Different mechanisms of actions of genistein and quercetin on spontaneous contractions of rabbit duodenum

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ABSTRACT

Flavonoids are known to relax precontracted intestinal smooth muscle and delay intestinal transit or intestinal peristalsis. The aim of this study was to determine the effects of genistein and quercetin on spontaneous contractions of rabbit duodenum in vitro in an organ bath. Genistein and quercetin (0.1-10 µM) reduced the amplitude of spontaneous contractions in the longitudinal and circular smooth muscle of rabbit duodenum, but they did not modify the frequency. Bay K8644 (L-type Ca²⁺ channel activator), apamin, charybdotoxin, and tetraethylammonium (K⁺ channel blockers) reverted the inhibition of amplitude of spontaneous contractions induced by genistein in longitudinal and circular smooth muscle. H-89 (protein kinase A inhibitor) antagonized the reduction of the amplitude of spontaneous contractions induced by quercetin in longitudinal and circular smooth muscle of duodenum, while 2,5-dideoxiadenosine (adenylyl cyclase inhibitor) reverted only the reduction of the amplitude in circular smooth muscle. In conclusion, genistein and quercetin reduce the spontaneous contractions in the duodenum by different mechanisms of actions. The effect of genistein would be mediated by Ca²⁺ and K⁺ channels, while the effect of quercetin would be mediated by cAMP and protein kinase A.

Key words: Genistein. Quercetin. Duodenum. Spontaneous contractions. Rabbit.

INTRODUCTION

Phenolic compounds, or polyphenols, are widely distributed in the plant kingdom and are an integral part of the human diet, with significant amounts found in vegetables and fruits (1). These compounds exhibit a wide range of biological effects including antibacterial, anti-inflammatory, and vasodilator actions. Many of these biological functions have been attributed to their free radical scavenging and antioxidant activity (2) and have motivated their use against gastrointestinal tract diseases such as the inflammatory bowel disease, gastric ulcers or diarrheas (3-4). Flavonoids are phenolic compounds with effects on the gastrointestinal tract such as slowing of intestinal transit, stomach and intestinal smooth muscle relaxation, and inhibition of intestinal peristalsis (5-7). However, the mechanisms of action of flavonoids are not clearly known. The objectives of this work were to evaluate the effects of the flavonoids genistein and quercetin on spontaneous contractions of rabbit duodenum and compare the mechanisms of action of these compounds.

MATERIALS AND METHODS

Chemicals

Genistein was obtained from Tocris (Madrid, España). Quercetin, Bay K8644 (a L-type Ca²⁺ channel activator), apamin (a blocker of small conductance Ca²⁺-activated K⁺ channels), charybdotoxin (a selective blocker of intermediate and large conductance Ca²⁺-activated K⁺ channels), glibenclamide (an ATP-sensitive K⁺ channels blocker), tetraethylammonium chloride (TEA, a non-specific K⁺ channels blocker), 2,5-dideoxiadenosine (DOA, an adenylyl cyclase inhibitor), and H-89 dihydrochloride hydrate (H-89, a specific protein kinase A inhibitor) were obtained from Sigma-Aldrich (Madrid, Spain).

Animals

The rabbits were humanely handled and put down in accordance with the Spanish Policy for Animal Protection RD53/2013 and the European Union Directive 2010/63/EU with the procedure PI10/11 approved by the Ethic Commission of the University of Zaragoza.

Acknowledgements: This work was funded by a grant from Gobierno de Aragón, Spain (B61) and Fondo Social Europeo.
Male New Zealand rabbits, weighing 2.0-2.5 kg, were fed with standard rabbit food and given free access to water.

**Preparation of duodenal segments and experimental protocols**

Segments of rabbit duodenum (10 mm long) were removed, washed, and freed from mesenteric attachment. Isometric recordings of duodenal motility were studied in an organ bath as previously described (8). After the adaptation period, the spontaneous contractions of the duodenum were recorded in a Krebs solution and considered as the control. Non-cumulative concentration-response curves of genistein or quercetin (0.1-10 µM) for 90 min were performed. The inhibitors were added to the bath 15 min before the addition of genistein or quercetin for 90 min. Each experimental protocol was systematically performed on four longitudinal and four circular smooth muscle segments taken from the same rabbit, and repeated in three or four animals. The effect per se of K+ channel inhibitors and Bay 8644 has been previously described (9,10).

**Analysis of data**

The amplitude (in mN) and the frequency (contractions per minute, cpm) of spontaneous contractions were calculated as previously described (8). Data are expressed as a mean percentage with respect to control ± SEM. Data sets were compared using one-way variance analysis (ANOVA) tests and p-values were determined using the Scheffé F test. Differences with p-values < 0.05 were considered statistically significant.

**RESULTS**

**Effects of genistein and quercetin**

Genistein (0.1-10 µM) or quercetin (0.1-10 µM) reduced the amplitude of spontaneous contractions in the longitudinal and circular smooth muscle of rabbit duodenum, but they did not have any effect on the frequency of spontaneous contractions (Table I).

**The mechanisms of action of genistein and quercetin**

Bay K8644 0.01 µM (a L-type Ca2+ channel activator), apamin 0.1 µM (a blocker of small conductance Ca2+-activated K+ channels), charybdotoxin 1 µM (a selective blocker of intermediate and large conductance Ca2+-activated K+ channels), and TEA 5 mM (a non-specific K+ channels blocker) reverted the inhibition of the amplitude of spontaneous contractions induced by genistein 1 µM in longitudinal and circular smooth muscle (Table II).

The quercetin induced reduction (1 µM) on the amplitude of spontaneous contractions in the longitudinal and circular muscle was antagonized by H-89 0.1 µM (a specific protein kinase A inhibitor), while DOA 1 µM (an adenylyl cyclase inhibitor) reduced only the inhibition of the amplitude in the circular smooth muscle (Table II). However, Bay K8644, apamin, charybdotoxin, glibenclamide, and TEA did not modify the effect of quercetin on the spontaneous contractions in the longitudinal and circular smooth muscle (Table II).

**DISCUSSION**

Intestinal motility is coordinated by the contraction of the longitudinal and circular smooth muscle layers (11). Ca2+ and other factors participate in the amplitude and frequency of spontaneous contractions in the small intestine (12). Signal transduction in the intestinal smooth muscle is mediated by G protein-coupled membrane receptors and the activation of several enzymes. The activation of these enzymes is different in the longitudinal and circular smooth muscle, generating the mobilization of Ca2+ and other messengers (13). In this study, genistein and quercetin reduced the amplitude of spontaneous contractions in the longitudinal and circular smooth muscle of the duodenum, but they did not modify the frequency. This result agrees with the effect of melatonin, an antioxidant, in the duodenum (14). Our results with genistein or quercetin agree with previous findings showing that flavonoids

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**Table I. Amplitude and frequency of spontaneous contractions in longitudinal and circular smooth muscles of rabbit duodenum incubated for 90 min in Krebs solution or in the presence of genistein (G, 0.1-10 µM) or quercetin (Q, 0.1-10 µM)**

<table>
<thead>
<tr>
<th></th>
<th>Longitudinal muscle</th>
<th>Circular muscle</th>
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<tbody>
<tr>
<td></td>
<td>Amplitude</td>
<td>Frequency</td>
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<tr>
<td>G 0.1 µM</td>
<td>79.0 ± 2.3 (8)***</td>
<td>94.6 ± 3.2 (12)</td>
</tr>
<tr>
<td>G 1 µM</td>
<td>73.8 ± 4.6 (15)*</td>
<td>100.1 ± 1.9 (15)</td>
</tr>
<tr>
<td>G 10 µM</td>
<td>69.8 ± 7.3 (12)**</td>
<td>96.4 ± 3.7 (12)</td>
</tr>
<tr>
<td>Q 0.1 µM</td>
<td>63.1 ± 4.2 (8)**</td>
<td>102.8 ± 12.7 (8)</td>
</tr>
<tr>
<td>Q 1 µM</td>
<td>76.9 ± 8.9 (28)*</td>
<td>105.6 ± 1.6 (21)</td>
</tr>
<tr>
<td>Q 10 µM</td>
<td>76.7 ± 8.5 (10)*</td>
<td>105.0 ± 3.5 (11)</td>
</tr>
</tbody>
</table>

The values are the mean ± SEM. Data are expressed as a percentage of the amplitude or frequency of spontaneous contractions with respect to control conditions. The number of segments taken from four rabbits is in parentheses.*p < 0.05. **p < 0.01. *** p < 0.001 vs. control.
The effect of quercetin in the vascular muscle is attributed to the Ca\(^{2+}\) sensitization or protein kinases (15). This agrees in part with the effect of quercetin in the duodenum, which is reverted with a protein kinase A inhibitor and with an adenylyl cyclase inhibitor.

In conclusion, the inhibition of genistein and quercetin on the spontaneous contractions of rabbit duodenum presents different mechanisms of action. Genistein would act on Ca\(^{2+}\) and K\(^{+}\) channels, and quercetin on cAMP and protein kinase A.

**REFERENCES**

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