Plant polyphenols

## 1. Introduction

Flavonoids belong to the large group of plant polyphenols which are ingested by man and animals with their regular diet. Many studies conducted in recent years point to a potentially important role of this class of dietary substances in the prevention of cardiovascular disease, several cancer forms, and inflammatory diseases [1–5]. A prominent feature of several flavonoids is their antioxidant effect *in vitro*, which is even larger, at least for certain compounds, than that of alpha-tocopherol, ascorbate, or glutathione [2,6,7].

Flavonoids are the most common and widely distributed group of plant phenolics. Their basic structure consists of two aromatic rings (rings A and B, Fig. 1) linked through three carbons that usually form an oxygenated heterocycle (ring C, Fig. 1). Variations in the heterocyclic ring C give rise to different classes: flavones, flavonols, flavanones, flavanonols, flavanols, anthocyanidines, and isoflavones (Fig. 1) [1,3,5,8]. Other flavonoid classes such as chalcones, aurones, biflavonoids, or proanthocyanidins differ from the basic structure shown in Fig. 1. The rings A and B allow a multitude of substitution patterns within each class of flavonoids: phenolic hydroxyls, glycosides, methoxy groups, sulfates, and glucuronides. Over 4000 different naturally occurring flavonoids have been described, most of which are commonly found as glycoside derivatives in plants [5].

Little is known about the influence of flavonoids on the mucosa of the gastrointestinal tract, the tissue these compounds come in contact with immediately after oral intake. In a previous study, we demonstrated that the most abundant flavonol, quercetin, induces a $Cl^-$ secretion in the small and large intestine of rats [9]. With the exception of the isoflavonoid genistein [9–12], nothing is known about the effects of other flavonoids on electrolyte transport in native intestinal epithelium. We conducted this study in order to elucidate structural principles required for the secretory activity observed with quercetin. Therefore, we tested the effect of certain representatives of several flavonoid classes and of other flavonols as well as of glycosylated and methoxylated quercetin derivatives on rat colon epithelium mounted in Ussing chambers.

* Corresponding author. Tel.: +41-1-63-58-809; fax: +41-1-63-58-932.

*E-mail address:* cermak@vetphys.unizh.ch (R. Cermak).

*Abbreviations:* $G_t$ tissue conductance; and $I_{sc}$ short-circuit current.

0006-2952/01/$ – see front matter 
PII: S0006-2952(01)

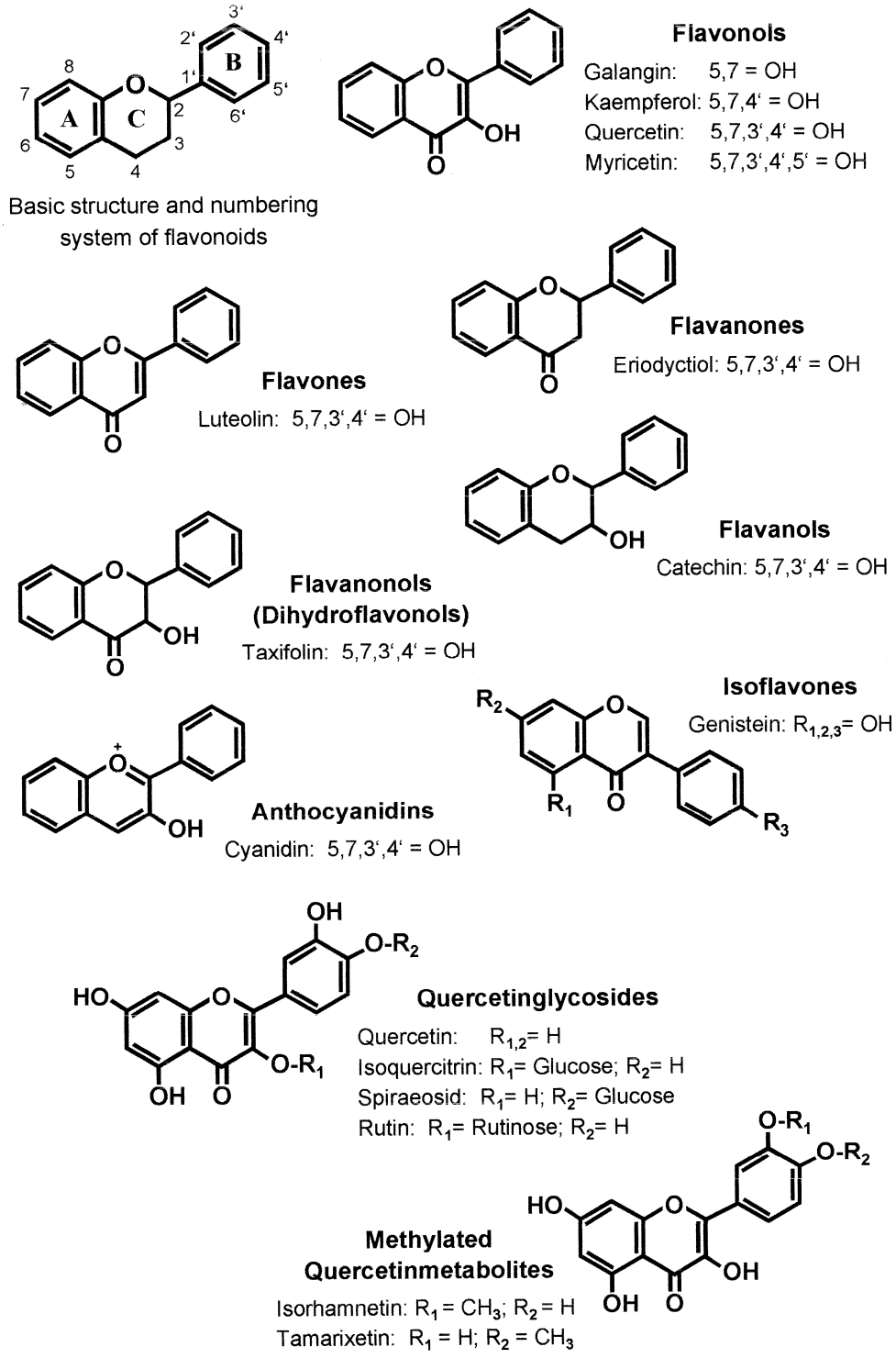


Fig. 1. Structure of different flavonoids, flavonoid glycosides, and methylated flavonoids.

## 2. Materials and methods

### *2.1. Tissue preparation*

Male Sprague–Dawley rats (RCC Ltd.) with a body weight of 200–250 g were used. 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 to the head and killed by exsanguination. The proximal and distal colon were taken out immediately and the serosa and muscularis were removed.

### 2.2. Determination of electrophysiological parameters

Two sheets of tissue from each proximal and distal colon were mounted in a modified Ussing chamber and bathed with a volume of 4-mL buffer solution on both sides of the epithelium. The buffer solution contained (in mM) NaCl 107, KCl 4.5, $NaHCO_3$ 25, $Na_2HPO_4$ 1.8, $NaH_2PO_4$ 0.2, $CaCl_2$ 1.25, $MgCl_2$ 1, and glucose 12; it was gassed with 5% $CO_2$ in 95% $O_2$ and kept at 37°; pH was adjusted to 7.4. The epithelium was continuously short-circuited by an automatic voltage clamp device (Aachen Microclamp, AC Copy Datentechnik) with correction for solution resistance. The exposed surface of the tissue was 1 $cm^2$. In 1-min intervals, a current of $\pm 100$ $\mu$A ($I$) was applied to the tissue and the change in voltage ($U$) measured. The tissue conductance ($G_t$) was calculated from these values according to Ohm's law ($G_t = I/U$). The values for $G_t$ and the continuously applied short-circuit current ($I_{sc}$) were printed out every minute. Before the addition of flavonoids, there was an equilibration time of at least 50 min to stabilize basal values. Control experiments were performed with tissue from the same animals. The baseline of the electrical parameters was determined as the mean over a 5-min period (5 values) immediately prior to administration of a drug. The maximal change in $I_{sc}$ induced by a flavonoid was expressed as the difference from the former baseline ($\Delta I_{sc}$). The viability of the tissue was routinely checked with forskolin (1 $\mu$M) 60 to 120 min after addition of the flavonoids at the end of the experiments. Data from tissues which did not respond to forskolin with an increase in $I_{sc}$ of at least 1 $\mu$Eq $h^{-1}$ $cm^{-2}$ were discarded.

### 2.3. Reagents

Gossypol, luteolin, and (±)-taxifolin were obtained from Sigma; galangin, kaempferol, myricetin, and (±)-catechin were from Fluka. Cyanidin chloride, eriodyctiol, isoquercitrin, isorhamnetin, spiraeosid, and tamarixetin were purchased from Extrasynthese. All compounds were dissolved in DMSO; the final DMSO concentration never exceeded 0.1% (v/v).

### 2.4. Statistics

Data are presented as mean values ± standard error of the mean (S.E.). Mean values of the tissue preparations receiving the respective drug were compared with mean values from control tissues derived from the same animals receiving only the vehicle. In some experiments, single tissues had to be omitted because basal $G_t$ was too high or because they failed the viability test. Therefore, statistical significance of the effects was determined using the unpaired $t$-test.

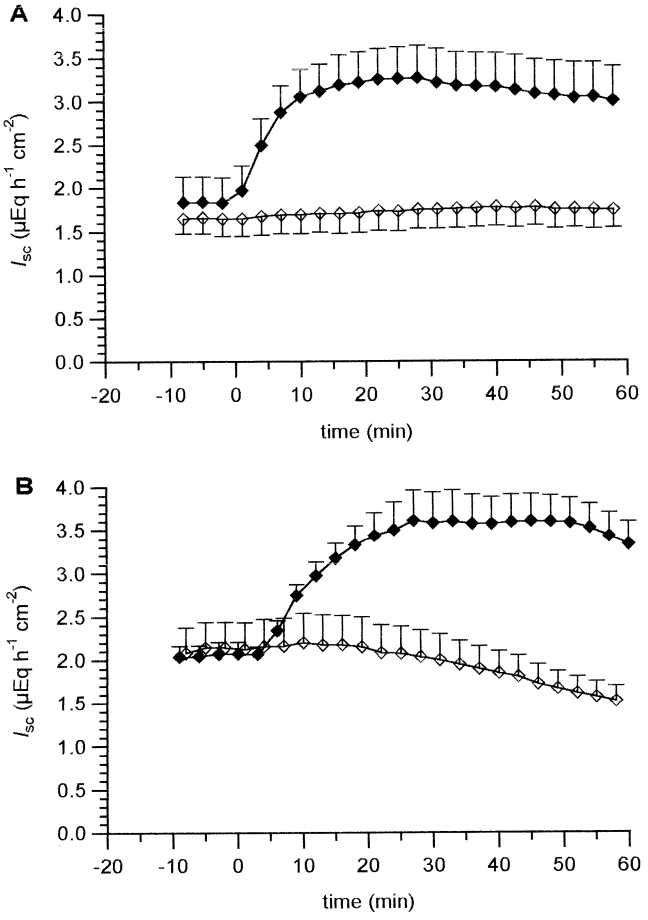


Fig. 2. Time-course of luteolin-induced increase in short-circuit current ($I_{sc}$) across rat proximal (A) and distal (B) colon. Luteolin (◆, 100 $\mu$M) was added at time 0 to both sides of the tissue (N = 6 in A and B); controls (◇) received vehicle only (N = 5 in A, N = 6 in B).

## 3. Results

All compounds were added at a concentration of 100 $\mu$M to the mucosal and serosal side of the tissue. For particular flavonoids that induced a prominent increase in $I_{sc}$, experiments were repeated with the addition of these flavonoids to the mucosal side only.

First, several compounds from different flavonoid classes were tested. They were selected according to the criterion that they had the same hydroxylation pattern as quercetin in ring A (positions 5 and 7) and ring B (positions 3′ and 4′); therefore, they differed only in their heterocyclic ring C, which determines their class specifity (Fig. 1).

The flavone luteolin, which differs from the flavonol quercetin only in the missing hydroxyl group at position 3, induced a significant increase in $I_{sc}$ in the proximal and distal colon (Fig. 2) which was similar in size and time-course as that induced by quercetin in earlier studies [9,13]. Luteolin also increased $G_t$ in both proximal ($\Delta G_t = 4.9 \pm 0.4$ mS $cm^{-2}$, N = 6) and distal colon ($\Delta G_t = 3.2 \pm 1.1$ mS $cm^{-2}$, N = 6) compared to controls (proximal colon: $\Delta G_t = 2.0 \pm 0.8$ mS $cm^{-2}$, N = 5, $P < 0.01$) (distal colon: $\Delta G_t =$

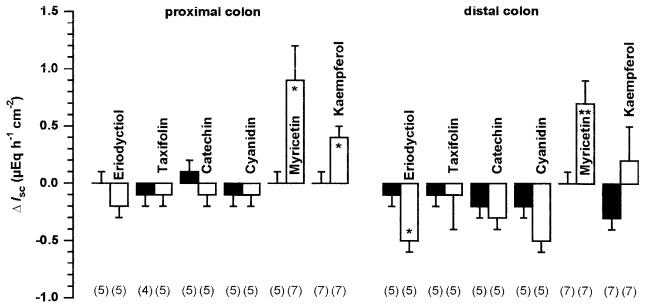


Fig. 3. Effect of various flavonoids on short-circuit current ($\Delta I_{sc}$) across rat proximal and distal colon. All compounds were added at a concentration of 100 $\mu$M to both sides of the tissue. Black bars are control values, open bars are values in the presence of the respective substance. Numbers in parentheses indicate the number of experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ versus respective controls.

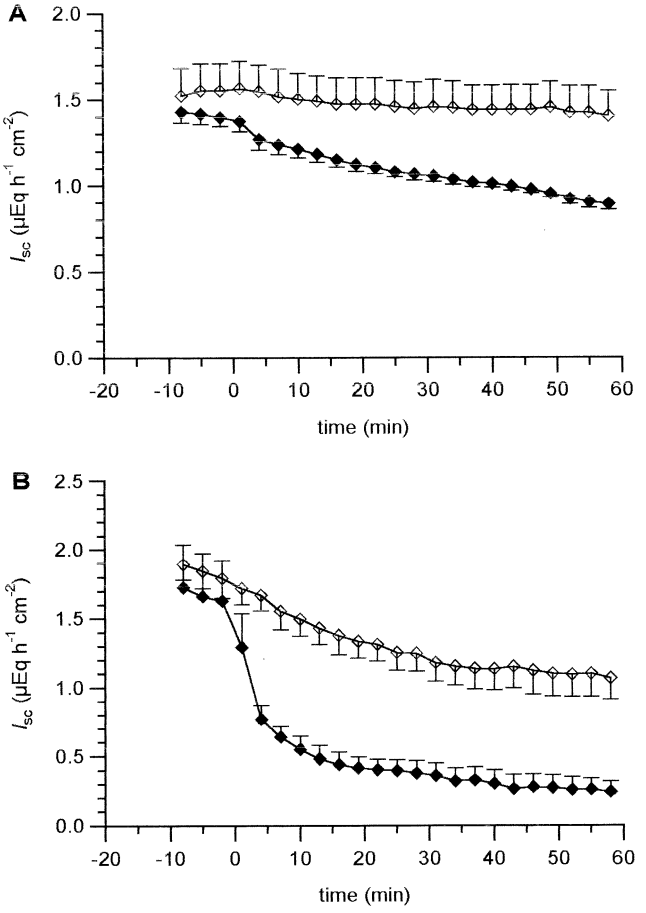


Fig. 4. Time-course of galangin-induced decrease in short-circuit current ($I_{sc}$) across rat proximal (A) and distal (B) colon. Galangin (◆, 100 $\mu$M) was added at time 0 to both sides of the tissue (N = 6 in A and B); controls (◇) received vehicle only (N = 6 in A and B).

$0.7 \pm 0.1$ mS cm$^{-2}$, N = 6, $P < 0.05$). The flavone also increased $I_{sc}$ in the distal segment when it was added only to the mucosal side by $0.9 \pm 0.4$ $\mu$Eq h$^{-1}$ cm$^{-2}$ (control: $\Delta I_{sc} = -0.1 \pm 0.1$ $\mu$Eq h$^{-1}$ cm$^{-2}$, both N = 6, $P < 0.05$), although this effect seemed to be smaller compared to bilateral addition (not significant). In parallel to the short-circuit current, $G_t$ increased slightly but this was not significant in this experimental series. In contrast to the distal segment, mucosal luteolin had neither an effect on $I_{sc}$ nor on $G_t$ in the proximal colon.

The flavanone eriodyctiol, which lacks the double bond between C2 and C3 in comparison to luteolin, failed to increase $I_{sc}$ or $G_t$ in the proximal colon. Remarkably, eriodyctiol induced a transient decrease in $I_{sc}$ (Fig. 3) and in $G_t$ ($\Delta G_t = -0.8 \pm 0.1$ mS cm$^{-2}$, N = 5; controls: $\Delta G_t = -0.2 \pm 0.1$ mS cm$^{-2}$, N = 5, $P < 0.01$) in the distal colon. The flavanonol taxifolin, which is hydroxylated at position 3 in comparison to eriodyctiol, showed no effect on $I_{sc}$ or $G_t$ at all (Fig. 3). The flavanol catechin, which lacks the 4-oxo group in comparison to taxifolin, was similarly ineffective (Fig. 3). The same was true for the anthocyanidine cyanidin chloride (Fig. 3).

Next, flavonols were investigated which differed from quercetin in the hydroxylation pattern of ring B. The flavonol galangin lacks hydroxyl groups in ring B, whereas kaempferol is hydroxylated at position 4′ and myricetin at positions 3′, 4′, and 5′. Similar to quercetin and luteolin, myricetin also increased the $I_{sc}$ in proximal and distal colon (Fig. 3). The time-course of this increase, however, was more transient than that induced by luteolin or quercetin; around 30 min after addition of myricetin, the $I_{sc}$ had returned to basal values. $G_t$ was also increased significantly by myricetin in both colonic segments (proximal colon: $\Delta G_t = 1.8 \pm 0.2$ mS cm$^{-2}$, N = 7; control: $\Delta G_t = 0.2 \pm 0.2$ mS cm$^{-2}$, N = 5, $P < 0.001$) (distal colon; $\Delta G_t = 1.1 \pm 0.4$ mS cm$^{-2}$; control: $\Delta G_t = 0.0 \pm 0.1$ mS cm$^{-2}$, both N = 7, $P < 0.05$).

Kaempferol induced in the proximal colon only a small, but significant increase in $I_{sc}$ (Fig. 3) and $G_t$ (control: $\Delta G_t = 0.2 \pm 0.1$ mS cm$^{-2}$, N = 7; kaempferol: $\Delta G_t = 2.2 \pm 0.5$ mS cm$^{-2}$, N = 7, $P < 0.01$). In the distal segment, kaempferol had no significant effect. The flavonol galangin, which is devoid of any hydroxyl groups at ring B, had the opposite effect to the other flavonols; it decreased $I_{sc}$ clearly in both colon segments (Fig. 4). Remarkably, the addition of galangin led to a marked increase in $G_t$ by $19.1 \pm 11.0$ mS cm$^{-2}$ in the proximal and by $57.4 \pm 14.6$ mS cm$^{-2}$ in the distal colon, which could indicate a severe disturbance of the epithelial integrity by this compound.

Since most flavonoids occur naturally as glycosides in plants, two common glycosides of quercetin were additionally tested: isoquercitrin (quercetin-3-*O*-glucoside) and spiraeosid (quercetin-4′-*O*-glucoside). Whereas isoquercitrin increased $I_{sc}$ in proximal colon only, spiraeosid did so in both segments (Fig. 5). The latter glucoside also increased $G_t$ significantly in the proximal colon by $4.9 \pm 0.8$ mS cm$^{-2}$ (control: $\Delta G_t = 1.4 \pm 0.7$ mS cm$^{-2}$, both N = 6, $P < 0.01$) as well as in the distal colon by $1.5 \pm 0.3$ mS cm$^{-2}$ (control: $\Delta G_t = 0.3 \pm 0.2$ mS cm$^{-2}$, both N = 6, $P < 0.01$). Isoquercitrin, on the other hand, did not alter tissue conductance at all in both segments.

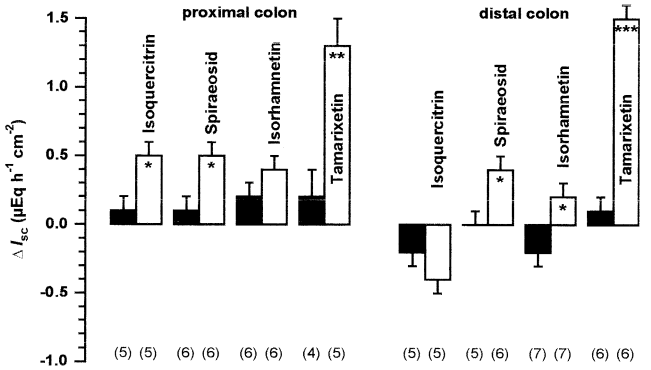


Fig. 5. Effect of quercetin glucosides and methylated quercetin metabolites on short-circuit current ($\Delta I_{sc}$) across rat proximal and distal colon. All compounds were added at a concentration of 100 $\mu$M to both sides of the tissue. Black bars are control values, open bars are values in the presence of the respective substance. Numbers in parentheses indicate the number of experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus respective controls.

Two metabolites of quercetin, which are often found in plasma and bile after oral intake of this flavonol, are isorhamnetin (3′-*O*-methyl-quercetin) and tamarixetin (4′-*O*-methyl-quercetin) [14–17]. Therefore, these derivatives of quercetin were also investigated. Isorhamnetin induced a very small $I_{sc}$ increase which was only significant in the distal colon (Fig. 5). $G_t$ was not altered at all by this quercetin metabolite. In contrast, tamarixetin increased $I_{sc}$ distinctly in both colonic segments (Fig. 5). The observed increases in $G_t$ were not significant, though. Because of the clear effect on $I_{sc}$, tamarixetin was added in another series of experiments to the mucosal side of the tissue only. Again, a rise in $I_{sc}$ could be observed which was significant in proximal colon (control: $\Delta I_{sc} = 0.1 \pm 0.1$ $\mu$Eq h$^{-1}$ cm$^{-2}$, N = 7; tamarixetin: $\Delta I_{sc} = 0.5 \pm 0.1$ $\mu$Eq h$^{-1}$ cm$^{-2}$, N = 8, $P < 0.05$) as well as in the distal segment (control: $\Delta I_{sc} = -0.3 \pm 0.1$ $\mu$Eq h$^{-1}$ cm$^{-2}$, N = 8; tamarixetin: $\Delta I_{sc} = 0.7 \pm 0.3$ $\mu$Eq h$^{-1}$ cm$^{-2}$, N = 8, $P < 0.01$). $G_t$ was not significantly altered in these experiments.

The non-flavonoid gossypol, which is found in cottonseed, also induced a significant increase in $I_{sc}$ in proximal (Fig. 6) and in distal colon ($\Delta I_{sc} = 2.3 \pm 0.4$ $\mu$Eq h$^{-1}$ cm$^{-2}$, N = 6; controls: $\Delta I_{sc} = 0.0 \pm 0.1$ $\mu$Eq h$^{-1}$ cm$^{-2}$, N = 6, $P < 0.001$) when it was added to both sides of the tissue at a concentration of 100 $\mu$M. Remarkable was the rise in $G_t$ by 17.6 ± 2.8 mS cm$^{-2}$ ($P < 0.01$) in the proximal and by 52.4 ± 4.0 mS cm$^{-2}$ ($P < 0.001$) in the distal segment after addition of the polyphenol. Gossypol had a somewhat lesser effect when added exclusively to the mucosal side. In proximal colon, it increased $I_{sc}$ by 1.1 ± 0.4 $\mu$Eq h$^{-1}$ cm$^{-2}$ (N = 7) (controls: $\Delta I_{sc} = 0.0 \pm 0.1$ $\mu$Eq h$^{-1}$ cm$^{-2}$, N = 7, $P < 0.05$) and $G_t$ by 4.5 ± 2.2 mS cm$^{-2}$ (controls: $\Delta G_t = 0.6 \pm 0.9$ mS cm$^{-2}$; $P > 0.05$). In the distal segment, mucosal gossypol increased $I_{sc}$ by 1.8 ± 0.8 $\mu$Eq h$^{-1}$ cm$^{-2}$ (N = 7) (controls: $\Delta I_{sc} = 0.1 \pm 0.1$ $\mu$Eq h$^{-1}$ cm$^{-2}$, N = 7, $P > 0.05$) and $G_t$ by 31.4 ± 8.5 mS cm$^{-2}$ (controls: $\Delta G_t = -1.1 \pm 0.7$ mS cm$^{-2}$, $P < 0.01$).

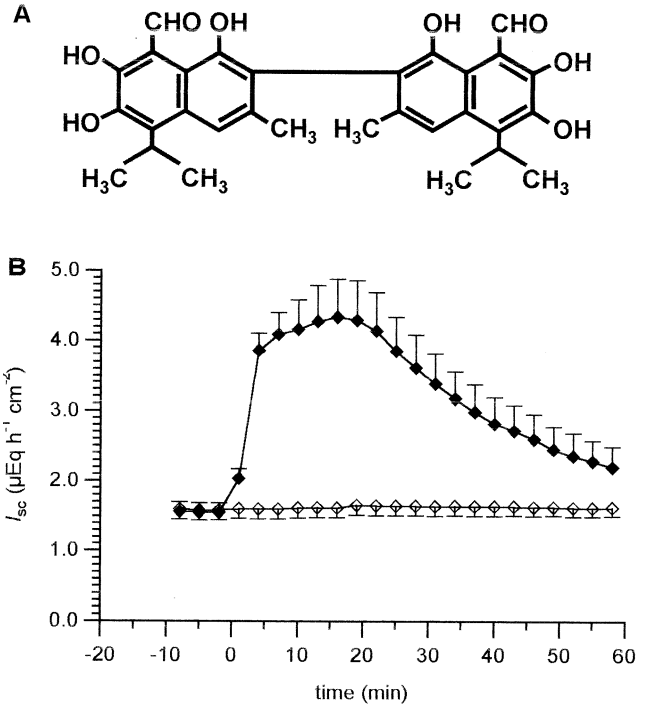


Fig. 6. (A) Molecular structure of the plant polyphenol gossypol. (B) Time-course of gossypol-induced increase in short-circuit current ($I_{sc}$) across rat proximal colon. Gossypol (◆, 100 $\mu$M) was added at time 0 to both sides of the tissue (N = 6); controls (◇) received vehicle only (N = 7).

## 4. Discussion

In previous studies, it was demonstrated that the flavonol quercetin induces a $Cl^-$ secretion in rat intestine [9,13]. We therefore tested some quercetin derivatives and other flavonoids in order to determine the structural requirements necessary for the secretory activity of these compounds in intestinal epithelium. A concentration of 100 $\mu$M was used to compare these compounds at the maximal effective dose of quercetin. In the small intestine, most of the fluid is absorbed, whereas the absorption of flavonoids seems to be rather limited [3,15–17]. In addition, flavonoids absorbed from the small intestine are conjugated and partially secreted back into the intestinal lumen directly by the enterocytes and via the bile [18]. Furthermore, considering the facts that the bioavailability of most flavonoids is rather low and plants contain almost exclusively various flavonoid glycosides from which the carbohydrate moiety is split off by bacterial glycosidases mainly in the large intestine [19], an increase in the flavonoid concentration might be expected to occur in the lumen of the large intestine. Although exact local concentrations cannot be calculated from daily intake, micromolar concentrations of certain flavonoids may be achieved in this segment. It was demonstrated that the flavonol quercetin already induces a significant secretion in the low micromolar range [9].

Since the effect of the flavone luteolin was very similar to that of quercetin, it may be concluded that the hydroxyl

group at position 3 in the heterocyclic C ring does not play a role in the secretory activity of flavonoids. Luteolin was also effective from the mucosal side, a property that was also demonstrated for the flavonol quercetin in a previous study [9]. However, this observation does not exclude the possibility that luteolin (or quercetin) acts via cytosolic sites after cellular uptake or at the basolateral membrane of enterocytes after paracellular or transepithelial uptake. It was demonstrated that the flavone is absorbed from the small intestine and, therefore, might also be capable of passing the large intestinal mucosa [20,21].

On the other hand, the failure to induce secretion of the tested flavanone, flavanonol, flavanol, and anthocyanidine, which all lack the 2,3-double bond and/or the 4-oxo group in the heterocyclic C ring, clearly point out that these two structural features are mandatory for the secretory effect of flavonoids. These structures also occur in isoflavones like genistein, which differ from other flavonoids by the substitution of the aromatic B ring at the 3-position (Fig. 1). In fact, many studies have demonstrated the secretory activity of genistein; this was also confirmed in native intestinal epithelium [9–12].

The observation that galangin decreased $I_{sc}$ leads to the conclusion that the B ring has to be hydroxylated in order to induce secretion. The decrease in $I_{sc}$ could indicate a cation secretion or, more likely, an inhibition of basal $CI^-$ secretion. The drastic increase in $G_t$ induced by galangin probably indicates a severe disintegration of the epithelium by this compound, with the reason being unknown at present. A single hydroxylation site, like that seen in kaempferol, induced a relatively weak secretion that was only significant in the proximal colon. In contrast, myricetin with three hydroxylation sites in ring B showed a larger effect in both segments. However, in comparison to the secretion induced by quercetin or luteolin, the effect of myricetin was only transient. An *o*-dihydroxy structure in the B ring seems, therefore, favourable for the secretory activity.

Two common glycosides of quercetin found in edible plants, isoquercitrin and spiraeosid, also induced an increase in $I_{sc}$ in the proximal colon. However, isoquercitrin failed to do so in the distal segment. Recently, it was demonstrated that human, rat, and sheep small intestine possess $\beta$-glucosidases which are capable of hydrolyzing flavonoid glucosides including isoquercitrin and spiraeosid [22–24]. Although such an enzymatic activity has yet not been demonstrated in the hindgut, we cannot exclude hydrolysis of the glycosides due to $\beta$-glucosidase or glucuronidase activity, because uptake of the glycosides themselves can be expected to be rather limited in the large intestine. It is noteworthy that small intestinal $\beta$-glucosidases were not able to hydrolyse the quercetin glycoside rutin (quercetin-3-*O*-rutinoside) [22–24]. Interestingly, rutin failed to exert any effect on $I_{sc}$ in a previous study [9].

Quercetin and other flavonoids are extensively metabolized in the intestinal wall during absorption. Glucuronidation and *O*-methylation occur in the small intestine which possesses UDP glucuronosyltransferase and catechol-*O*-methyltransferase activity [20,21,25,26]. Two metabolites commonly found in the plasma of various species after oral intake of quercetin are isorhamnetin and tamarixetin [14–17]. Isorhamnetin induced a slight increase in $I_{sc}$ only in the distal colon. However, tamarixetin had a marked effect comparable with that of quercetin in both segments. Tamarixetin was also effective after addition to the mucosal side only. This is of importance since metabolites of quercetin are partly released into the intestinal lumen by enterocytes of the small intestine or secreted via the bile [18]. Such compounds could reach the large intestine. Therefore, it could be possible that at least part of the secretion observed after addition of quercetin or quercetin glycosides is due to the activity of metabolites such as tamarixetin.

Since many flavonoids are potent antioxidants *in vitro*, we compared the structural requirements for this antioxidant activity with those mandatory for the secretory activity. A previous study concluded that the following structural features explain the potent antioxidative effect of certain flavonoids: (i) the presence of a 3′,4′-dihydroxy structure in the B ring; (ii) the presence of a 2,3-double bond in conjunction with a 4-oxo group in the C ring, which are responsible for electron delocalization; (iii) the presence of 3- and 5-hydroxyl groups for maximal radical-scavenging potential [7]. In further studies the importance of the 3- and 5-hydroxyl groups was questioned [6]. In addition, not criterion (ii) per se, but rather the conjugation between rings A and B via a planar C ring is likely the decisive feature for the ability of electron delocalization. This can explain the potent antioxidant activity of quercetin and cyanidin in those studies [6,7]. Although, at first sight, these criteria seem to be similar to those found in our study, they do not apply to the secretory activity since cyanidin showed no effect at all on $CI^-$ secretion. Furthermore, eriodyctiol, taxifolin, catechin, and the quercetin glycoside rutin showed an antioxidant effect still larger than that of alpha-tocopherol, ascorbate, or glutathione *in vitro* [6,7], whereas these flavonoids were ineffective in the present or a former study [9], respectively. Thus, we conclude that the secretory activity of flavonoids in intestinal epithelium is a quality which is distinct from their antioxidative properties.

A plant polyphenol with a structure different from flavonoids, gossypol, was able to induce an effect similar to that induced by quercetin and luteolin. Gossypol, which consists of two linked naphthalene rings, lacks a heterocyclic ring and a fixed 2,3 double bond. Besides hydroxyl groups, aldehyde, methyl, and isopropyl groups serve as substituents. At present, we cannot say if the mechanism by which gossypol induces secretion in rat colon is the same as that used by flavonols and flavones. In contrast to the secretory flavonols and flavones, gossypol drastically increased $G_t$, which indicates a severe disturbance of epithelial integrity with probably large increases in paracellular permeability. This observation points to a different mode of action.

At present, we can only speculate about the biological significance of this observed effect. Principally, the secretion evoked by some of the investigated polyphenols could help to solubilize the ingesta and support fermentation processes. An increased secretion could also help to dilute pathogenic and toxic material at the epithelial barrier of the intestine.

In summary, flavonoids with a γ-pyrone ring and a hydroxylated B ring are capable of inducing secretion in intestinal tissues. The same characteristic is also found with certain glycosides and methoxylated metabolites of quercetin. However, this quality requires different structural features than those mandatory for the antioxidative properties of flavonoids.

## References


[1] Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol 1995;33:1061–80.

[2] Frankel EN, Kanner J, German JB, Parks E, Kinsella JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet 1993;341:454–7.

[3] Hollman PC, Katan MB. Dietary flavonoids: intake, health effects and bioavailability. Food Chem Toxicol 1999;37:937–42.

[4] Duthie GG, Duthie SJ, Kyle JA. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutr Res Rev 2000; 13:79–106.

[5] Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52:673–751.

[6] Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res 1995;22:375–83.

[7] Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: determination of radical scavenging efficiencies. Methods Enzymol 1990;186:343–55.

[8] Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 1998;56:317–33.

[9] Cermak R, Föllmer U, Wolffram S. Dietary flavonol quercetin induces chloride secretion in rat colon. Am J Physiol 1998;275:G1166–72.

[10] Diener M, Hug F. Modulation of $CI^-$ secretion in rat distal colon by genistein, a protein tyrosine kinase inhibitor. Eur J Pharmacol 1996; 299:161–70.

[11] Goddard CA, Evans MJ, Colledge WH. Genistein activates CFTR-mediated $CI^-$ secretion in the murine trachea and colon. Am J Physiol 2000;279:C383–92.

[12] Mall M, Wissner A, Seydewitz HH, Hübner M, Kuehr J, Brandis M, Greger R, Kunzelmann K. Effect of genistein on native epithelial tissue from normal individuals and CF patients and on ion channels expressed in *Xenopus oocytes*. Br J Pharmacol 2000;130:1884–92.

[13] Cermak R, Vujicic Z, Kuhn G, Wolffram S. The secretory response of the rat colon to the flavonol quercetin is dependent on $Ca^{2+}$-calmodulin. Exp Physiol 2000;85:255–61.

[14] Ueno I, Nakano N, Hirono I. Metabolic fate of [$^{14}$C]quercetin in the ACI rat. Jpn J Exp Med 1983;53:41–50.

[15] Manach C, Morand C, Crespy V, Demigné C, Texier O, Régérat F, Rémésy C. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. FEBS Lett 1998;426: 331–6.

[16] Manach C, Texier O, Régérat F, Agullo G, Demigné C, Rémésy C. Dietary quercetin is recovered in rat plasma as conjugated derivates of isorhamnetin and quercetin. J Nutr Biochem 1996;7:375–80.

[17] Ader P, Wessmann S, Wolffram S. Bioavailability and metabolism of the flavonol quercetin in the pig. Free Radic Biol Med 2000;28:1056–67.

[18] Crespy V, Morand C, Manach C, Besson C, Demigné C, Rémésy C. Part of quercetin absorbed in the small intestine is conjugated and further secreted in the intestinal lumen. Am J Physiol 1999;277: G120–6.

[19] Erlund I, Meririnne E, Alfthan G, Aro A. Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. J Nutr 2001;131:1235–41.

[20] Shimoi K, Okada H, Furugori M, Goda T, Takase S, Suzuki M, Hara Y, Yamamoto H, Kinae N. Intestinal absorption of luteolin and luteolin 7-*O*-β-glucoside in rats and humans. FEBS Lett 1998;438: 220–4.

[21] Spencer JP, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK, Rice-Evans CA. The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Lett 1999;458:224–30.

[22] Ioku K, Pongpiriyadacha Y, Konishi Y, Takei Y, Nakatani N, Terao J. β-Glucosidase activity in the rat small intestine toward quercetin monoglucosides. Biosci Biotechnol Biochem 1998;62:1428–31.

[23] Day AJ, Dupont MS, Ridley S, Rhodes M, Rhodes MJ, Morgan MR, Williamson G. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver β-glucosidase activity. FEBS Lett 1998;436:71–5.

[24] Day AJ, Cañada FJ, Díaz JC, Kroon PA, McLauchlan WR, Faulds CB, Plumb GW, Morgan MR, Williamson G. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS Lett 2000;468:166–70.

[25] Piskula MK, Terao J. Accumulation of (−)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. J Nutr 1998;128:1172–8.

[26] Kuhnle G, Spencer JP, Schroeter H, Shenoy B, Debnam ES, Srai SK, Rice-Evans CA, Hahn U. Epicatechin and catechin are *O*-methylated and glucuronidated in the small intestine. Biochem Biophys Res Commun 2000;277:507–12.