INHIBITORY EFFECT OF SILDENAFIL ON RAT DUODENAL CONTRACTILITY IN VITRO: PUTATIVE cGMP INVOLVEMENT

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SUMMARY

1. Sildenafil citrate (Viagra™; Pfizer, Sandwich, Kent, UK), a phosphodiesterase 5 inhibitor, raises cGMP levels in smooth muscle cells. It relaxes both vascular and visceral smooth muscle. In order to assess the intestinal effects of sildenafil, we decided to investigate its actions on rat duodenal motor activity in vitro.

2. In isolated duodenal segments maintained in Tyrode’s solution, sildenafil exhibited a concentration-dependent anti-spasmodic effect on acetylcholine (ACh)-induced phasic contractions, with an IC₅₀ value of 26.7 μmol/L (95% confidence interval (CI) 2.0–55.3 μmol/L).

3. Sildenafil also relaxed the carbamylcholine (CCh)-induced sustained contraction with an IC₅₀ of 16.2 μmol/L (95% CI 9.5–27.6 μmol/L). Sildenafil produced significant additional relaxation of 25.2 ± 8.1% of the CCh-induced contraction, beyond basal tone.

4. Sildenafil reduced the amplitude of spontaneous duodenal contractions with an EC₅₀ of 9.6 μmol/L (95% CI 5.7–16.2 μmol/L). This effect was significantly more potent than the effects of zaprinast and papaverine, which were significantly increased to 39.0 μmol/L (95% CI 23.9–63.4 μmol/L) and 43.8 μmol/L (95% CI 24.5–78.3 μmol/L), respectively.

5. In preparations treated previously with methylene blue (10 μmol/L) or 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 10 μmol/L), the EC₅₀ values for the sildenafil effect were significantly increased to 39.0 μmol/L (95% CI 23.9–63.4 μmol/L) and 43.8 μmol/L (95% CI 24.5–78.3 μmol/L), respectively. These values were significantly greater than those obtained with sildenafil alone.

6. In conclusion, sildenafil has myorelaxant and anti-spasmodic effects on rat duodenal segments in vitro. The contractile inhibitory effect of sildenafil on rat isolated duodenum is probably due to intracellular cGMP accumulation as a result of its decreased degradation.

Key words: guanylate cyclase, intestinal motility, peristalsis, phosphodiesterase inhibitors, sildenafil citrate.

INTRODUCTION

Sildenafil citrate (Viagra™; Pfizer, Sandwich, Kent, UK) has received widespread public interest owing to its therapeutic efficacy in erectile dysfunction. Sildenafil effectively blocks selectively the phosphodiesterase (PDE) 5-mediated hydrolysis of cGMP in vascular smooth muscle cells.1-3

The smooth muscle cells of the gastrointestinal tract also contain cGMP and PDE5 enzyme.4-5 Once inhibitory neurotransmitters, such as nitric oxide (NO) or atrial natriuretic peptide, are released, they increase intracellular cGMP levels, which decrease cytosolic Ca²⁺ content, and smooth muscle relaxation occurs.6,7 Recent evidence indicates that sildenafil modifies gastrointestinal motility both in animals and humans. Sildenafil was reported to inhibit the antroduodenal motility in fasting healthy volunteers8-9 and to delay gastric emptying and the gastrointestinal transit of a liquid meal in awake rats,10 although it did not change either gastric emptying or the post-prandial frequency of antral contractions in healthy volunteers.11

Gastric emptying is considered to be the result of a complex coordination between proximal stomach tonus and pylorus–duodenal relaxations.12-14 In order to assess the effects of sildenafil on small intestine contractile activity, we decided to investigate its effects on spontaneous and chemically stimulated motor behaviour of rat duodenal segments.

METHODS

Animals and tissue preparation

Twenty-eight male Wistar rats (180–250 g) were obtained from the Federal University of Ceará’s Central Housing Station and killed by cervical dislocation. A 2 cm long segment of the proximal rat duodenum was removed and placed into a Petri dish containing Tyrode’s solution (composition (in mmol/L): NaCl 136.0; KCl 5.0; MgCl₂ 0.98; CaCl₂ 2.0; NaH₂PO₄ 0.36; NaHCO₃ 11.9; glucose 5.5). After further dissection, the luminal contents were washed out with the physiological solution. Duodenal segments were placed under 1 g resting tension in a glass organ bath filled...
with 10 mL Tyrode’s solution. The solution was maintained at 37°C, pH 7.4, and bubbled continuously with air. Longitudinal muscle tension was recorded on a computer-coupled data-acquisition system (Dataq Instruments, Akron, OH, USA) by means of an isometric force transducer (model FT.03; Grass, Quincy, MA, USA). One hour was allowed for equilibration.

**Solutions and drugs**

1H-(1,2,4)Oxadiazolo(4,3-a)quinazolin-1-one (ODQ), 1,4-dihydro-5-(2-proxopyphenyl)-7H-1,2,3-triazo[4,5-d]pyrimidine-7-one (zaprinast), methylene blue, papaverine, acetylcholine and carbamylcholine were purchased from Sigma Chemical (St Louis, MO, USA), whereas sildenafil citrate was kindly provided by Pfizer (Sandwich, Kent, UK). Solutions were prepared by adding the pure substance directly to Tyrode’s solution.

**Experimental protocols**

In each assay, concentration–effect curves for sildenafil were obtained by exposing the preparation to increasing concentrations, added cumulatively to the bath chamber (5 min for each concentration or, when necessary, 10 min to observe the plateau response). To test the antispasmodic effect of sildenafil, preparations were first exposed to sildenafil for 5 min and were then challenged with acetylcholine (ACh; 1 μmol/L), still in the presence of sildenafil. To verify the relaxing effect of sildenafil on muscle tonus, preparations were precontracted by carbamylcholine (CCh; 1 μmol/L) and, during the stationary phase of the contraction (tonic component), sildenafil was added cumulatively to the bath chamber. The difference between the peak and valley records at plateau contraction in the presence of sildenafil was considered as the maximal effect of sildenafil-induced relaxation. The activity of sildenafil was also tested in the presence of two guanyl cyclase inhibitors, namely methylene blue and ODQ, in order to evaluate the effect of cGMP synthesis on the responses to sildenafil. In order to verify tissue vitality, duodenal responses to the contractile agents ACh and CCh, were tested again 30 min after withdrawal of sildenafil.

**Statistical analysis**

Data are expressed as the mean±SEM, with n the number of experiments. The EC50 and IC50 values (i.e. the concentration of agonist or antagonist at which 50% of the response was observed) were calculated by interpolation from semilogarithmic plots and are given as geometric means (95% confidence interval (CI)). Univariate analysis of variance (ANOVA) was performed by a multiple comparison test (as appropriately indicated in Results) were used to investigate the significance of differences between means. Statistical significance was accepted when P < 0.05.

**RESULTS**

**Antispasmodic and myorelaxant effects of sildenafil on ACh- and CCh-induced contractions of rat duodenal segments**

Sildenafil demonstrated a concentration-dependent antispasmodic effect on ACh-induced phasic contractions in rat duodenum with an IC50 value of 26.7 μmol/L (95% CI 2.0–55.3 μmol/L; n = 6; P < 0.001, ANOVA; Fig. 1a,c). Sildenafil also relaxed the tonic component of the CCh-induced sustained contraction of duodenal preparations with an IC50 value of 16.2 μmol/L (95% CI 9.5–27.6 μmol/L; n = 8; Fig. 1b,c). Interestingly, sildenafil (100 and 300 μmol/L) brought about significant additional relaxation, corresponding to 25.2 ± 8.1% of the CCh-induced contraction (Fig. 1b), which was beyond the basal tone of the preparation (P < 0.05, Dunnett’s test; Fig. 1c). Moreover, the inhibitory effect of sildenafil was reversible after consecutive washings. The amplitude of the ACh- and CCh-induced contractions after sildenafil withdrawal corresponded to 76.4 ± 10.4 and 90.9 ± 17.4% of that obtained in control preparations (P > 0.05, Dunnett’s test).

**Inhibitory effects of sildenafil on spontaneous activity of rat duodenal segments**

Isolated duodenal segments showed oscillatory spontaneous activity with a mean force corresponding to 496.2 ± 43.1 mg (n = 46). Sildenafil (0.1–300 μmol/L) reduced the amplitude of these spontaneous contractions in a concentration-dependent manner, with an EC50 of 9.6 μmol/L (95% CI 5.7–16.2 μmol/L; n = 9; P < 0.001, ANOVA) and maximal effect at a concentration of 300 μmol/L, which almost abolished spontaneous duodenal contractions (Fig. 1c). Moreover, the inhibitory effect of sildenafil was reversible after consecutive washings. The amplitude of the ACh- and CCh-induced contractions after sildenafil withdrawal corresponded to 76.4 ± 10.4 and 90.9 ± 17.4% of that obtained in control preparations (P > 0.05, Dunnett’s test).

![Fig. 1](image-url)  
**Fig. 1** Antispasmodic effects of sildenafil on acetylcholine (ACh)- and carbamylcholine (CCh)-induced contractions in rat duodenum. Typical traces are shown of an experiment showing the inhibitory effect of sildenafil on (a) phasic ACh (1 μmol/L)-induced contractions (n = 6) and (b) sustained CCh (1 μmol/L)-induced contractions (n = 8) in rat duodenal segments. The exposure time for sildenafil and CCh is indicated by the lines above the experimental traces and the moment of addition of each concentration of sildenafil is indicated by the dots. The dashed line in (a) indicates that sildenafil was removed with washing (empty triangles) of the preparation, whereas the unbroken line indicates that sildenafil addition was cumulative. Recovery was recorded 30 min later. (c) Graph of mean values of the inhibitory effect of sildenafil on ACh- (△) and CCh-induced (▲) contractions. *P < 0.05 compared with control agonist-induced amplitude; †P < 0.05 compared with basal tone (dotted line a,b).
contractions (Fig. 2). This effect was more potent ($P < 0.05$, Dunnett’s test) than those of well known PDE inhibitors, such as zantrapast and papaverine, which also decreased the duodenal contractions with EC$_{50}$ values of 91.6 μmol/L (95% CI 46.0–182.2 μmol/L; $n = 8$) and 78.5 μmol/L (95% CI 37.1–166.3 μmol/L; $n = 6$), respectively.

**Effects of guanylyl cyclase inhibitors on the action of the sildenafil on rat isolated duodenum**

When preparations were treated previously with methylene blue (10 μmol/L), a non-specific guanylyl cyclase blocker, the EC$_{50}$ value for the inhibitory effect of sildenafil was significantly increased to 39.0 μmol/L (95% CI 23.9–63.4 μmol/L; $n = 11$; Fig. 3). In the presence of ODQ (10 μmol/L), a specific guanylyl cyclase inhibitor, the EC$_{50}$ value for sildenafil-induced decreases of spontaneous contractions was increased significantly to 43.8 μmol/L (95% CI 24.5–78.3 μmol/L; $n = 8$; Fig. 3b). These values were significantly different ($P < 0.05$, Dunnett’s test) compared with values obtained for sildenafil alone (9.6 μmol/L; 95% CI 5.7–16.2 μmol/L; $n = 9$).

**DISCUSSION**

The introduction of sildenafil as a treatment for male erectile dysfunction can be considered as a revolution for men’s life style. Apart from its well-defined relaxant effect on penile corpus cavernosum, via selective PDE5 inhibition, recent studies have indicated that sildenafil inhibits contractility while enhancing the relaxation of other smooth muscle tissues, including the gastrointestinal tract. The present study assessed the possible effect of sildenafil on intestinal contractile behaviour using rat duodenal preparations *in vitro*. Over the concentration range used in the present study (0.1–300 μmol/L), sildenafil showed potent myorelaxant and antispasmodic actions, probably because of inhibition of intestinal PDE activity.

The antispasmodic activity of sildenafil is a consequence of its ability to reversibly block both phasic and sustained cholinergic contractions. Moreover, incubation of duodenal segments with sildenafil also produced a significant relaxation of basal tone, concomitant with a concentration-dependent decrease in amplitude of intestinal contractions compared with control periods. These effects were similar to those obtained with papaverine and zantrapast, other unrelated substances able to inhibit PDE, despite differences in potencies. These results corroborate the early studies of Bortolotti et al. in humans and Rosalmeida et al. in rats indicating that sildenafil inhibits gastroduodenal motility.

Nitric oxide is considered one of the most important relaxing agents of the gastrointestinal wall. It acts either directly on smooth muscle cells or indirectly by modulating neuronal reflexes (e.g. in neurotransmitter release from enteric nerve terminals). Nitric oxide drives cGMP formation through a NO–guanylyl cyclase pathway, which, in turn, causes the activation of cGMP-dependent protein kinase, leading to relaxation. Activation of soluble guanylate cyclase and the subsequent elevation of intracellular

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Fig. 2 Inhibitory effect of sildenafil on spontaneous contractions of rat isolated duodenal preparations. (a) Typical record trace showing the diminished contractility of the rat duodenum induced by cumulative additions of sildenafil ($n = 9$). The addition of sildenafil is indicated by dots. The moment of washing is indicated by the empty triangle. Recovery of the spontaneous activity is indicated in real time without record interruption. (b) Graph showing the mean values of the effects of sildenafil (•), papaverine ($n = 6$; □) and zantrapast ($n = 8$; ○) on spontaneous contractions of rat duodenal strips.

Fig. 3 Effects of methylene blue and 1H-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one (ODQ) on the inhibitory actions of sildenafil on spontaneous contractions of rat isolated duodenal preparations. (a) Typical record trace showing the effect of sildenafil on the contractility of the rat duodenum in the presence of methylene blue (10 μmol/L; $n = 11$). The exposure of the preparation to methylene blue is indicated by the upper line in the figure. The addition of sildenafil is indicated by dots. The moment of washing is shown by an empty triangle. (b) Graph showing the mean values of sildenafil-induced inhibition on spontaneous contractions of rat duodenal strips alone (●) and in the presence of 10 μmol/L methylene blue (○) or 10 μmol/L ODQ ($n = 8$; †).
cGMP levels are considered to be the primary modes of action of NO. It should be emphasized that termination of the smooth muscle relaxation is accomplished as a result of cGMP hydrolysis by PDEs.

The cyclic nucleotide PDEs play an important role in signal transduction by regulating the intracellular concentration of cyclic nucleotides. They hydrolyse the intracellular second messengers cAMP and cGMP to their corresponding monophosphates. Phosphodiesterase 5, the target of sildenafil, with other components of the cGMP signalling cascade is primarily located in smooth muscle tissues, including the gastrointestinal tract. This type of drug produces PDE5 inhibition, increasing intracellular concentrations of cGMP and enhancing NO-induced smooth muscle relaxation. Therefore, substances that increase cellular levels of cGMP may lead to a reduced smooth muscle tone and inhibited responsiveness to contractile agonists.

In accordance with this hypothesis, sildenafil diminished the cholinergic agonist-induced contractions while relaxing the basal tone to below control levels. Moreover, the concentration–effect curve for sildenafil-induced inhibition of spontaneous contractions was significantly shifted to the right when segments were pretreated with methylene blue or ODQ, two well-known guanylyl cyclase blockers. Comparisons of EC50 values indicate that the sildenafil-decreased duodenal contractility was related, at least in part, to a functionally active enzyme. In other tissues, it has been demonstrated that methylene blue also led to a suppression of the inhibitory effects of sildenafil on agonist-evoked rat uterine contractions, whereas in human vas deferens the inhibition induced by sildenafil was not modified by ODQ. These data show that the effects of sildenafil are tissue dependent.

Thus, the results of the present study show that sildenafil has myorelaxant and antispasmodic effects on rat duodenal smooth muscle in vitro. The study also confirms that, as in the carvenosum vascular tissue, the inhibitory effects of sildenafil may be because of decreased PDE-induced cGMP degradation.

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**REFERENCES**
