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Research Paper

Proton pump inhibitors omeprazole, lansoprazole and pantoprazole induce relaxation in the rat lower oesophageal sphincter

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Abstract

Objectives We aimed to investigate effects of the proton pump inhibitors (PPIs) omeprazole, lansoprazole and pantoprazole, which are currently used for the treatment of hyperacidity and gastro-oesophageal reflux, on the reactivity of the isolated rat lower oesophageal sphincter.

Methods Omeprazole, lansoprazole and pantoprazole (all 10^{-9}–10^{-3} M, cumulatively) were tested on carbachol-induced (10^{-6} M) contraction. In addition, the effects of PPI preincubation (all 10^{-3} M) on the contractions induced by cumulative carbachol (10^{-9}–10^{-5} M), angiotensin-2 (10^{-9}–10^{-5} M) or electrical field stimulation (EFS; 40 V, 32 Hz, 1 ms, 10 s) were assessed. Finally, the effects of PPI on the spontaneous contractile activity of the tissue were also evaluated.

Key findings PPI relaxed precontracted lower oesophageal sphincter in a concentration-dependent manner and suppressed carbachol-, angiotensin- and EFS-induced contractions. Furthermore, PPI attenuated spontaneous contractile activity of the tissue.

Conclusions Omeprazole, lansoprazole and pantoprazole had a suppressor effect on lower oesophageal sphincter contractions.

Keywords gastro-oesophageal reflux; lansoprazole; lower oesophageal sphincter; omeprazole; pantoprazole

Introduction

Proton pump inhibitors (PPI), namely omeprazole, lansoprazole and pantoprazole, are widely prescribed as first-order treatments of gastric hyperacidity and gastro-oesophageal reflux disease (GORD) – the reflux of gastric contents into the oesophagus leading to oesophagitis, which causes long-term complications and impairs life quality.[1] Most GORD cases can be efficiently treated by PPI, but a substantial proportion of cases are refractory to the drugs.[2]

The lower oesophageal sphincter (LOS), part of the oesophago-gastric junction, acts as the main physiological barrier for preventing reflux of gastric contents into the oesophagus.[3] The dysfunction of the LOS is the primary pathophysiological mechanism involved in GORD. Although the underlying causes of GORD are not yet known, transient relaxation of the LOS is believed to be the primary mechanism.[1]

It has been reported that PPI induces relaxation in different types of smooth muscle preparations, for instance guinea-pig gallbladder,[4] guinea-pig and human airway smooth muscles,[5] rabbit prostatic strips,[6] rat corpus cavernosum,[7] rat vas deferens[8] and human myometrial smooth muscle.[9] However, effects of PPI have yet to be investigated on the reactivity of LOS, the disfunction of which may cause GORD where PPI are frequently prescribed. We therefore investigated the effects of several PPIs, namely omeprazole, lansoprazole and pantoprazole, on the contractions induced by carbachol, angiotensin or electrical field stimulation (EFS). Spontaneous rhythmic activity was also assessed.

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Materials and Methods

Animals

This study was conducted in accordance with the guide for the Care and Use of Laboratory Animals of the Mersin University Centre for Experimental Medicine. The protocol of this work was approved by the local ethics committee of the medical faculty at Mersin University. Adult Wistar rats were caged separately under a 12-h light/12-h dark photoperiod and a constant temperature (23 ± 1°C) and received standard mice chow *ad libitum*.

Drugs and chemicals

Carbachol, angiotensin-2 and dimethyl sulfoxide were obtained from Sigma-Aldrich (St Louis, USA). Y-27632 [(+)-(R)-*trans*-4-(1-aminoethyl)-N-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate] was obtained from Tocris Cookson Ltd (Bristol, UK). Omeprazole, lansoprazole and pantoprazole were kindly donated by Mustafa Nevzat Pharm. Inc. (Istanbul, Turkey) as pure powders. All chemicals were dissolved in distilled water except PPI, which was dissolved in DMSO. Vehicle-control studies were performed.

Tissue preparation

The rats were euthanised with carbon dioxide asphyxia and exsanguinated. Thereafter, abdomen and thorax were opened immediately. In order to isolate the LOS, the stomach was dissected and opened along the great curvature through to the oesophagus. Afterwards, the oesophagus proximal to the gastroesophageal junction was immediately excised as an approximately 2 mm-wide strip. The strip was placed longitudinally in an organ bath (10 ml) filled with Tyrode's solution, the composition of which was (mmol/l) NaCl 136, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaH_2PO_4H_2O$ 0.42, $NaHCO_3$ 11.9 and glucose 5.5. The chamber was continuously gassed with 95% O_2/5% CO_2 and maintained at 37°C. Responses of the LOS were recorded by a force transducer (MP35) connected to a Biopac data acquisition system (BIOPAC, CA, USA). The initial tension of strips was 1 g, which was readjusted to the initial level 15 min before the beginning of the experiment. During 1 h of equilibration, the chamber was washed out with fresh Tyrode solution every 15 min.

Organ bath experiments

Following equilibration, the LOS strips were maximally contracted by 80 mM KCl (generally reaching a steady state within 15 min), after which the organ bath was refilled with fresh Tyrode's solution three times. Following incubation in Tyrode's solution for 1 h, EFS (40 V, 32 Hz, 1 ms, 10 s intervals) was applied ten times. Subsequently the bath medium was replaced with fresh Tyrode's solution three times and strips were incubated in the presence or absence of PPI for 60 min. Thereafter, EFS was applied in the same manner as before.

In another series of experiments, we investigated the possible relaxant effects of cumulative PPI (10^{-9} –10^{-3} M) on the tonic contractions induced by carbachol (10^{-6} M). For these purposes, carbachol was applied to the tissue in basal tone to induce half-maximal tonic contraction (generally reaching steady state within 5 min) and then PPI were cumulatively added to the organ bath to induce relaxation.

In the other experimental series, the contractions induced by carbachol (10^{-9}–10^{-3} M, cumulatively) or angiotensin-2 (10^{-9}–10^{-5} M, cumulatively) were assessed in the absence or presence of PPI (10^{-3} M).

Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance followed by Dunnet's post-hoc test was used for statistical comparison. The pEC50 is defined as the negative logarithm of the EC50 value, the concentration that leads to 50% maximal response. $P < 0.05$ was considered significant.

Results

Effects of PPI on EFS-induced contractions and spontaneous contractile activity of LOS strips

Lansoprazole (10^{-3} M) markedly reduced spontaneous contractile activity of the LOS strips as well as decreasing resting tone (Figure 1a). The effects of both omeprazole and pantoprazole on spontaneous contractions of LOS strips were similar (Figure 1a). All PPIs (10^{-3} M) substantially suppressed the EFS-induced contractions (40 V, 32 Hz, 1 ms, 10 times, 10 s, $n = 5$, Figure 1b) of LOS strips.

Effect of PPI on carbachol and angiotensin-2 induced contractions

The effects of cumulative carbachol (10^{-9}–10^{-3} M) and angiotensin-2 (10^{-9}–10^{-5} M) application were evaluated in the absence and presence of PPIs (10^{-3} M). Carbachol and angiotensin-2 brought about significant contractions of LOS strips in a concentration-dependent manner. Carbachol-induced contractions were markedly attenuated by the PPIs (all 10^{-3} M, $n = 5$, Figure 2a). Furthermore, angiotensin-2 induced contractions were eliminated in the presence of PPIs (10^{-3} M, $n = 4$, Figure 2b). In the experiments where the possible relaxant effects of PPIs were investigated, the LOS strip was precontracted with carbachol (10^{-6} M, $n = 4$, Figure 3). All of the PPIs relaxed the LOS strips in a concentration-dependent manner. The EC50 values of omeprazole, lansoprazole and pantoprazole were 3.9 ± 0.13, 4.6 ± 0.21 and 4.9 ± 0.17, respectively.

Effect of the selective Rho-kinase inhibitor Y-27632 on carbachol-induced contractions

The selective rho-kinase (ROCK) inhibitor Y-27632 [(+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride] (10^{-5} M, $n = 4$) markedly decreased the carbachol-induced contractions of rat LOS (Figure 4).

Discussion

In the present study, we investigated the effects of the PPIs omeprazole, lansoprazole and pantoprazole on the contractions induced by EFS, carbachol and angiotensin-2 in isolated rat LOS strips. Spontaneous contractile activity was also assessed in the presence of PPIs.

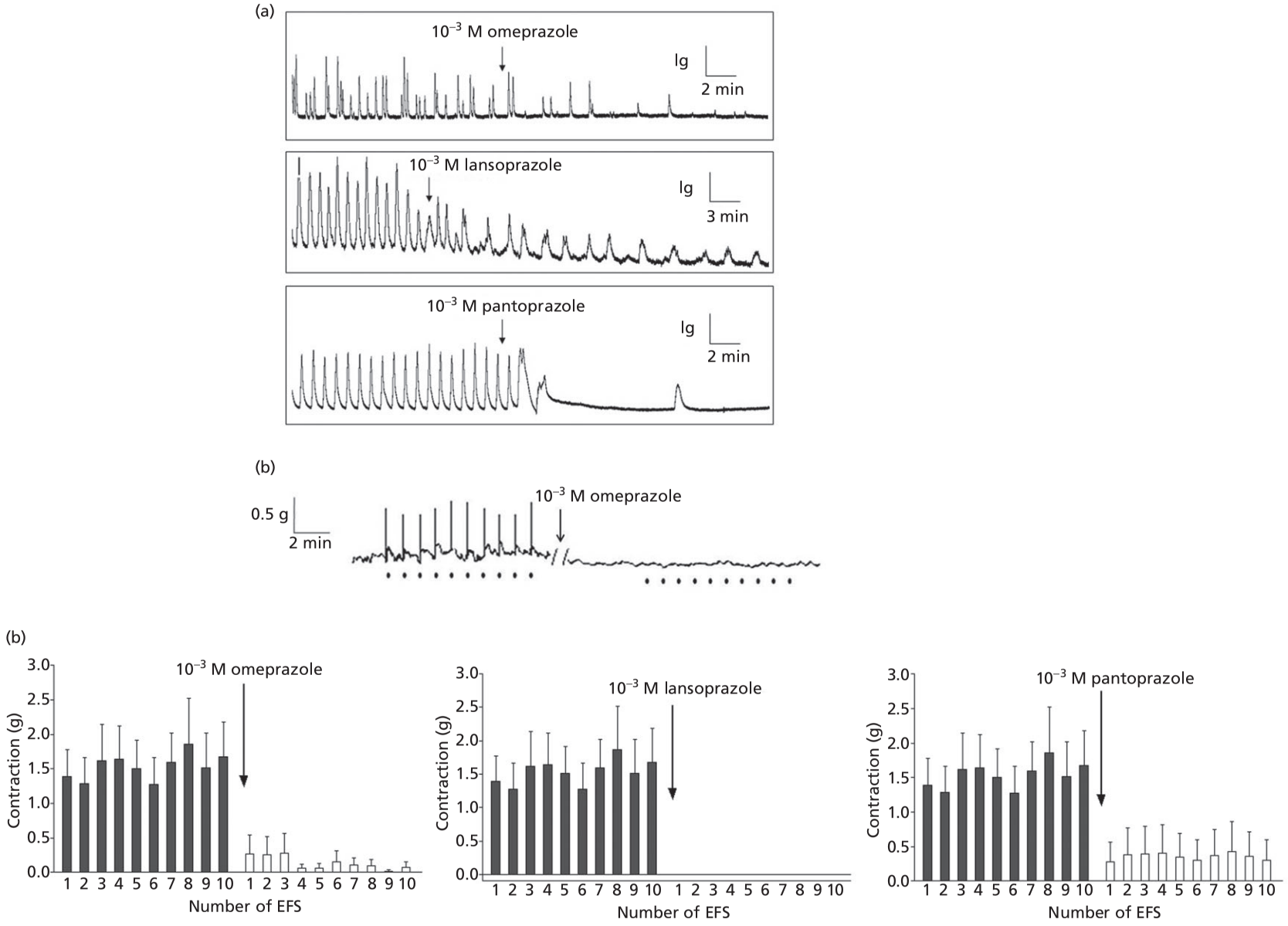

Figure 1 Effects of PPIs on spontaneous contractile activity and EFS-induced contractions of the rat LOS. (a) Original trace showing the effect of omeprazole (10^{-3} M), lansoprazole (10^{-3} M) and pantoprazole (10^{-3} M) on the spontaneous tone of rat lower oesophageal sphincter. Note that PPIs reduced not only the contractile activity but also the resting tone. (b) Effects of lansoprazole (10^{-3} M), omeprazole (10^{-3} M) and pantoprazole (10^{-3} M) on the EFS (40 V, 32 Hz, 1 ms,10 times,10 s intervals)-elicited contractions of rat LOS strips (the original trace of the effect of omeprazole on the EFS-induced contractions is on the upper panel). Data represent means ± SEM. Comparison was made by one-way ANOVA, followed by Dunnet's post-hoc test. $P < 0.001$ for the effects of all three PPIs.

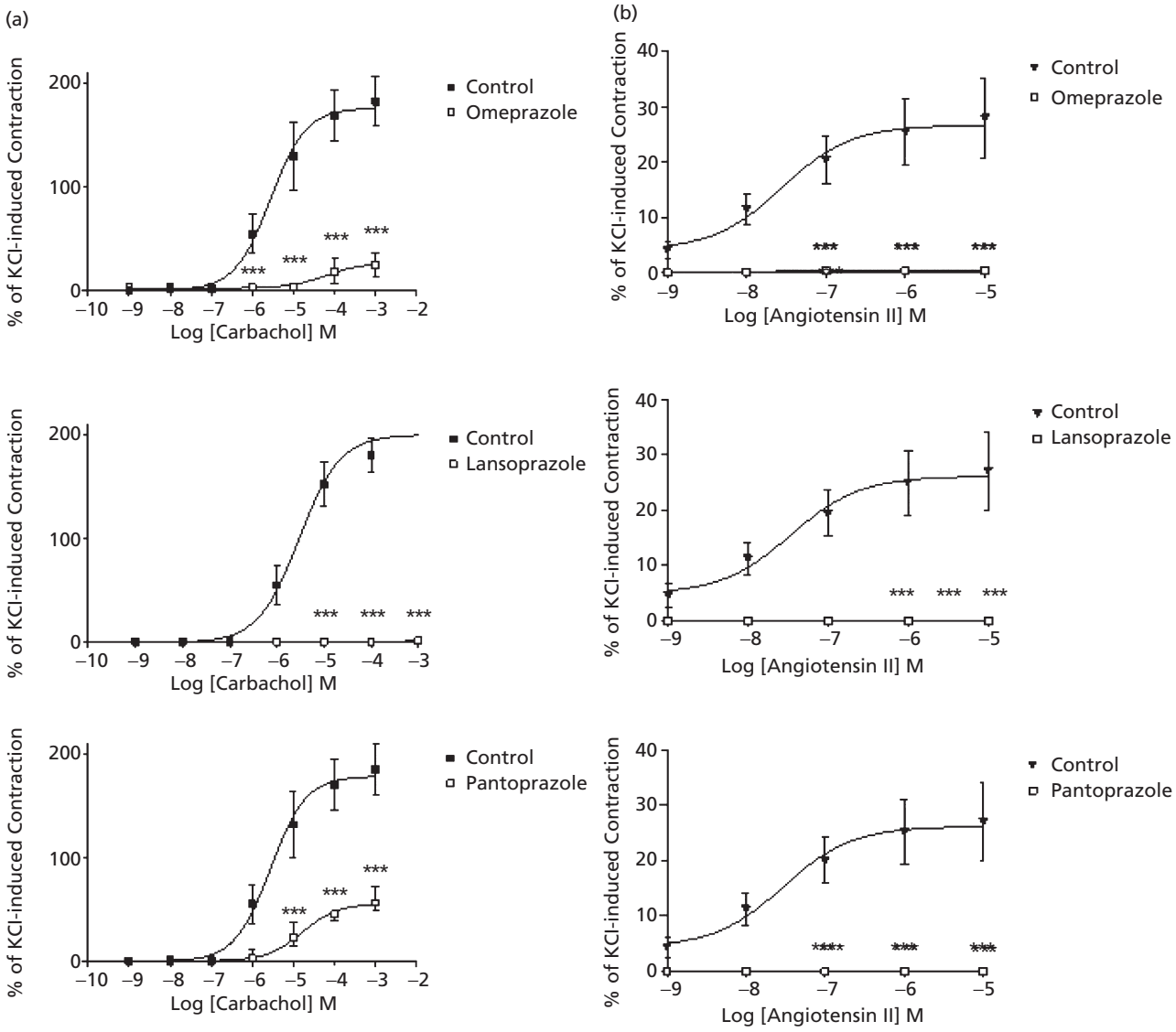

Figure 2 Effects of carbachol and angiotensin-2 on rat LOS strips in the absence and presence PPI. Effects of (a) carbachol (10^{-9}–10^{-3} M) and (b) angiotensin-2 (10^{-9}–10^{-5} M) on rat LOS strips in the absence and presence of the PPIs omeprazole, lansoprazole or pantoprazole (10^{-3} M). Note that carbachol-induced contraction was eliminated by lansoprazole. Contractions were expressed as percentage of KCl (80 mM)-induced contractions. Data represent means ± SEM. Comparison was made by one-way ANOVA, followed by Dunnet's post-hoc test. ***$P < 0.001$ for the effects of all three proton pump inhibitors.

Normally the LOS prevents retrograde passage of the gastric content into the oesophagus by producing a tonic pressure of 15–30 mmHg above the intragastric pressure.[3] In GORD, acidic stomach contents regurgitate into the oesophagus, leading to tissue damage.[10,11] The pathophysiological mechanisms of GORD are transient LOS relaxation[12–14] or LOS hypotension (<10 mmHg).[10,14] Although PPIs are widely prescribed as the first-order treatments of GORD, a substantial proportion of cases may be resistant to therapy.[2] About 30% of GORD patients remain symptomatic in spite of receiving long-term PPI treatment.[15] It has been suggested that transient lower oesophageal relaxation and nocturnal acid breakthrough during PPI treatment are the underlying mechanisms of refractory GORD.[16] There is some evidence that PPIs may induce relaxation of smooth-muscle preparations, including guinea pig gallbladder,[4] human myometrial smooth muscle,[9] human internal mammary and radial arteries *in vitro*,[17] rat aorta,[18] rabbit corpus cavernosum,[7] rabbit prostatic strips[6] and rat vas deferens.[8] However, the mechanism of PPI-induced relaxation of smooth muscles remains unclear.

Inhibition of smooth muscle tone by PPI may not be due to H^+,K^+-ATPase inhibition, since relaxant responses to omeprazole and lansoprazole are not altered by changing or even removing extracellular K^+. However, it has been shown that H^+,K^+-ATPase activity is inhibited in K^+-free solutions. In addition, acidification of the bath solution does not change the relaxant responses of human myometrium and rabbit corpus cavernosum strips to PPIs.[7,9] Moreover, the PPIs are prodrugs and need an acidic milieu to be converted to their protonated active forms, which bind covalently and inhibit activity of the

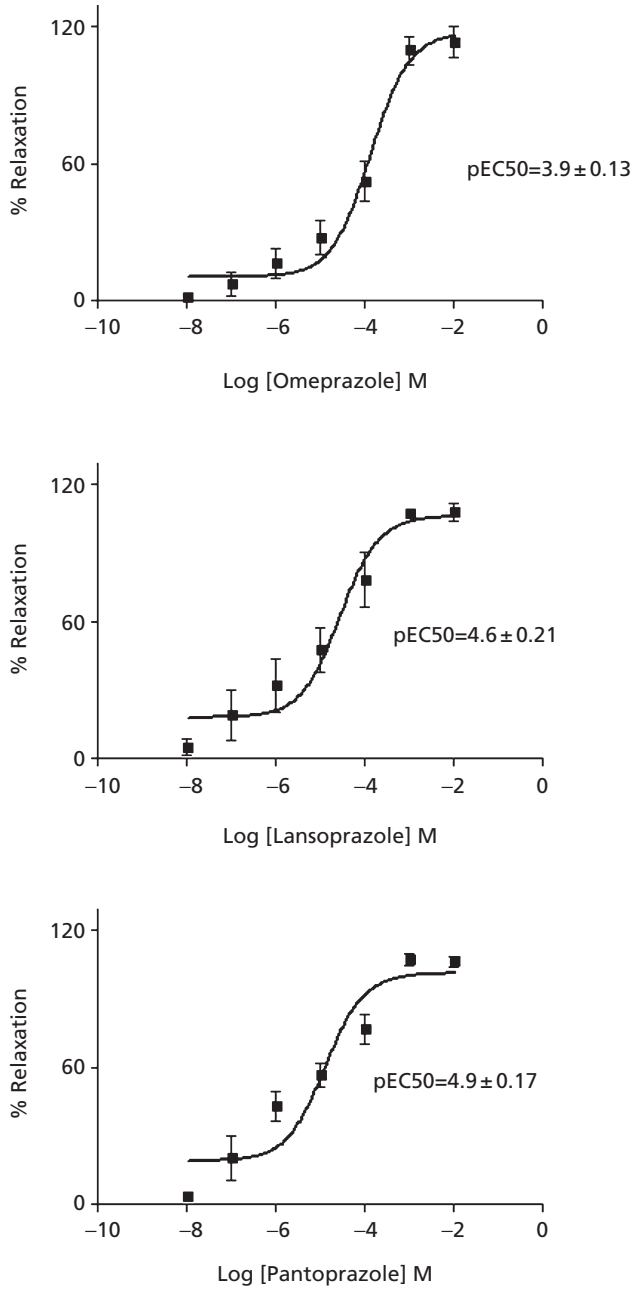

Figure 3 Relaxant effects of PPIs on carbachol-induced contraction in rat LOS strips. Relaxant effects of PPIs (10^{-9}–10^{-3} M) on carbachol (10^{-6} M)-precontracted rat LOS strips. Relaxation was expressed as a percentage of carbachol-induced tonic contraction. Data represent means ± SEM. The pEC50 is defined as the negative logarithm of the EC50 value, the concentration that leads to 50% maximal response.

H^+,K^+-ATPase.[19] However, all experimental protocols took place in Tyrode's solution at neutral pH (7.4), which is not an optimum pH for the conversion of these drugs to their active forms. Obviously, the fact that conditions were sub-optimal for the conversion and thus activation of the PPIs in almost all previous reports raises the possibility that there might be other mechanisms of action of PPI. One possible alternative seems to be the activation of Na^+/K^+-ATPase for smooth muscle

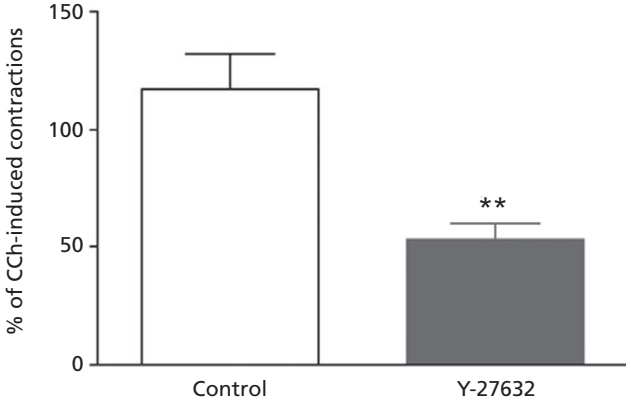

Figure 4 Effect of the selective Rho-kinase inhibitor Y-27632 on carbachol-induced contractions of the rat LOS. The effect of the selective Rho-kinase inhibitor, Y-27632 (10^{-5} M) was tested on carbachol-induced contractions in the rat LOS. Y-27632 dramatically suppressed the contraction induced by carbachol. Data represent means ± SEM. Comparison was made by Student's *t*-test for unpaired observations. $^{**}P < 0.01$.

relaxation. In accordance with this suggestion, in a recent study omeprazole and lansoprazole relaxed isolated human arteries precontracted with ouabain, an inhibitor of Na^+,K^+-ATPase.[17] However, it has been reported that two PPIs, namely omeprazole and SK&F96067, can inhibit Na^+,K^+-ATPase at high concentrations.[20,21] Another possible mechanism for smooth muscle relaxation may be the activation of K^+ channels. Unfortunately, it has been found that K^+ channel activation is not involved in omeprazole-induced relaxation of the rat aorta.[18] Furthermore, omeprazole induces relaxation in the rabbit corpus cavernosum, and this was not modified by glibenclamide and tetraethylammonium chloride (TEA).[22] Moreover, relaxation of rabbit prostatic strips induced by lansoprazole and omeprazole was not impaired by TEA and glibenclamide, implying that the relaxant effects of PPI in the smooth muscles are independent of K^+ channel activation.[6]

One possible mechanism for the suppressor effects of PPI in smooth muscle contractions could be the inhibition of ROCK, which has recently been reported to be involved in the regulation of smooth muscle cell contraction.[23] It has been demonstrated that Rho/Rho-kinase pathway is involved in the contractile activity of several smooth muscles, including mouse corpus cavernosum,[24] mouse gastric fundus,[25] mouse vas deferens,[26] rat urinary bladder,[27] sheep ureter[28] and human uterus.[29] A recent study has indicated that spontaneously contracted muscles such as the internal anal sphincter and the LOS have higher levels of the Rho/Rho-kinase.[30] Moreover, it was found that EFS-induced contractions of the feline oesophagus were inhibited by C3 exoenzyme (a RhoA inactivator),[31] indicating that the Rho/Rho-kinase pathway may be involved in these contractions. In support of this idea, it has been suggested that RhoA/Rho-kinase pathway-mediated $Ca^{[2+]}$ sensitisation has an important role in LOS tone.[32] In this study we confirmed that Y-27632, a selective Rho-kinase inhibitor (10^{-5} M), inhibited the carbachol-induced contractions of the rat LOS (Figure 4). However, the inhibition of Rho-kinase by PPIs remains to be demonstrated

by Western blotting in this tissue. In parallel, PPI also suppressed angiotensin-2-induced contractions, which are mediated by Rho/Rho-kinase in the rat LOS.[33]

Possible dissynergic effects of PPIs on the pyloric sphincter should be investigated since bile acids may be regurgitated into the stomach and back to the lower part of the oesophagus, which may then worsen the prognosis of GORD treatment with PPIs.

Conclusions

The findings of the present study indicate that the PPIs omeprazole, lansoprazole and pantoprazole reduce spontaneous contractile activity and EFS-, carbachol- or angiotensin-2-induced contractions in the rat LOS. Moreover, these results may presumably explain refractory cases of GORD to treatment with PPIs. Furthermore, based on this finding, precautions must be taken when prescribing these agents in the treatment of hyperacidity and GORD.

Declarations

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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