Inhibition of Guinea Pig Intestinal Peristalsis by the Flavonoids Quercetin, Naringenin, Apigenin and Genistein

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Key Words
Flavonoids • Quercetin • Naringenin • Apigenin • Genistein • Intestinal peristalsis • Enteric nervous system • Distension sensitivity • Motor performance

Abstract
Flavonoids are known to relax precontracted intestinal smooth muscle and to delay intestinal transit. We therefore investigated the effects of quercetin, naringenin, apigenin and genistein on intestinal peristalsis in vitro. Peristalsis in fluid-perfused segments of the guinea pig small intestine was recorded through the intraluminal pressure changes associated with the peristaltic waves. Alterations of distension sensitivity were reflected by changes in the peristaltic pressure threshold and alterations of peristaltic performance by changes in the maximal acceleration, amplitude and residual baseline pressure of the peristaltic waves. Quercetin, naringenin, apigenin and genistein (10–300 μmol/l) depressed intestinal peristalsis in a structure- and concentration-dependent manner. The flavonoid-evoked changes in peristalsis parameters made it possible to distinguish between two patterns of peristaltic motor inhibition: a decrease in distension sensitivity and peristaltic performance (apigenin and genistein) and a decrease in distension sensitivity without a major change in peristaltic performance (quercetin and naringenin). The antiperistaltic effect of quercetin was partially prevented by apamin (0.5 μmol/l), N-nitro-L-arginine methylester (100 μmol/l) and naloxone (0.5 μmol/l), whereas the effect of genistein was hardly affected by these drugs. Peristaltic motor activity suppressed by quercetin (300 μmol/l), but not genistein (100 μmol/l), was partially restored by apamin. In contrast, neostigmine (0.3 μmol/l) caused a significant recovery of peristalsis from blockade by genistein but failed to reverse peristaltic motor blockade due to quercetin. These observations suggest that naringenin and quercetin inhibit peristalsis by facilitating inhibitory enteric pathways, whereas apigenin and genistein interfere with muscle excitation or excitation-contraction coupling.

Introduction

Over 4,000 structurally unique flavonoids have been identified in plant sources [1]. Flavonoids are phenylbenzopyrones with an assortment of structures based on a common three-ring nucleus. This basic structure is comprised of two benzene rings (A and B) linked through a heterocyclic pyran or pyrone (with a double bond) ring (C)
in the middle (fig. 1). These substances have long been recognized to possess a wide spectrum of biological activities [2], and the effects of polyphenolic compounds on the gastrointestinal tract have been the object of numerous reports. It is widely accepted that flavonoids and tannins show gastroprotective effects against several necrotizing agents [3–5]. Another important activity of flavonoids is their ability to reduce small and large intestinal transit in mice [6, 7], to cause intestinal smooth muscle relaxation [8] and to prevent castor oil-induced diarrhea [9]. These properties are consistent with the use of flavonoids as antidiarrheal drugs in traditional medicine. Moreover, several flavonoids were shown to relax isolated segments of ileum contracted by acetylcholine, KCl, histamine and prostaglandin E2 [8, 10–12]. Similar effects were reported for vascular and tracheal smooth muscle preparations [13–16].

Intestinal peristalsis is a highly coordinated pattern of longitudinal and circular muscle activity, which propagates in an aboral direction to propel the intestinal contents. This motor pattern is governed by excitatory and inhibitory pathways within the enteric nervous system [17]. While the ascending excitatory pathways give rise to contraction of the muscle, the descending inhibitory pathways cause relaxation of the muscle, both pathways playing a crucial role in the coordination and propagation of peristaltic waves [18, 19]. In the guinea pig intestine, two mechanisms of non-adrenergic non-cholinergic (NANC) transmission between the inhibitory enteric motor neurons and the circular muscle can be distinguished [20]. One mechanism relies on fast inhibitory junctional potentials that are blocked by apamin and most probably mediated by adenosine triphosphate or a related purine [21, 22]. The apamin-insensitive transmission depends on slow inhibitory junction potentials [23] that are brought about by vasoactive intestinal peptide and nitric oxide [21]. Acetylcholine is the major transmitter of excitatory enteric motor neurons that are part of the ascending neural pathways regulating propulsive activity [24, 25].

The mechanism by which flavonoids inhibit intestinal propulsion is not fully understood. Apart from their possible interaction with intracellular mediators of smooth muscle contractility [26], there is no information about the possible effects of flavonoids on enteric neurotransmission. We therefore set out to study the action of flavonoids on peristaltic motor activity in the guinea pig iso-

![Fig. 1. Chemical structure of the flavonoids under study.](image-url)
lated small intestine, because this motor pattern is entirely dependent on enteric nerve activity. Firstly, we examined the possible inhibitory effect of some flavonoids (quercetin, naringenin, apigenin, genistein; for their structure see figure 1) on peristalsis. Having seen that the test substances differentially inhibit intestinal peristalsis we, secondly, attempted to elucidate the site of action whereby quercetin and genistein, two representative molecules, suppress peristaltic motor activity. In particular, we wanted to know whether the flavonoids inhibit peristalsis by interfering with excitatory or inhibitory enteric pathways subserving peristalsis. The possibility that flavonoids act on inhibitory motor pathways was addressed by the use of apamin which inhibits fast inhibitory junction potentials. N-nitro-L-arginine methylester (L-NAME) which prevents nitric oxide formation and naloxone which blocks the effect of endogenous opioids. A possible effect of flavonoids on excitatory motor pathways was tested with neostigmine, dimethyl phenylpiperazinium (DMP) and cerulein which stimulate peristalsis through enforcing or mimicking cholinergic neurotransmission.

Methods

Recording of Peristalsis

The care and handling of the guinea pigs and the research protocol were in accordance with the institutional guidelines for the use of experimental animals. Adult guinea pigs of either sex (TRIK strain) and 450–650 g body weight were stunned and bled. The small intestine (jejunum and ileum) was excised and divided into eight segments, each being 10 cm long. Four intestinal segments were set up in parallel and secured horizontally in organ baths containing 30 ml of Tyrode solution at 37 °C as described previously [27]. The composition of the Tyrode solution was (mmol/l): NaCl 136.9, KCl 2.7, CaCl2 1.8, MgCl2 1.0, NaHCO3 11.9, NaH2PO4 0.4, and glucose 5.6. In order to trigger repetitive peristalsis, prewarmed Tyrode solution was continuously infused into the lumen of the segments at a rate of 0.5 ml/min. The intraluminal pressure at the aboral end of the segments was measured with a pressure transducer whose signal was, via an analog/digital converter, fed into a personal computer and recorded and analyzed with the software Peristal 1.0 (Heinemann Scientific Software, Graz, Austria). The fluid passing through the gut lumen was directed into a vertical outlet tubing which ended 4.1 cm above the fluid level in the organ bath. After baseline peristaltic activity had been recorded for a period of 30 min, the drugs to be tested were added to the bathing medium. Each drug was tested on at least six segments from 6 different animals.

In the initial experiments, the concentration-dependent effects of quercetin (10–300 μmol/l), naringenin (0.1–20 μmol/l), apigenin (1–100 μmol/l) and genistein (1–100 μmol/l) on peristalsis were studied. The drugs were added to the bath in a cumulative manner at 20-minute intervals which had been found to be long enough for the drug effects to reach a maximum. Control segments were treated with corresponding volumes of the vehicle for each substance (DMSO, serial dilutions of 1 mol/l NaOH in 0.9% NaCl or Tyrode solution). When it was necessary to use pure DMSO or 1 mol/l NaOH, the volumes added to the bath did not exceed 0.1% of the bath volume. At these volumes the vehicle solutions were devoid of any significant effect on peristalsis.

In order to elucidate the mechanism of action whereby flavonoids inhibit peristalsis, three experiments with quercetin and genistein as representative molecules were performed. Firstly, concentration-response curves for the effects of quercetin and genistein were constructed after exposure of the intestinal segments to apamin (0.5 μmol/l), L-NAME (100 μmol/l) or naloxone (0.5 μmol/l). These drugs were added to the bath 15 min before the recording of the quercetin and genistein concentration-response curves was begun. Secondly, it was tested whether apamin (0.5 μmol/l). L-NAME (100 μmol/l) or naloxone (0.5 μmol/l) was able to restore peristalsis inhibited by quercetin (300 μmol/l) or genistein (100 μmol/l). Apamin, L-NAME and naloxone were administrated to the bath 20 min after the intestinal segments had been pretreated with quercetin or genistein. Thirdly, it was examined whether neostigmine (0.003–0.3 μmol/l), DMP (0.1–1 μmol/l) or cerulein (0.1–1 nmol/l) was capable of restoring peristalsis after blockade by quercetin (300 μmol/l) and genistein (100 μmol/l). Neostigmine, DMP and cerulein were added to the bath in a cumulative manner at 15-minute intervals 20 min after the segments had been pretreated with quercetin or genistein.

Evaluation of Peristalsis

The recordings of peristalsis were analyzed with the software Peristal 1.0 with regard to four parameters: the peristaltic pressure threshold (PPT), the maximal acceleration, the amplitude (maximal pressure) and the residual baseline pressure of the peristaltic waves [27]. PPT (Pa) is the intraluminal pressure at which a peristaltic wave is triggered. Inhibition of peristalsis was associated with an increase of PPT, and abolition of peristalsis manifested itself in a lack of propulsion in spite of an intraluminal pressure of 400 Pa. Although in this case PPT exceeded 400 Pa, abolition of peristalsis was expressed quantitatively by assigning PPT a value of 400 Pa in order to obtain numerical results suitable for further statistical evaluation. The maximal acceleration (Pa/s2) and amplitude (Pa) of the peristaltic waves are determined not only by the speed with which the muscle contracts but also by the speed with which the contraction moves aborally to empty the segments. The residual baseline pressure (Pa) corresponds to the minimal intraluminal pressure that is achieved after completion of each peristaltic wave and thus reflects a measure of the emptying capacity of the peristaltic waves [27].

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Drugs and Solutions

The flavonoids used here (fig. 1) as well as apamin, naloxone, neostigmine, N,N-dimethyl-N-phenyl piperazinium (DMPP) iodide and L-NAME were obtained from Sigma (St. Louis, Mo., USA). Stock solutions of quercetin (250 mmol/l) and naringenin (10 mmol/l) were made in 1 mol/l NaOH, while apigenin (100 mmol/l) and genistein (100 mmol/l) were dissolved in dimethyl sulfoxide (DMSO). L-NAME (30 mmol/l) and naloxone (0.5 mmol/l) were dissolved in distilled water, whereas the stock solution of apamin (1 mmol/l) was prepared in 0.5 mol/l acetic acid and diluted with water. Neostigmine (0.3 mmol/l) and DMPP (1 mmol/l) were dissolved in 0.9% NaCl. Cerulein (TakusR, Pharmacia, Erlangen, Germany) was provided as stabilized aqueous solution of ceruletide Tris-(diethylamine) salt (20 µg/ml) and diluted with 0.9% NaCl to a concentration of 10 µmol/l.

Data Calculation and Statistics

The parameters of three consecutive peristaltic waves were averaged to determine the characteristics of peristalsis at baseline immediately before administration of a drug. The same procedure was applied to calculate the peak values of drug-induced changes in peristaltic indices, unless peristalsis was abolished in which case PPT was assigned a value of 400 Pa. Data are presented as means ± SEM of n experiments, n referring to the number of guinea pigs used in the test. The results were evaluated statistically with the paired or two-sample Student’s t-test or one-way analysis of variance followed by Dunnett’s test. A probability value of p < 0.05 was regarded as significant.

Results

Concentration-Dependent Effect of Flavonoids on Peristalsis

The peristaltic waves at baseline were characterized by the following parameters: PPT 77 ± 3 Pa, maximal acceleration 336 ± 17 Pa/s², maximal pressure 763 ± 24 Pa and residual baseline pressure 14 ± 1 Pa (n = 24).

Addition of quercetin, apigenin, naringenin and genistein to the bathing medium increased PPT in a concentration-dependent manner, the peak changes usually occurring within 10 min (fig. 2). Naringenin and genistein were more potent than apigenin and quercetin, given that naringenin and genistein caused a significant rise of PPT (p < 0.05) at a concentration of 10 µmol/l, whereas a concentration of 100 µmol/l apigenin and quercetin was needed to increase PPT. The highest concentration of genistein tested here (100 µmol/l) abolished peristaltic motor activity. Solubility limitations prevented us from examining the other flavonoids at concentrations high enough to suppress peristalsis.

Analysis of all four peristalsis parameters under study revealed that quercetin, apigenin, naringenin and genistein altered propulsive motility in a differential manner (table 1). While all flavonoids under study increased PPT, although at different concentrations, only apigenin (100 µmol/l) and genistein (30 µmol/l) significantly (p < 0.05) reduced the maximal acceleration and maximal pressure of the peristaltic waves, whereas quercetin (300 µmol/l) and naringenin (20 µmol/l) failed to significantly alter these two indices of peristaltic motor effectiveness (table 1). The residual baseline pressure was enhanced by apigenin and genistein to a larger extent than by quercetin and naringenin (table 1).

Influence of Apamin, L-NAME and Naloxone on the Peristaltic Motor Responses to Quercetin and Genistein

The peristalsis parameters under study were not significantly altered after a 15-minute exposure to apamin (0.5 µmol/l), L-NAME (100 µmol/l) or naloxone (0.5 µmol/l), although a small and transient decrease of PPT and the residual baseline pressure occurred immediately after exposure to L-NAME and apamin. Apamin, L-NAME and naloxone inhibited the antiperistaltic motor action of quercetin to a significant degree so that the highest concentration (300 µmol/l) of quercetin under study had only a small influence on PPT (fig. 3). In contrast, the antiperistaltic motor action of genistein was not
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Fig. 3. Effect of quercetin on the peristaltic pressure threshold (PPT) in the presence of vehicle, apamin (0.5 μmol/l), L-NAME (100 μmol/l) and naloxone (0.5 μmol/l). These drugs were added to the bath 15 min before quercetin was administered in a cumulative manner at 20-minute intervals. The values are means ± SEM, n = 6. *p < 0.05 vs. respective values recorded in the presence of vehicle.

Table 1. Effect of quercetin, naringenin, apigenin and genistein on peristaltic motor parameters

<table>
<thead>
<tr>
<th>Peristaltic motor parameters</th>
<th>Flavonoids</th>
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<tbody>
<tr>
<td></td>
<td>control</td>
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<tr>
<td>Peristaltic pressure threshold, Pa</td>
<td>85 ± 11</td>
</tr>
<tr>
<td>Maximal acceleration, Pa/s²</td>
<td>297 ± 36</td>
</tr>
<tr>
<td>Maximal pressure, Pa</td>
<td>705 ± 48</td>
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<tr>
<td>Residual baseline pressure, Pa</td>
<td>16 ± 3</td>
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</table>

This table compares the peristaltic motor effects of approximately equipotent flavonoid concentrations, i.e., concentrations that enhanced the peristaltic pressure threshold to a similar degree but did not abolish peristalsis. The values are means ± SEM, n = 6.

* p < 0.05 vs. control (pooled data).

significantly altered by apamin and L-NAME (fig. 4). Only naloxone was able to attenuate (p < 0.05) the ability of 10 and 30 μmol/l genistein to increase PPT but failed to prevent 100 μmol/l genistein from abolishing peristalsis (fig. 4).

Fig. 4. Effect of genistein on the peristaltic pressure threshold (PPT) in the presence of apamin (0.5 μmol/l), L-NAME (100 μmol/l) and naloxone (0.5 μmol/l). These drugs were added to the bath 15 min before genistein was administered in a cumulative manner at 20-minute intervals. The values are means ± SEM, n = 6. *p < 0.05 vs. respective values recorded in the presence of vehicle.

Effect of Apamin, L-NAME and Naloxone to Restore Peristalsis from Inhibition by Quercetin and Genistein

The intestinal segments were first exposed to concentrations of quercetin (300 μmol/l) and genistein (100 μmol/l) which inhibited and abolished peristalsis, respectively. Concentrations of quercetin higher than 300 μmol/l could not be employed owing to solubility limitations. Twenty minutes after treatment with quercetin

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or genistein, vehicle, apamin, L-NAME or naloxone was added to the bath to test whether these drugs restored peristalsis from flavonoid-induced blockade. Compared with vehicle, apamin (0.5 μmol/l) was able to cause a partial but significant recovery (p < 0.05) of peristalsis from quercetin-induced inhibition, whereas L-NAME (100 μmol/l) and naloxone (0.5 μmol/l) were without statistically significant effect (fig. 5). The apamin-evoked restoration of peristalsis from quercetin-induced inhibition manifested itself primarily in a reduction of PPT (fig. 5) whereas the other parameters of peristalsis were little improved (data not shown). When peristalsis was blocked by genistein, apamin, L-NAME and naloxone failed to bring about any recovery of peristaltic motor activity (n = 6, data not shown).

**Effect of Neostigmine, DMPP and Cerulein to Restore Peristalsis from Inhibition by Quercetin and Genistein**

Following inhibition of peristalsis by a 20-minute exposure to quercetin (300 μmol/l) or genistein (100 μmol/l), increasing concentrations of neostigmine (0.003–0.3 μmol/l), DMPP (0.1–1 μmol/l) or cerulein (0.1–1 μmol/l) were added to the bath to test whether these drugs reverse flavonoid-induced suppression of peristaltic motor activity. The concentrations of neostigmine, DMPP and cerulein were chosen such that they stimulated peristalsis but did not induce non-propulsive spastic contractions. Neostigmine, DMPP and cerulein were unable to restore peristalsis from quercetin-induced blockade (n = 6, data not shown). Following suppression by genistein, peristaltic motor activity likewise did not recover in response to DMPP and cerulein (n = 6, data not shown). In contrast, cumulative administration of neostigmine to a total concentration of 0.3 μmol/l in the bath restored peristalsis following blockade by genistein (fig. 6). Recuperation of peristalsis was evident from a complete normalization of PPT and a partial restoration of the residual baseline pressure, maximal acceleration and maximal pressure of the peristaltic waves (fig. 6).

**Discussion**

The major results of this study were as follows: (1) Quercetin, naringenin, apigenin and genistein impaired distension-triggered peristalsis in the guinea pig isolated small intestine in a structure- and concentration-related fashion, although their potency was fairly low. (2) While the distension sensitivity of peristalsis was attenuated by all four flavonoids under study, peristaltic motor effectiveness was depressed by apigenin and genistein only. (3) The antiperistaltic effect of quercetin was partially prevented by pretreatment of the intestinal segments with apamin, L-NAME and naloxone, whereas the effect of genistein was little affected by these drugs. (4) Peristalsis blocked by quercetin, but not genistein, was partially restored by apamin. (5) Neostigmine rescued peristaltic motor activity from blockade by genistein, but not quercetin.

The inhibitory effect of flavonoids on intestinal peristalsis is consistent with their inhibition of spontaneous, agonist- and electrically-induced contractions of the guinea pig and rat isolated ileum [8, 10, 28–30]. The myorelaxant effect of flavonoids has been attributed to alterations in the availability of Ca²⁺ to the contractile machinery of smooth muscle, but effects on crucial enzyme systems of intracellular signaling pathways need also to be taken into account. Thus, modulation of protein tyrosine kinase-, protein kinase A- and protein kinase C-dependent pathways by certain flavonoids has been reported in different cell systems [31–34].
The overall aim of this in vitro study was to examine the influence of flavonoids on intestinal peristalsis with respect to their site of action on the intestinal nerve-muscle circuitry subserving propulsive motility. This goal was addressed by two experimental approaches. Firstly, we analyzed which parameters of intestinal peristalsis were primarily modified by the flavonoids under study. Secondly, we tried to define the site of flavonoid action with pharmacological agents known to interfere with certain transmission relays in the enteric nerve pathways of peristalsis. The refined analysis of intestinal peristalsis with regard to four parameters permits some conclusions to be made as to whether peristalsis-modifying drugs interfere primarily with enteric nerve or intestinal muscle activity [27]. Changes in PPT reflect alterations in the sensitivity of the nerve-muscle circuitry to distension, whereas changes in the maximal acceleration, maximal pressure and/or residual baseline pressure of the peristaltic waves indicate modifications of peristaltic motor performance [27].

Detailed analysis of the flavonoid-induced modifications of the peristaltic parameters enabled us to differentiate between two patterns of peristaltic motor impairment. The first pattern was exemplified by quercetin and naringenin which appear to act primarily on enteric nerve neurons to reduce distension sensitivity, because they did not alter the maximal acceleration and maximal pressure of the peristaltic waves and caused only a moderate rise of the residual baseline pressure. The second pattern was typified by apigenin and genistein which attenuated both distension sensitivity and peristaltic performance and thus are likely to interfere with smooth muscle activity, possibly in addition to an action on enteric nerve activity. In view of these findings quercetin and genistein were chosen as test substances for the pharmacological study of flavonoid-induced peristaltic motor inhibition.

The pharmacological analysis of drug effects on propulsive motility is complicated by the multiplicity of sites at which drugs can interfere with the neural and muscular effector systems of peristalsis [19]. Theoretically, flavonoids may inhibit peristaltic motility because they shift
the balance between the excitatory and inhibitory neural pathways of peristalsis towards a prevalence of inhibition. Motor inhibition could result from stimulation of inhibitory enteric pathways or blockade of excitatory enteric pathways. The former possibility was tested with the help of the K+ channel blocker apamin (0.5 μmol/l), the nitric oxide synthase inhibitor L-NAME (100 μmol/l) and the pan-opioid receptor antagonist naloxone (0.5 μmol/l). At the concentrations used here, these drugs have previously been established to effectively block inhibitory NANC transmission [18–21, 23] and opioid receptor activation [25, 35, 36] in the guinea pig small intestine.

The observation that the antiperistaltic action of quercetin was partially prevented by apamin and L-NAME (15-minute contact time), whereas that of genistein was not significantly altered by these drugs, suggests that inhibitory NANC transmission plays a role in the peristaltic motor inhibition due to quercetin. It is very unlikely that apamin and L-NAME attenuated the quercetin-induced peristaltic motor inhibition by virtue of their transient facilitation of baseline peristalsis, an effect [19] that did not attain statistical significance in the present experiments. We therefore hypothesize that quercetin impairs propulsive motility by preferentially enforcing descending inhibitory pathways of peristalsis. Apamin-sensitive mechanisms appear to be particularly important because apamin, unlike L-NAME and naloxone, was also able to partially reverse quercetin-induced blockade of peristalsis. In nanomolar concentrations, apamin is a blocker of low conductance Ca2+-activated K+ channels in smooth muscle, while at higher concentrations it also acts as an antagonist of adenosine triphosphate at P2 purinoreceptors [21, 37, 38]. Through these actions, apamin depresses NANC inhibitory neuromuscular transmission, NANC inhibitory junction potentials and NANC relaxation in gastrointestinal smooth muscle [20, 39–42].

The pan-opioid receptor antagonist naloxone ameliorated the peristaltic motor inhibition caused by both quercetin and genistein when it was added to the bath prior to the flavonoids. However, this effect was no longer observed when naloxone was added after peristalsis had been blocked by quercetin or genistein. It is very improbable that naloxone counteracted the flavonoid-induced peristaltic motor inhibition by virtue of its transient facilitation of baseline peristalsis, since this action [25] was insignificant in the current study. It would appear, therefore, that flavonoids can activate opioidergic enteric neurons and that this action contributes to their antiperistaltic activity. The role of this opioidergic mechanism, however, seems to be limited because naloxone failed to restore peristalsis once flavonoid-induced blockade of peristalsis was established.

Since the antiperistaltic action of genistein was largely spared by apamin, L-NAME and naloxone it can be deduced that this flavonoid blocked peristaltic motor activity by a mechanism other than facilitation of inhibitory enteric pathways of peristalsis. We therefore postulated that genistein suppressed propulsive motility by blockade of excitatory enteric pathways or inhibition of muscle activity and sought to obtain pharmacological evidence for this contention. The pharmacological possibilities are limited, however, because blockade of excitatory pathways with the acetylcholine receptor antagonists atropine and hexamethonium per se inhibits or abolishes peristaltic motor activity [24, 25, 35]. We reasoned, therefore, that stimulation of excitatory neural pathways by cerulein, DMPP or neostigmine should be able to restore flavonoid-inhibited peristalsis if flavonoids were to inhibit propulsive motility by interfering with cholinergic transmission processes. Cerulein, DMPP and neostigmine were chosen because they act through three different mechanisms. Cerulein is a cholecystokinin-like decapeptide which at subnanomolar concentrations facilitates intestinal peristalsis and circular muscle activity through stimulation of cholinergic neurons and release of acetylcholine [43, 44]. DMPP (<1 μmol/l) activates nicotinic acetylcholine receptors on excitatory enteric motor pathways and thereby elicits nerve-mediated muscle contractions [45], while neostigmine promotes cholinergic transmission through inhibition of acetylcholine esterase.

The failure of cerulein, DMPP and neostigmine to rescue propulsive motility from quercetin-induced blockade indicates that this flavonoid does not interfere with the excitatory enteric pathways of peristalsis. In contrast, the genistein-induced abolition of peristaltic motor activity was reversed by neostigmine, but not DMPP and cerulein. Since DMPP and cerulein act on enteric neurons only, whereas neostigmine facilitates neuropeuronal as well as neuromuscular transmission via acetylcholine, we conclude that genistein interferes with cholinergic activation of smooth muscle. Our results do not allow us to differentiate whether genistein inhibits the release or action of acetylcholine at the neuromuscular junction or interferes with downstream signaling mechanisms in the muscle cells. We favor this latter possibility, given that certain flavonoids can depress depolarization-induced contractions of smooth muscle through interference with Ca2+ flux or metabolism [32, 46] and inhibit intracellular signaling cascades involving cyclic nucleotides and protein.
kinases that act directly on the contractile apparatus in smooth muscle [26].

In summary, the current experiments have demonstrated that flavonoids depress peristaltic motility in the guinea pig small intestine through a variety of mechanisms. Quercetin and naringenin reduce distension sensitivity and peristaltic performance appears to result from inhibition of muscle activation or muscle activity. In a wider perspective, the results of this study indicate that flavonoids possess antiperistaltic and antidiarreal activity, which may explain the wide use of flavonoid-containing plants for this type of pathology.

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