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ORIGINAL PAPERS

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 Ca^{2+} channel activator), apamin, charybdotoxin, and tetraetylammonium (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 Ca^{2+} 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 Ca^{2+} channel activator), apamin (a blocker of small conductance Ca^{2+}-activated K^+ channels), charybdotoxin (a selective blocker of intermediate and large conductance Ca^{2+}-activated K^+ channels), glibenclamide (an ATP-sensitive K^+ channels blocker), tetraetylammonium 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.

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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 Ca^{2+} channel activator), apamin 0.1 μM (a blocker of small conductance Ca^{2+}-activated K^+ channels), charybdotoxin 1 μM (a selective blocker of intermediate and large conductance Ca^{2+}-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). Ca^{2+} 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 Ca^{2+} 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

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)

	Longitudinal muscle		*Circular muscle*	
	Amplitude	*Frequency*	*Amplitude*	*Frequency*
G 0.1 μM	79.0 ± 2.3 (8)***	94.6 ± 3.2 (12)	79.8 ± 9.1 (9)*	102.3 ± 6.9 (10)
G 1 μM	73.8 ± 4.6 (15)*	100.1 ± 1.9 (15)	79.8 ± 6.8 (15)*	100.6 ± 5.5 (15)
G 10 μM	69.8 ± 7.3 (12)**	96.4 ± 3.7 (12)	72.2 ± 5.3 (8)**	100.1 ± 8.2 (8)
Q 0.1 μM	63.1 ± 4.2 (8)**	102.8 ± 12.7 (8)	62.8 ± 4.3 (8)***	99.4 ± 5.0 (8)
Q 1 μM	76.9 ± 8.9 (28)*	105.6 ± 1.6 (21)	75.5 ± 9.1 (18)*	95.5 ± 4.1 (17)
Q 10 μM	76.7 ± 8.5 (10)*	105.0 ± 3.5 (11)	75.3 ± 8.3 (9)*	102.3 ± 4.6 (11)

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.

Table II. Effect of the incubation for 90 min with Krebs or genistein (G, 1 µM) or quercetin (Q, 1 µM) on amplitude and frequency of spontaneous contractions in the longitudinal and circular smooth muscles from rabbit duodenum

	Longitudinal muscle		*Circular muscle*	
	Amplitude	*Frequency*	*Amplitude*	*Frequency*
Krebs	93.0 ± 5.8 (8)	96.6 ± 3.0 (8)	95.6 ± 4.6 (8)	97.1 ± 4.6 (8)
G 1 µM	73.8 ± 4.6 (15)*	100.1 ± 1.9 (15)	79.8 ± 6.8 (15)*	100.6 ± 5.5 (15)
Bay + G 1 µM	96.8 ± 10.4 (8)	109.5 ± 2.7 (8)	93.5 ± 5.8 (8)	109.7 ± 18.6 (8)
Ap + G 1 µM	93.7 ± 17.0 (8)	104.4 ± 2.6 (8)	111.9 ± 20.2 (8)	110.2 ± 8.9 (8)
ChTX + G 1 µM	97.9 ± 10.8 (10)	129.2 ± 4.4 (10)	103.9 ± 11.5 (9)	122.7 ± 11.8 (11)
GB + G 1 µM	59.2 ± 9.0 (8)*	116.8 ± 4.1 (8)	70.3 ± 5.8 (8)*	105.1 ± 9.7 (8)
TEA + G 1 µM	87.1 ± 14.3 (12)	102.0 ± 11.6 (12)	104.2 ± 27.0 (8)	108.4 ± 13.3 (9)
H-89 + G 1 µM	75.7 ± 10.6 (8)*	117.5 ± 5.9 (8)	76.6 ± 14.2 (8)*	116.0 ± 8.3 (8)
Q 1 µM	76.9 ± 8.9 (28)*	105.6 ± 1.6 (21)	75.5 ± 9.1 (18)*	95.5 ± 4.1 (17)
Bay + Q 1 µM	72.7 ± 4.0 (8)*	120.4 ± 7.3 (8)	74.6 ± 10.7 (8)*	112.4 ± 14.6 (8)
Ap + Q 1 µM	79.8 ± 11.9 (8)*	112.1 ± 3.4 (8)	78.2 ± 12.0 (8)*	122.0 ± 7.2 (8)
ChTX + Q 1 µM	73.8 ± 7.5 (8)*	124.2 ± 6.0 (8)	66.4 ± 10.8 (8)*	107.1 ± 4.3 (8)
Gb + Q 1 µM	72.9 ± 7.2 (9)*	117.0 ± 5.1 (12)	67.9 ± 3.3 (9)*	117.1 ± 8.3 (8)
TEA + Q 1 µM	65.5 ± 15.3 (7)*	118.7 ± 4.0 (8)	76.5 ± 8.3 (8)*	108.3 ± 7.1 (8)
DOA + Q 1 µM	74.1 ± 13.7 (8)*	111.1 ± 3.5 (8)	89.3 ± 15.4 (7)	125.6 ± 11.6 (7)
H-89 + Q 1 µM	116.8 ± 10.4 (8)	116.7 ± 3.0 (8)	106.9 ± 13.1 (8)	102.4 ± 5.1 (8)

The effect of Bay K8644 (Bay, 0.01 µM), apamin (Ap, 0.1 µM), charybdotoxin (ChTX, 0.01 µM), glibenclamide (Gb, 0.1 µM), tetraetylammonium (TEA, 5 mM), 2,5 dideoxiadenosine (DOA, 1 µM), or H-89 (0.1 µM) added 15 min before genistein (1 µM) or quercetin (1 µM) on amplitude and frequency of spontaneous contractions. Results are mean percentage values with respect to spontaneous contractions in Krebs (control) ± SEM. The number of segments taken from four rabbits is in parentheses. *p < 0.05, **p < 0.01, *** p < 0.001 *vs.* Krebs

relax precontracted intestinal smooth muscles and delay the intestinal transit and peristalsis (6,7). It has also been described that flavonoids have vasorelaxing effects in isolated rat aortic rings (15,16). Genistein and quercetin produce a concentration-dependent relaxation on the gastric tone in mouse isolated stomach and inhibit intestinal peristalsis in guinea pig (5,7). This agrees in part with our results of genistein and quercetin, as they act on longitudinal and circular smooth muscle. It has been proposed that flavonoids produce relaxant effects in the smooth muscle acting on K^+ channels or the muscular excitation-contraction coupling (7). Peristaltic motor activity suppressed by quercetin is partially restored by apamin (7). However, in this study, the relaxant effect of genistein is reduced by the inhibitors of K^+ channels, apamin, charybdotoxin, and TEA, and these compounds did not reduce the quercetin inhibition on the rabbit duodenum. Genistein and quercetin have relaxant effects in the mouse stomach, but they are not dependent on neural actions potentials, NO/prostaglandin production, or activation of K^+ channels (5). These results are consistent with the effects of quercetin but not genistein in duodenum. K^+ and Ca^{2+} channels participate in the effect of the antioxidant Trolox on duodenal contractility (10). These results agree with the reversion of the genistein effects in the rabbit duodenum. The inhibitory 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

1. Soobrattee MA, Neergheen VS, Luximon-Ramma A, et al. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat Res 2005;579(1-2):200-13. DOI: 10.1016/j.mrfmmm.2005.03.023
2. Middleton E Jr., 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.
3. Somani SJ, Modi KP, Majumdar AS, et al. Phytochemicals and their potential usefulness in inflammatory bowel disease. Phytother Res 2015;29:339-50. doi: 10.1002/ptr.5271. DOI: 10.1002/ptr.5271
4. Alvarez-Suarez JM, Dekanski D, RistićS, et al. Strawberry polyphenols attenuate ethanol-induced gastric lesions in rats by activation of antioxidant enzymes and attenuation of MDA increase. PLoS One 2011;6:e25878. DOI: 10.1371/journal.pone.0025878
5. Amira S, Rotondo A, Mule F. Relaxant effects of flavonoids on the mouse isolated stomach: Structure-activity relationships. Eur J Pharmacol 2008;599:126-30. DOI: 10.1016/j.ejphar.2008.09.021

6. Formica JV, Regelson W. Review of the biology of Quercetin and related bioflavonoids. Food Chem Toxicol 1995;33:1061-80. DOI: 10.1016/0278-6915(95)00077-1
7. Gharzouli K, Holzer P. Inhibition of guinea pig intestinal peristalsis by the flavonoids quercetin, naringenin, apigenin and genistein. Pharmacology 2004;70:5-14. DOI: 10.1159/000074237
8. Fagundes DS, Gonzalo S, Grasa L, et al. Trolox reduces the effect of ethanol on acetylcholine-induced contractions and oxidative stress in the isolated rabbit duodenum. Rev Esp Enferm Dig 2011;103:396-401. DOI: 10.4321/S1130-01082011000800002
9. Lamarca V, Grasa L, Fagundes DS, et al. K+ channels involved in contractility of rabbit small intestine. J Physiol Biochem 2006;62:227-36. DOI: 10.1007/BF03165751
10. Fagundes DS, Grasa L, Gonzalo S, et al. Mechanism of action of Trolox on duodenal contractility. J Physiol Pharmacol 2013;64:705-10.
11. Sanders KM, Koh SD, Ro S, et al. Regulation of gastrointestinal motility -insights from smooth muscle biology. Nat Rev Gastroenterol Hepatol 2012;9:633-45. DOI: 10.1038/nrgastro.2012.168
12. Grasa L, Rebollar E, Arruebo MP, et al. The role of Ca2+ in the contractility of rabbit small intestine in vitro. J Physiol Pharmacol 2004;55:639-50.
13. Makhlouf GM, Murthy KS. Signal transduction in gastrointestinal smooth muscle. Cell Signal 1997;9:269-76. DOI: 10.1016/S0898-6568(96)00180-5
14. Fagundes DS, Gonzalo S, Arruebo MP, et al. Melatonin and Trolox ameliorate duodenal LPS-induced disturbances and oxidative stress. Dig Liver Dis 2010;40:40-4. DOI: 10.1016/j.dld.2009.04.014
15. Duarte J, Perez-Vizcaino F, Zarzuelo A, et al. Inhibitory effects of quercetin and staurosporine on phasic contractions in rat vascular smooth muscle. Eur J Pharmacol 1994;262:149-56. DOI: 10.1016/0014-2999(94)90038-8
16. Calderone V, Chericoni S, Martinelli C, et al. Vasorelaxing effects of flavonoids: Investigation on the possible involvement of potassium channels. Naunyn Schmiedebergs Arch Pharmacol 2004;370:290-8. DOI: 10.1007/s00210-004-0964-z