


Quercetin relaxes human gastric smooth muscles directly through ATP-sensitive potassium channels and not depending on the nitric oxide pathway

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Abstract

Background: Quercetin has recently become a remarkably popular subject of research due to its broad beneficial pharmacological properties. The goal of our study was to observe its effects on contractility of human gastric smooth muscles in reference to the NO pathway and direct influence of potassium channels.

Methods: Tissues were obtained from patients undergoing sleeve gastrectomy due to morbid obesity (n = 10 aged 24–56; BMI 47.16 ± 1.84). The following parameters were evaluated in the recordings: area under the curve (AUC), average baseline muscle tone, and relative change in muscle contraction.

Key Results: Quercetin induced noticeable, dose-dependent relaxation of the carbachol treated gastric strips. The substantial effect was noted at concentrations higher than 10⁻⁷ mol/L and maximal at 10⁻⁴ mol/L (81.82 ± 3.32%; n = 10; p < 0.0001) of the control. Neither NOS blockers nor guanylyl cyclase blockers had inhibitory effects on the relaxation of strips induced by examined polyphenol. Glibenclamide inhibited the relaxing effect of quercetin, significant at concentrations higher than 5·10⁻⁵ mol/L. Preincubation with charybdotoxin or apamin extended the relaxing effect of quercetin (from 10⁻⁶ mol/L). Tamoxifen, in turn, significantly increased muscle relaxation at all quercetin concentrations.

Conclusions & Inferences: In conclusion, the current study was the first to show that quercetin-induced relaxation of human gastric smooth muscle occurs directly through K⁺_{ATP} channels and independently to NO pathways. The present results suggest that quercetin is a potential nutraceutical in the treatment of functional gastrointestinal dyspepsia and other minor gastric muscle motility disturbance.

KEYWORDS

gastric motility, gastric smooth muscles, nitric oxide, potassium channels, quercetin

Abbreviations: AUC, area under the curve; BK_{Ca}, high conductance Ca²⁺-dependent potassium channels; cAMP, 3'-5'-cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CTX, charybdotoxin; DMSO, dimethyl sulfoxide; GI, gastrointestinal; K⁺_{ATP}, ATP-sensitive potassium channels; L-NAME, N^G-methyl-L-arginine; L-NNA, N^G-nitro-L-arginine; MAPK, mitogen-activated protein kinase; NANC, non-adrenergic non-cholinergic; NO, nitric oxide; NOS, nitric oxide synthase; ODQ, 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one; sGC, soluble guanylyl cyclase; SK_{Ca}, small conductance apamin-sensitive Ca²⁺-dependent potassium channels.

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1 | INTRODUCTION

Phenolic substances including flavonoids, for example, quercetin, have gained significant attention over the years for their potential health benefits. Quercetin is a naturally occurring, plant-derived polyphenol. It can be found in the form of a glycoside in various vegetables, fruits, nuts, seeds, red wine, and tea.¹ Numerous biological effects of this substance have been published in a great number of studies based on animals and human experiments. Among others, quercetin is presumed to have antioxidant, anti-inflammatory, anticarcinogenic, antidiabetic, and immunoprotective effects.²⁻⁶ Moreover, promising experimental studies induced oral supplementation of quercetin in many clinical trials.³

Supplementary intake of quercetin was found to lower the blood pressure of patients suffering from hypertension, smokers, or type 2 diabetes mellitus patients.²⁻⁴ Furthermore, quercetin has been reported to have beneficial effects on neurodegenerative diseases, for example, Alzheimer's or Parkinson's disease.^{1,5} In addition, it also inhibits fat accumulation in mature human fat cells and triggers apoptosis in existing fat cells.⁶ Moreover, quercetin has shown to have antiulcer effect due to decreased gastric acid secretion and increased gastric mucus production.⁷

Quercetin is known for its very broad spectrum of biological activities; however, little attention has been attributed to quercetin-induced relaxation of smooth muscle of the human gastrointestinal (GI) tract.⁸⁻¹⁰ Even though flavonoids are largely consumed in the diet, the plasma concentrations of flavonoids are lower than their effective therapeutic doses due to poor bioavailability. Human kinetic studies recovered total concentrations of quercetin (including free and sulfated/glucuronidated metabolites) reached plasma levels ~15–450 µg/L. Therefore, high concentrations of these nutraceuticals may exist especially within the digestive tract and express local rather than systemic effects after oral administration.^{11,12}

GI motility is defined by the contraction of the muscles of the digestive system, and the transit of the contents within it. It is regulated by a complex interactions of nervous and endocrine systems.¹³ Symptoms related to motility problems may have various causes, but the main is gastric motility dysfunction. What is more, sex hormones, estrogens in particular may contribute to GI disorder and irritable bowel syndrome.⁸⁻¹⁰ Quercetin is known to exert strong estrogenic¹⁴ and antiestrogenic¹⁵ effects; hence, this estrogen receptor regulator has been classified as a phytoestrogen.¹⁶ Moreover, quercetin has been reported to inhibit GI tract contractility in mice and guinea pigs.^{8,10} Despite the increasing attention to the effect of nutraceuticals on smooth muscles, little is known about the mechanisms of these effects. Although the flavonol quercetin is among the most frequently investigated flavonoids, with both beneficial and harmful effects on diverse cell types being thoroughly described, its mechanism of action remains unclear.^{17,18}

Neurons that regulate the motor function of the GI tract are located in myenteric plexus between the inner circular and outer longitudinal layers. They undergo relaxant responses to non-adrenergic

non-cholinergic (NANC)¹³ and purinergic co-neurotransmission.¹⁹ Inhibitory neuromuscular transmission in the GI tract therefore involves at least two inhibitory co-transmitters: a purine and nitric oxide (NO). NO is synthesized from L-arginine by neuronal isoform of NO synthase (nNOS). The classic target for NO is soluble guanylyl cyclase (sGC), which produces a second messenger cyclic guanosine monophosphate (cGMP).²⁰ Increased level of cGMP leads to activation of potassium channels: high conductance Ca²⁺-dependent (BK_{Ca}) and small conductance (SK_{Ca}) apamin-sensitive or ATP-sensitive K⁺ (K_{ATP}).^{10,19,21,22} Furthermore, NO can also directly activate cGMP-independent K⁺-channels and inhibit L-type Ca²⁺-channels that cause smooth muscle relaxation.^{23,24} *In vitro* studies show that quercetin may exert multiple actions on the NO-guanylyl cyclase pathway and causes both NO-dependent and NO-independent vasodilatation.²⁵

Our study aimed to observe the effects of quercetin on the contractility of human gastric smooth muscles in reference to the NO pathway and direct influence of potassium channels. Additionally, the results may support the potential use of quercetin for the treatment of functional dyspepsia and other minor gastric muscle motility disturbances. A better understanding of the physicochemical properties of this bioactive nutraceutical would be beneficial for the development of an optimized system for oral administration.

2 | MATERIALS AND METHODS

2.1 | Sample processing

The study was conducted in accordance with the Helsinki Declaration principles, the International Conference on Harmonisation Guideline for Good Clinical Practice, and the laws and regulations of Poland, and with the approval from the Ethical Committee of Medical University of Białystok (No.R-I-002/304/2018). Tissues were obtained from patients undergoing sleeve gastrectomy due to morbid obesity (n = 10: women = 2, man = 8; aged 24–56; BMI 47.16 ± 1.84). The surgical procedure involved a longitudinal resection of the stomach on the greater curvature from the antrum up to the angle of His.²⁶ The experiment did not affect the course of the operation. Written informed consent was achieved from the patients before processing and analyzing the specimens.

Tissue samples were removed immediately after surgical resection of the stomach and cooled on ice cold Tyrode's buffer, bubbled with carbogen (95% O₂ +5% CO₂), and transferred directly to the laboratory wherein they were processed as previously described.²⁷ After removal of mucosa and submucosa, muscle strips (10 × 3 × 1.5 mm) were cut in the direction of the longitudinal muscle layer and mounted to organ bath (20 ml) of isometric force transducer system. The temperature of bath solution was thermostatically maintained at 37°C and continuously bubbled with carbogen. Each strip was stretched passively to resting tension after 1–1.5 hours of equilibration. The bath solution was replaced every 20 min of incubation. Contractile responses of the strip to the high K⁺ (80 mmol/L, 10 min) was then repeated two or three times until the responses were reproducible.

2.2 | Experimental protocol

After the equilibration period, direct smooth muscle contractions were obtained by addition of carbachol (10^{-6} mol/L) to the organ bath, and then, the muscle strips contractions were subsequently checked. Only the strips exhibiting stable response to the administered agonist were used in further experiments. The contractile activity of strips incubated only with carbachol was considered as control after reaching the plateau. To examine concentration-response relationships, quercetin was added cumulatively to the organ chambers (range 10^{-7} – 10^{-4} mol/L) at 10-min intervals, and the isolated human muscle strips relaxations were measured. To eliminate the effect of quercetin solvent, a DMSO group was established. To verify the role of NOS in antispasmodic effect of quercetin, 10^{-5} mol/L of N^G-nitro-L-arginine (L-NNA) and N^G-Methyl-L-arginine (N-Nitroarginine methyl ester, L-NAME), NO synthase (NOS) blockers, and 10^{-6} mol/L cystamine,^{28,29} and 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (soluble guanylyl cyclase blockers, sGCs) were used. Concentration-response curves to quercetin were also constructed in the absence and presence of: 10^{-7} mol/L charybdotoxin (CTX), an inhibitor of BK_{Ca} and intermediate (IK_{Ca}) conductance calcium-activated potassium channels; 10^{-6} mol/L SK_{Ca} blocker—apamin or 10^{-6} mol/L glibenclamide, an K⁺_{ATP} channel blocker, and 10^{-6} mol/L tamoxifen—a selective estrogen response modifier, protein kinase C inhibitor. As far as possible, experiments were performed with strips from the same stomach sample and were studied in parallel. Appropriate controls were run under similar experimental conditions obtained from the same patient. In addition, vehicle-matched control experiments were conducted under similar experimental conditions with strips obtained from the patient.

2.3 | Measurement of contraction parameters

Gastric muscle activity was recorded by an isometric force transducer with digital output (BIO-SYS-TECH, Białystok, Poland) and calculated with the DASyLab software unit (version 9.0; Laboratory Data Acquisition System, SuperLogics, Waltham, MA, USA). The following parameters were evaluated in the recordings: area under the curve (AUC), average baseline muscle tone, and relative change in muscle contraction. Values from three to four strips from each sample were averaged at each time point for each drug dose. The AUC reflected the total change over time representing the contractile activity of stomach muscle responses before and after the administration of the given drug. The AUC was measured as the area under all recorded contractions over a 10-min interval before the addition of each agonist or antagonist, respectively.^{30,31} The AUC of contractions of each muscle strip over a 10-min interval before the addition of quercetin was treated as control (100%).

All results are expressed as mean \pm SEM of experiments performed on muscle strips from different patients or as percentage change [(effect size - baseline contractile response)/baseline contractile response \times 100]. When the same protocol was run on two strips from the same gastric specimen, the data were averaged. Mean

concentration-response curves to the used drugs were analyzed by fitting to a four parameter logistic equation using nonlinear regression. The AUC was evaluated by calculating the integral of the appropriate section of the curve. Concentration-response curves were fitted to the logistic equation using nonlinear regression $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogIC}_{50} - X) \cdot \text{Hillslope}})$ (PRISM 6.0, GraphPad Software Inc., San Diego, CA, USA). The maximal response (E_{max}) was expressed as a percentage of the contractile activity before administration of tested substances, whereas the concentrations of agents that resulted in a half-maximal effect were expressed as $-\log \text{IC}_{50}$.

2.4 | Chemicals

Quercetin (2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one) was purchased from abcr GmbH (Karlsruhe, Germany), while Carbamylcholine chloride ((2-Hydroxyethyl)trimethylammonium chloride carbamate; carbachol), N5-(Nitroamidino)-L-2,5-diaminopentanoic acid, N^G-NO₂-L-Arg (N^ω-Nitro-L-arginine, L-NNA), N^G-Methyl-L-Arg (N^ω-Nitro-L-arginine methyl ester hydrochloride, L-NAME), cystamine, pinacidil, 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), apamin, charybdotoxin (CTX), glibenclamide, and tamoxifen were purchased from the Sigma Chemical Company, were purchased from Sigma (St. Louis, MO).

Quercetin, ODQ, and glibenclamide were dissolved in dimethyl sulfoxide (DMSO) so that the final concentration of DMSO was never $>0.1\%$, which had no effect on basal contraction. Dilution series of quercetin were prepared on the day of experiment and were sustained at room temperature during the period of the experiment.

Stock solutions of carbachol, L-NNA, L-NAME, cystamine, pinacidil, apamin, CTX, and tamoxifen were prepared using bidistilled water. All substances were added directly to the organ bath containing a Tyrode's solution composed of (mmol/L): NaCl 139.6; KCl 2.68; MgCl₂ 1.05; NaH₂PO₄ 1.33; CaCl₂ 1.80; NaHCO₃ 25.0; and glucose 5.55.

2.5 | Statistical analysis

All series of data were checked for consistency with a Gaussian distribution in accordance with the D'Agostino-Pearson normality test. Dose-response was determined using one-way ANOVA or the Kruskal-Wallis test, where appropriate. Statistically significant differences between means were determined by Tukey's post hoc or a nonparametric Mann-Whitney *U* test, where appropriate. Values were considered to be statistically significant at $p < 0.05$. All analyses were performed using Prism 6 for Windows.

3 | RESULTS

In muscle strips isolated from human gastric specimens, 10^{-6} mol/L carbachol induced a marked and long-duration muscle contraction

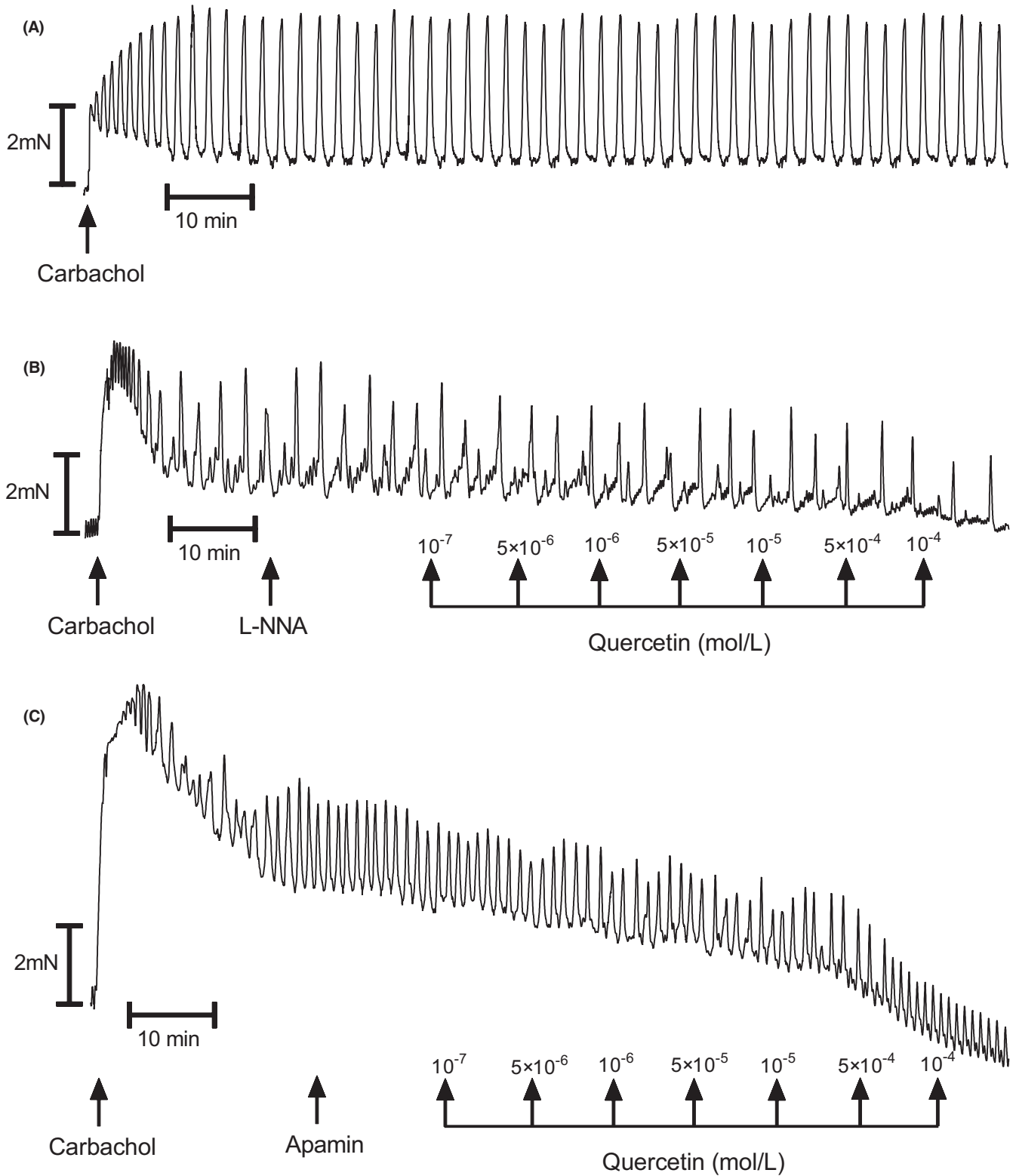


FIGURE 1 A typical recording of carbachol-induced contractile activity of the gastric strips and the effect of cumulatively administered quercetin range 10^{-7} – 10^{-4} mol/L. (A)—control; (B)—quercetin after preincubation with L-NNA (10^{-5} mol/L); (C)—quercetin after preincubation with apamin (10^{-6} mol/L)

(Figure 1A). Cumulatively applied quercetin induced a significant muscle relaxation of the carbachol treated gastric strips which was dose-dependent (Figures 2A and 4 and 5A and C). Typical tracings show the response to quercetin or pinacidil in body of stomach smooth muscle strips (Figures 2A). Quercetin caused substantial

relaxation at concentrations higher than 10^{-7} mol/L and maximal at 10^{-4} mol/L ($81.82 \pm 3.32\%$; $n = 10$; $p < 0.0001$) (Figures 4A-C and 5A). This polyphenol also dose-dependently decreased the resting tension in all concentrations, and the reduction was significant in all utilized concentrations of quercetin (Figures 3 and 4D-F and 5C).

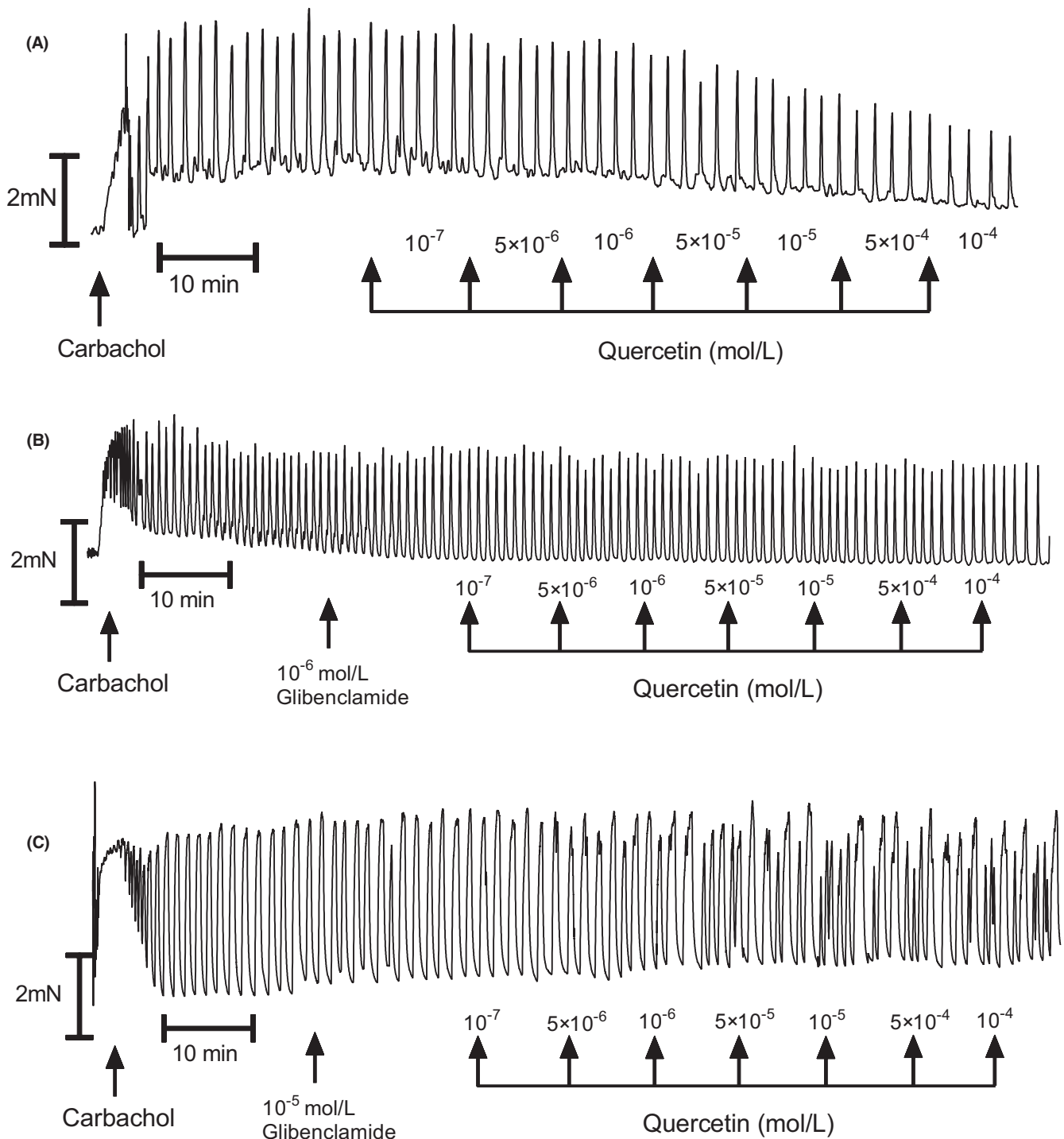


FIGURE 2 An original tracing of the effect of cumulatively administered quercetin range 10^{-7} – 10^{-4} mol/L on carbachol-induced contractile activity of the gastric strips (A)—quercetin alone; after preincubation with glibenclamide (B)— 10^{-6} mol/L, (C)— 10^{-5} mol/L

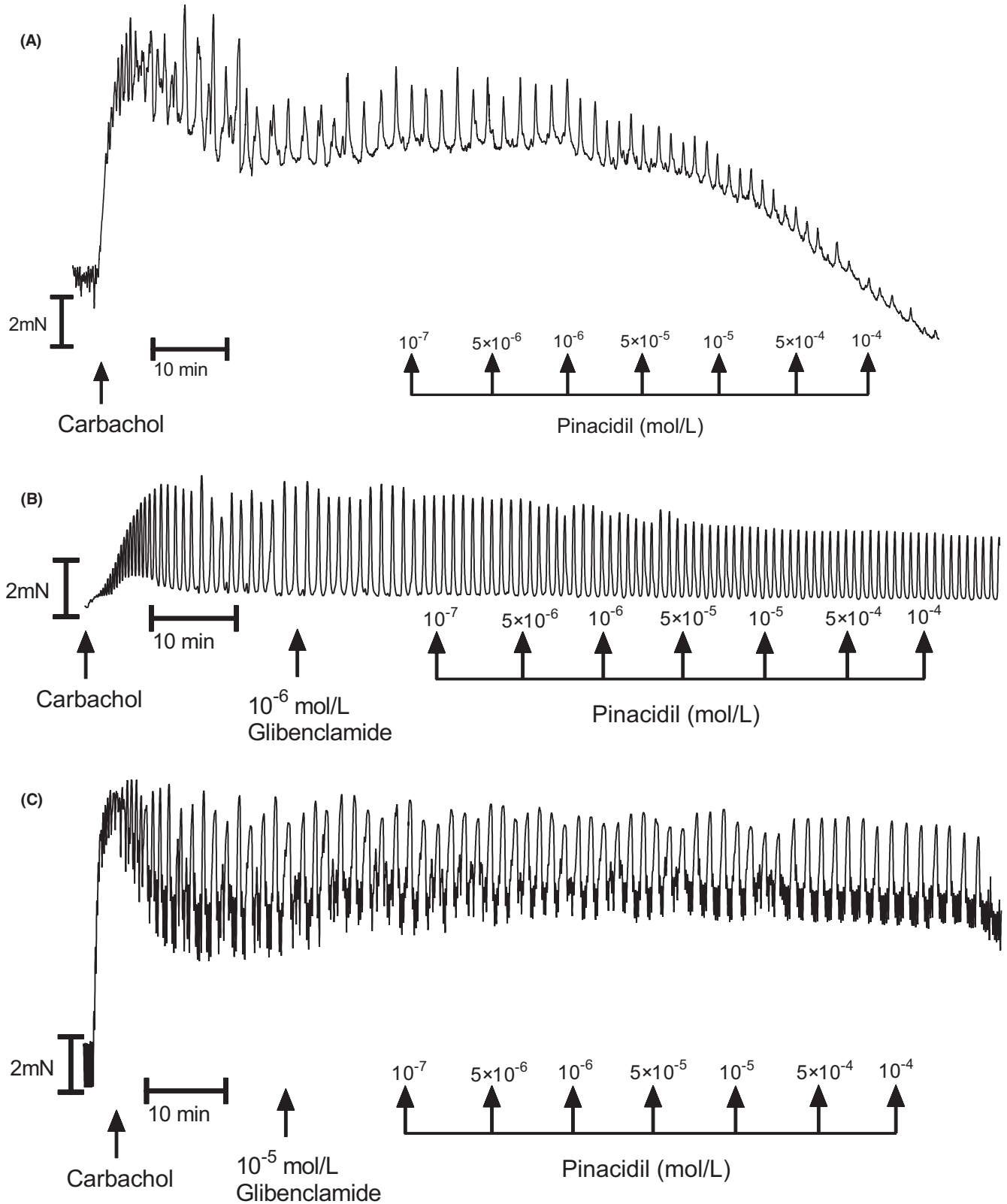


FIGURE 3 An original tracing of the effect of cumulatively administered pinacidil range 10⁻⁷–10⁻⁴ mol/L on carbachol-induced contractile activity of the gastric strips (A)—pinacidil alone; after preincubation with glibenclamide (B)—10⁻⁶ mol/L, (C)—10⁻⁵ mol/L

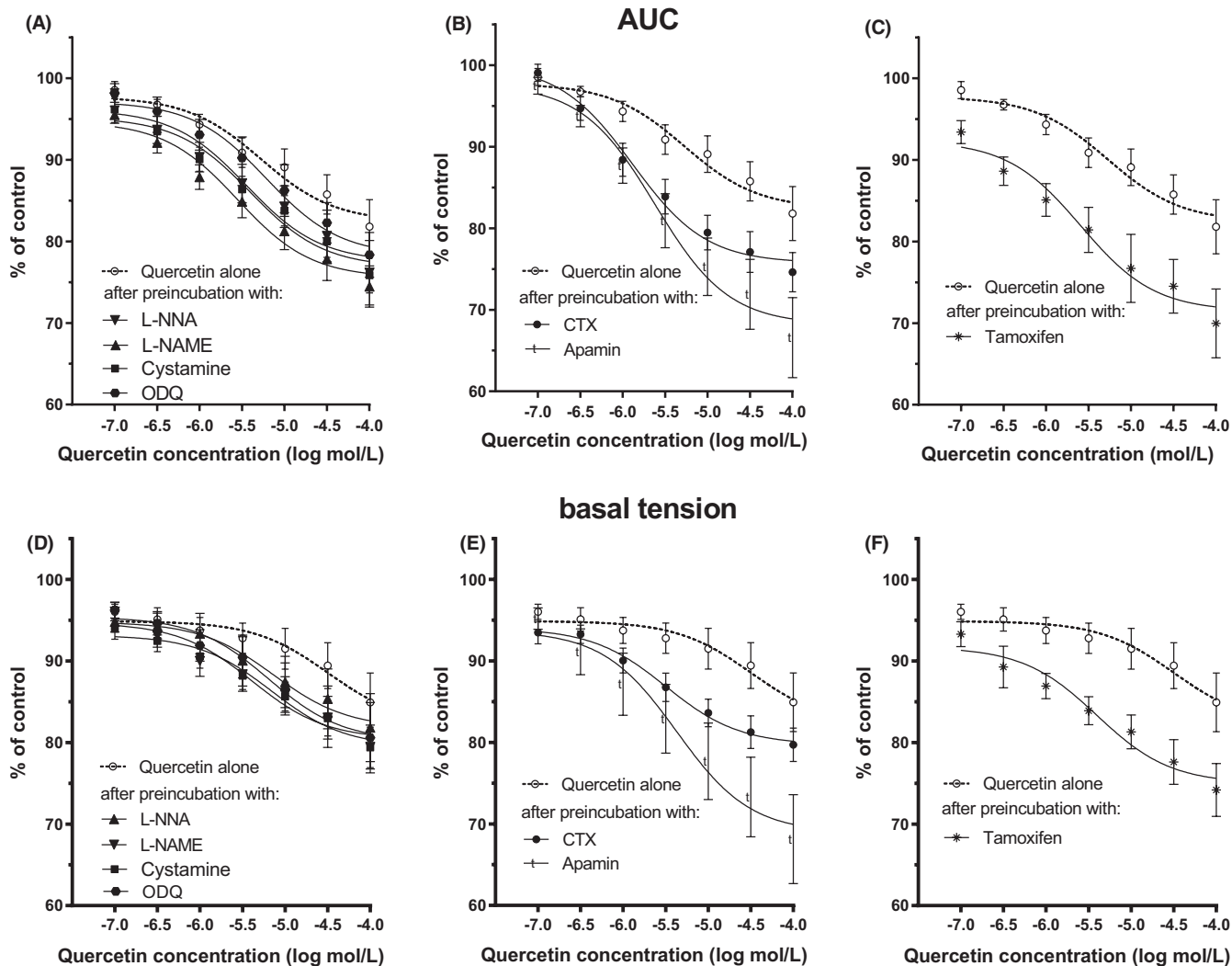


FIGURE 4 Effects of quercetin after preincubation with (A, D)—L-NNA, L-NAME, ODQ and cystamine; (B, E)—CTX or apamin; (C, F) tamoxifen on the gastric strips, as measured by AUC (A, B or D) and basal tension (D, E or F). Each point represents mean \pm SEM of values obtained from individual gastric strips ($n = 10$) from different patients. Contractions of the gastric strips before quercetin were treated as controls

3.1 | The influence of blockers on carbachol-induced contractility

Preincubation with a NOS inhibitor—L-NNA, a sGC inhibitor—ODQ, a SK_{Ca} channel blocker—apamin, a BK_{Ca} channel blocker—CTX, and ATP-sensitive K^+ blocker—glibenclamide (10^{-6} mol/L) did not alter carbachol-induced muscle contractions. In turn, the other NOS inhibitor—L-NAME (95.23 ± 0.62 ; $p < 0.0001$) and an estrogen receptor blocker—tamoxifen (89.77 ± 0.96 ; $p < 0.0001$) significantly inhibited contractility induced by carbachol. A sGC inhibitor—cystamine significantly reduced AUC (96.55 ± 0.27 ; $p < 0.0001$) but did not influence basal tension of muscle strips. Preincubation with 10^{-5} mol/L glibenclamide substantially extended basal tension (103.3 ± 1.14 ; $p = 0.0184$) without affecting AUC (Figure 6).

3.2 | The influence of blockers on quercetin-induced relaxation

Blockers of the nitric oxide pathway (L-NNA, L-NAME, ODQ, or cystamine) had no inhibitory effects on the relaxation of muscle strips induced by quercetin. Moreover, no obvious effect of preincubation with L-NNA, L-NAME, ODQ, or cystamine on the decrease of basal tension was observed Figure 4A and D and Table 1.

As shown in Figure 5A and C and Table 1, glibenclamide inhibited the relaxing effect of the tested polyphenol on human gastric muscle strips. Statistically significant effect was observed at concentrations of quercetin higher than $5 \cdot 10^{-5}$ mol/L for 10^{-6} mol/L glibenclamide and higher than $5 \cdot 10^{-6}$ mol/L for 10^{-5} mol/L glibenclamide.

Preincubation with CTX deepened the relaxing effect of quercetin observed as a decrease of AUC and basal tension

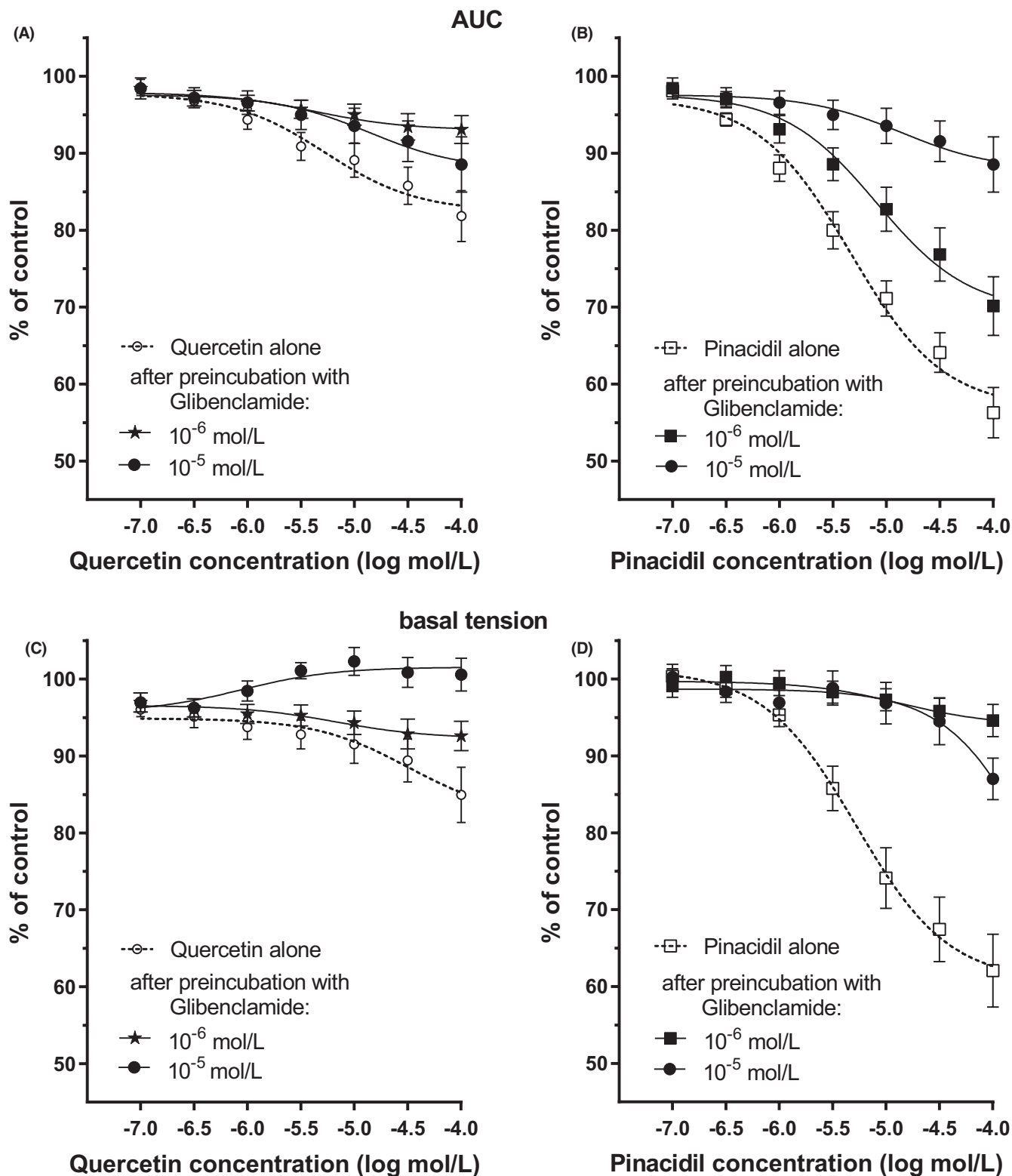


FIGURE 5 Effects of quercetin after preincubation with glibenclamide (A, C)– 10^{-6} mol/L, (B, D)– 10^{-5} mol/L on the gastric strips, as measured by AUC (A or B) and basal tension (C or D). Each point represents mean \pm SEM of values obtained from individual gastric strips ($n = 10$) from different patients. Contractions of the gastric strips before quercetin were treated as controls

substantial at quercetin concentrations, 10^{-6} mol/L and $5 \cdot 10^{-4}$ mol/L, respectively (Figure 4B and E and Table 1). Apamin also extended the effects of cumulatively applied quercetin

statistically significant at concentrations higher than 10^{-6} mol/L (AUC) and $5 \cdot 10^{-5}$ – 10^{-4} mol/L (basal tension) (Figure 4B and E and Table 1).

Preincubation with tamoxifen substantially increased the relaxation of muscle strips observed as a decrease of AUC and their basal tension at all quercetin concentrations (Figure 4C and F and Table 1).

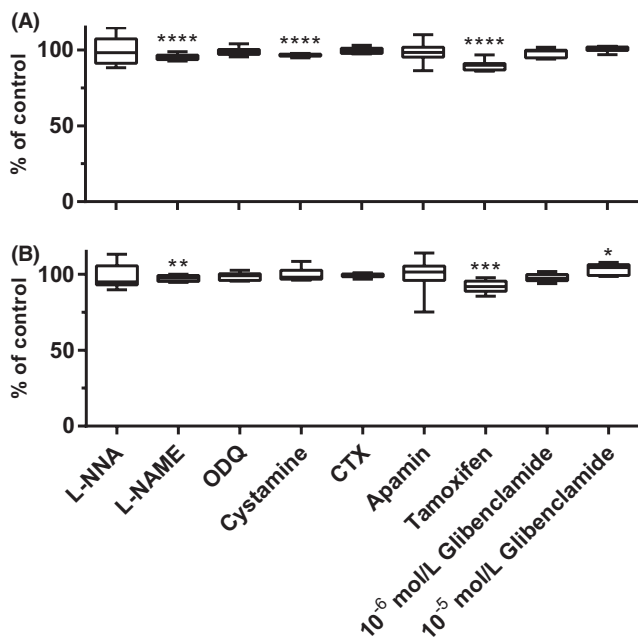


FIGURE 6 Effects of NOS inhibitors—L-NNA, L-NAME; a sGC inhibitor—ODQ or cystamine; a BK_{Ca} channel blocker—CTX; a SK_{Ca} channel blocker—apamin ADRB or an estrogen receptor blocker—tamoxifen; an ATP-sensitive K⁺ blocker—glibenclamide 10⁻⁶ mol/L and 10⁻⁵ mol/L as measured by AUC (A) and basal tension (B) on the carbachol-induced contractions in strips of gastric muscles. Carbachol-induced contractile activity was treated as control values. The values are mean ± SEM of n (n = 10) individual myometrial strips from different patients. Asterisk indicates values significantly different from a control. *p < 0.05; *** p < 0.001

TABLE 1 Log EC₅₀ and E_{max} for quercetin and pinacidil on carbachol-induced contractility of the human ventricle muscles. The values are mean ± SEM of n = 10 individual ventricle strips from different patients. *p < 0.05; ** p < 0.01; *** p < 0.001; versus quercetin alone; ^{xy} p < 0.01; ^{xyxy} p < 0.0001 versus pinacidil alone

	logEC ₅₀	p	E _{max} (%)	p
Quercetin alone	-5.27 ± 0.27		81.82 ± 3.32	
<i>Quercetin after preincubation with</i>				
L-NNA (10 ⁻⁵ mol/L)	-5.44 ± 0.28	0.1830	76.16 ± 3.95	0.3150
L-NAME (10 ⁻⁵ mol/L)	-5.55 ± 0.21	0.0199	74.46 ± 2.54	0.1051
ODQ (10 ⁻⁶ mol/L)	-5.21 ± 0.26	0.5977	78.37 ± 2.74	0.1763
Cystamine (10 ⁻⁶ mol/L)	-5.40 ± 0.20*	0.2466	75.85 ± 2.17	0.0892
Apamin (10 ⁻⁶ mol/L)	-5.62 ± 0.22**	0.0063	66.59 ± 4.91*	0.0288
CTX (10 ⁻⁷ mol/L)	-5.87 ± 0.18*	0.0131	74.61 ± 2.41*	0.0446
Glibenclamide (10 ⁻⁶ mol/L)	-5.38 ± 0.56	0.5985	93.06 ± 1.81**	0.0026
Glibenclamide (10 ⁻⁵ mol/L)	-4.86 ± 0.54*	0.0441	88.53 ± 3.60	0.1357
Tamoxifen (10 ⁻⁶ mol/L)	-5.59 ± 0.29*	0.0225	69.96 ± 4.22*	0.0433
Pinacidil alone	-5.36 ± 0.13	0.3798	56.31 ± 3.30***	0.0001
<i>Pinacidil after preincubation with</i>				
Glibenclamide (10 ⁻⁶ mol/L)	-5.09 ± 0.19 ^{xy}	0.0016	70.15 ± 3.83 ^{xy}	0.0092
Glibenclamide (10 ⁻⁵ mol/L)	-4.86 ± 0.53 ^{xy}	0.0096	88.53 ± 3.60 ^{xyxy}	<0.0001

4 | DISCUSSION

The relaxant property of polyphenols has been reported in a variety of organs including arteries,³² uterus,³³ bladder,³⁴ and GI tract.^{35,36} The studies were in most cases carried out on the tissues of various animal species: rats, guinea pigs, rabbits, or mice. In regard to potential clinical aspects, some of the previously conducted interventional studies include the oral administration of quercetin in combination with one or more other possibly biologically active substances.¹¹ It is worth noting that due to low oral bioavailability, quercetin in differently selected oral doses from 25 mg to even 5 g operates almost exclusively in the GI tract.^{12,37,38} Therefore, we have investigated the effect of this nutraceutical on the contractility of isolated human gastric smooth muscles.

We found that quercetin concentration dependently reduced the carbachol-induced contractions and the resting tension in all concentrations used in the isolated human gastric smooth muscle. This observation is consistent with those reported for the effects of flavonoids on GI smooth muscles of rats and guinea pigs.^{9,10,36,39,40}

Modulation of NO metabolism is one of the possible mechanisms that may contribute to the explanation of polyphenol activity observed *in vivo*. In the stomach, polyphenols in the diet may not only promote the reduction of nitrites toward NO, but also deal with a complex network of chemical reactions to produce NO with its signaling functions. Therefore, in this sense, the local and systemic effects of NO can be caused in this sense by dietary flavonoids.⁴¹ NO is considered to be the most important neurotransmitter in the GI tract. The relaxation of smooth muscles is regulated by many signaling pathways, including NO, cAMP, and cGMP. In the classic concept of nitrenergic or purinergic co-neurotransmission, NO produced from L-arginine by neuronal NO synthase (nNOS, NOS-1) in NANC neurons penetrates to the GI smooth muscle cells where its major target is sGC generating cGMP.^{19,20} Previous studies have shown that

quercetin-induced vasorelaxant activity was mainly associated with NO-related and NO-unrelated mechanisms and potassium channels.^{8,25,32} The role of NO is also not clearly defined in the literature on the influence of polyphenols on the GI tract. The inhibitory effect of flavonoids was also not significantly reduced in the presence of the NOS inhibitor L-NAME on the mouse stomach smooth muscles, suggesting that it is not related to NO production.³⁹ In this study, we showed that quercetin-induced relaxation was not blocked by inhibition of NO with the NOS inhibitor L-NNA and L-NAME or the sGC inhibitor cystamine. Our data indicate that quercetin induces human gastric muscle fiber relaxation independently of NO pathway. This result suggests that a nitrenergic or purinergic pathway may not be activated during the carbachol-induced contraction. The difference of quercetin-induced relaxation mechanisms between the rat, guinea pig, and human smooth muscle might be due to possible organ specificity. The ion channels may vary structurally according to the organ or may show different expression depending on the location of the smooth muscle, and more studies are needed to clarify the difference between them.

Potassium channels play an important role in regulating the excitability of the syncytium. Smooth muscle contraction depends on a balance of K^+ . The activation of membrane K^+ channels leads to hyperpolarization of the plasma membrane and further inhibits Ca^{2+} influx into the cell, resulting in relaxation of smooth muscle.⁴² Inhibition of peristalsis through facilitating inhibitory enteric pathways by some flavonoids has been declared in guinea pig small intestine or rat colon.^{8,35} To explore whether quercetin acts directly on the BK_{Ca} or SK_{Ca} channels, the effects of CTX or apamin on quercetin-induced relaxation were examined in this study. The usage of these blockers not only did not cause inhibition of induced by quercetin muscle relaxation; on the contrary, it deepened this effect. These results suggest that the majority of the response is independent of BK_{Ca} or SK_{Ca} channels or, alternatively, the involved BK_{Ca} or SK_{Ca} channels are apamin- or CTX-insensitive.¹⁹ Nevertheless, relaxation of the examined strips does not occur directly through the effects of quercetin on BK_{Ca} or SK_{Ca} channels. On the other hand, in future research, the nitrenergic component should be isolated from the purinergic component by more appropriate pharmacological tools that allow to distinguish between neurotransmission.^{19,43}

However, in the presence of glibenclamide, the relaxant effect of quercetin was significantly attenuated, confirming that ATP-sensitive K^+ channels are positively modulated by quercetin to block L-type Ca^{2+} -channels indirectly. Moreover, the lack of effect on gastric tone by glibenclamide alone might indicate a low activity of K^+ channels under the carbachol-induced condition. A number of studies have confirmed the presence of K^+ channels in GI smooth muscle.⁴⁴ Some research suggested that the inhibitory NANC neuromuscular transmission in GI smooth muscle does not involve the opening of K^+ channels on the mouse gastric fundus or rat ileum.⁴⁴ In turn, Yang *et al.* showed that the K^+ channel opener, pinacidil, induced a dose-dependent gastric relaxation and glibenclamide completely and the NOS inhibitor L-NAME partially blocked the pinacidil induced relaxation effect.³⁵ The difference may be

attributed to species or regional differences in the activity and the regulation of K^+ channels. Notably, this is the first study showing that quercetin can relax human gastric muscles directly via K^+ channels. The present, *in vitro* study demonstrated that quercetin may have the ability to act as an opener of K^+ channels and suggests the therapeutic possibility of this nutraceutical for GI motility disorders *in vivo*. Furthermore, its use as a potential K^+ channel opener could explain important benefit as a myocardial protector, an antihypertensive vasodilator, a bronchodilator, a bladder relaxant, and an antiepileptic factor. The therapeutic implications of quercetin should also be considered in the treatment of hyperinsulinemia and type 2 and type 1 diabetes.^{45,46}

Since, the chemical structures of quercetin and other bioflavonoids and the synthetic estrogen diethylstilbestrol are similar, we used tamoxifen, as an estrogen receptor antagonist, to explore whether an estrogen receptor is involved in quercetin-induced relaxation of human gastric smooth muscles. Tamoxifen was not able to inhibit the investigated quercetin-induced relaxation, suggesting that the estrogen receptor may not play a significant role in regulating smooth muscles in the human stomach. Furthermore, Al-Shboul *et al.* demonstrated that, in rats, estrogen relaxes gastric muscle cells via a NO- and cGMP-dependent mechanism.⁴⁷ In turn, our results indicate that quercetin causes human gastric muscle relaxation regardless of the NO pathway. If the relaxing effect of estrogen also occurs in humans via the NO metabolic pathway, this may explain the lack of inhibition of quercetin-induced action despite blocking the estrogen receptor.

In addition to assessing the effect of quercetin, the influence of the used blockers on the contractile activity of the stomach muscles was also considered. Preincubation of the gastric muscle with L-NNA did not significantly alter its contraction parameters; therefore, NO does not appear to be tonically active in the gastric fundus, which is consistent with the observation of Holzer-Petsche and Moser.⁴⁸ However, other researchers observed increases in amplitude and basal tension after incubation with L-NNA.^{49,50} In turn, L-NAME significantly inhibited contractility induced by carbachol. In the literature, no such effect can be found⁴⁸ and an increase in contractility is reported.⁵¹ The variety of the observed gastric muscle responses to L-NAME may be due to the fact that, despite its use as a NOS antagonist, there are numerous reports of L-NAME mediated reactions that are incompatible with NOS inhibition.⁵² Moreover, several electrophysiological studies have also been conducted showing that NO synthase inhibitors reduce or abolish NANC inhibitory induced by intestinal nerves.⁵³ As for GC blockers we observed that ODQ did not affect carbachol-induced contractility while cystamine significantly reduced AUC but did not influence basal tension of muscle strips. Cystamine was investigated to induce vasodilation in rat mesenteric small arteries by inhibiting the receptor-coupled TG2, leading to the opening of Kv channels and reduction of intracellular calcium, and the activation of the pathway sensitive to inhibitors of mitochondrial complexes I and III.⁵⁴

The blocker of an ATP-sensitive K^+ channel—glibenclamide at the concentration of 10^{-6} mol/L did not significantly

modify carbachol-induced muscle contractions, but at 10^{-5} mol/L—substantially increased the basal tension. Multiple *in vitro* studies have reported that glibenclamide ($\leq 10^{-6}$ mol/L), which blocks K^+_{ATP} channels, increases muscle tone and depolarizes vascular and non-vascular smooth muscles.⁵⁵ The attenuation of carbachol-induced muscle contraction by tamoxifen may be mediated by inhibition of the Ca^{2+} —independent mitogen-activated protein kinase (MAPK) pathway. Park et al. (2003) demonstrated in a rat aortic smooth muscle study that tamoxifen inhibited the ET-1-induced ERK1/2 and p38 MAPK activity.⁵⁶

To sum up, dietary polyphenols and/or their metabolites may exert valuable health effects directly during their passageway through the oral cavity and the entire GI tract at the local level. Many is known about possible therapeutic role of various polyphenols such as quercetin. However, due to their low bioavailability, main pharmacodynamics and/or adverse effects may affect the GI motility in first place. Thus, taking into account the physiology of the digestive system, a further interpretation of the effects of quercetin should be proposed. The presence of polyphenols in the lumen of the stomach and further parts of the GI tract leads to the assumption that these compounds may exert their beneficial effects *in situ*. However, currently biochemical modifications of quercetin have led to the formation of new semi-synthetic derivatives with improved bioavailability and solubility. These new compounds are still being studied.³ Therefore, nutraceuticals that enhance gastric relaxation to treat functional dyspepsia are worth studying.

5 | CONCLUSIONS

In conclusion, the current study was the first to show that quercetin-induced relaxation of human gastric smooth muscle occurs directly through K^+_{ATP} channels and independently to NO pathways. The present results suggest that quercetin is a potential nutraceutical in the treatment of functional gastrointestinal dyspepsia and other minor gastric muscle motility disturbance. Furthermore, its use as a potential K^+_{ATP} channel opener could explain important benefit as a myocardial protective agent, antihypertensive vasodilator, bronchodilator, bladder relaxant, islet cell protector, and antiepileptic drug.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

BM and KD conceptualized the study and involved in writing—original draft preparation. BM, PP, HRH, and TK involved in methodology. BM and TK validated and investigated the study. BM involved in informal analysis. BM, HRH, and KD collected resources.

BM, AK, KK, KD, and AC involved in data curation. BM, KD, and TK involved in writing—review and editing, and funding acquisition. BM, KK, and AK visualized the study. BM and HRH supervised the project. BM, PP, and HRH involved in project administration. All authors have read and agreed to the published version of the manuscript.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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