

EFFECT OF ERYTHROMYCIN ON CONTRACTILE RESPONSE OF UTERINE SMOOTH MUSCLE STRIPS IN NON-PREGNANT RATS

Heng Liu^{1,#}, Tianmin Zhu², Yongming Ma³, Songyi Qu³

¹Cell Biology Institute, Life Science School, Lanzhou University, Lanzhou 730000, Gansu, P.R.China, ²West China Center of Medical Science, Sichuan University, Chengdu 610041, Sichuan, P.R.China, ³Department of Physiology, Lanzhou Medical College, Lanzhou 730000, Gansu, P.R. China

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Objective. Erythromycin stimulates stomach smooth muscle contraction *via* action on motilin receptors, but the effects of erythromycin on non-pregnant uterine smooth muscle are unknown. The purpose of this study was to assess the effect of erythromycin on non-pregnant uterine smooth muscle and to examine the possible mechanism of its action.

Study Design. Uterine smooth muscle strips from rats were suspended in organ baths containing Krebs solution, and then isometric tension was measured. The response to erythromycin and the effect of hexamethonium, indomethacin, phentolamine, diphenhydramine, atropine, metoclopramide and verapamil on erythromycin-induced contraction were also assessed.

Results. The present study showed for the first time that erythromycin dose-dependently increased contractile frequency, and at a dose of 1.55×10^{-3} mol/l it also increased contractile tension in non-pregnant uterine smooth muscle strips in rats. These actions were not affected by pretreatment with hexamethonium, indomethacin, phentolamine, atropine and metoclopramide, but histamine H1 receptor blocker diphenhydramine and calcium channel blocker verapamil inhibited both responses induced by erythromycin.

Conclusion. Our results suggest that erythromycin could increase contractile frequency and tension of non-pregnant uterine smooth muscle *via* histamine H1 receptor and calcium channel.

Key words: erythromycin, non-pregnant uterine smooth muscle, diphenhydramine, verapamil, histamine H1 receptor, calcium channel, rat

[#] correspondence; e-mail: hliu_kong@yahoo.com

INTRODUCTION

Erythromycin was first isolated in the 1950s from a Philippine soil sample, and the derivatives of erythromycin A, called the macrolide antibiotics, have been used as effective antibacterial agents ever since [20]. Erythromycin can be considered the prototype of macrolide antibiotics. These drugs inhibit the ribosomal protein synthesis in bacteria and, thus, have a bacteriostatic and bactericidal effect. Erythromycin has a similar spectrum of action as penicillin and affects in particular many Gram-positive bacteria. Like the tetracyclines, erythromycin is also active against bacteria-like organisms (*Chlamydia trachomatis*, *Mycoplasma pneumoniae*, *Ureaplasma urealyticum*). Its primary side effects are nausea, vomiting, abdominal pain, bloating, and diarrhea.

In 1984, erythromycin has been shown to interact with gastrointestinal smooth muscle in a similar manner to motilin, and has been postulated to act as a motilin receptor agonist [8, 16]. Motilin is a gastrointestinal peptide containing 22 amino acid residues that was for the first time isolated from porcine duodenal mucosa [2, 17, 18]. *In vivo*, motilin stimulates contractile activity in the antrum and duodenum of several species [7, 23]. *In vitro*, it has been reported that it is only effective on rabbit and human intestinal smooth muscle strips, and this effect is insensitive to inhibitors of neural activity [23]. The physiological role of motilin consists in the induction during the interdigestive state of a periodic recurrent pattern of contractile activity that is known as phase III of the migrating motor complex (MMC) [6].

As hollow organs, uterus shares a number of similarities with the stomach, both belong to the group of smooth muscles that are spontaneously active, and both have the ability to maintain force as their volume increases and to expel its content, but even more differences exist between them. Whether the adjunctive administration of antibiotics, such as ampicillin and erythromycin, to women in preterm labor are beneficial to prolongation pregnancy and improvement of perinatal outcome has been studied by several investigators for a long time [12, 13, 19, 26]. But the data and the effect of erythromycin on the contraction of uterine smooth muscle are limited. Granovsky-Grisaru et al. [5] first reported that erythromycin produced a decrease in the pregnant rat myometrial activity *in vi-*

tro, independent of the stimulant. But no more information is available concerning the effect of erythromycin on non-pregnant uterine smooth muscle. So the objective of our study was to characterize *in vitro* the effect of erythromycin on the spontaneous contractility of the non-pregnant uterine smooth muscle strips in rats.

MATERIALS and METHODS

Animal preparation and contraction studies

Female non-pregnant Wistar rats, weighting 200–250 g, were purchased from Animal Room of Lanzhou Medical College. The College Committee on Use and Care of Animals approved all animal experiments. The animals were pretreated subcutaneously with estradiol benzoate (0.5 mgkg⁻¹) at 72 h before the experiments [21], fasted for 24 h and killed by stunning and exanginations, and then their uteri were rapidly dissected out. Smooth muscle strips (2 × 5 mm) were cut along the longitudinal axis of uterus.

Each strip was suspended horizontally between two parallel stainless steel hooks for the measurement of isometric tension in individual organ bath containing Krebs solution composed of (mmol/l) NaCl 120, KCl 5.9, NaH₂PO₄ 1.2, MgCl₂ 1.2, NaHCO₃ 15.4, CaCl₂ 2.5, and glucose 11.5, bubbled with 95% O₂ and 5% CO₂. Temperature was maintained at 37°C and pH = 7.4. The Krebs solution was changed every 20 min. Isometric tension generated by uterine smooth muscle was measured using a force transducer (JH-2) and recorded with BL-310 Experimental System of Biological Function (TME, China) through IBM computer. After 1 h of equilibration with 1 g tensions, erythromycin or antagonists were given to the tissue chamber separately or antagonists were added to the organ bath 5 min before erythromycin.

Drugs

The following drugs were used: erythromycin (Sigma), indomethacin (Jiangsu taicang, China), hexamethonium bromide (Sigma), verapamil (Sigma), phentolamine (The Thirteen Pharmaceutical Factory of Beijing, China), atropine (Jiangsu yan-cheng, China), diphenhydramine (Beijing shuang-jiao, China), metoclopramide (Shanghai tianfeng, China).

Calculations and data analysis

The results were presented as means \pm SEM. The baseline obtained during equilibration at 1 g tension was considered as zero tension, and the tension increase after erythromycin addition was calculated with the run-up distance of this zero tension baseline. Contractile frequency was calculated as the numbers of contractile waves per minute with n representing the number of rats. The data were statistically analyzed by Student's t -test, correlation coefficients (r) were calculated, and they were considered significant if $p < 0.05$.

RESULTS

The effect of erythromycin on the spontaneous contraction of uterine smooth muscle *in vitro*

Figure 1 shows the effect of erythromycin on the spontaneous contraction of uterine smooth muscle *in vitro*. Erythromycin dose-dependently increased the contractile frequency of the rat uterine smooth muscle *in vitro* ($r = 0.7609$, $p < 0.001$, Tab. 1). Erythromycin at 1.55×10^{-3} mol/l also sig-

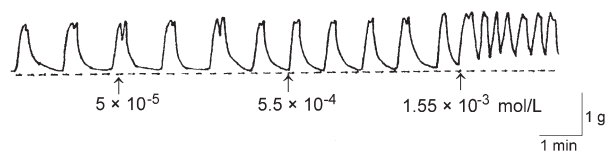


Fig. 1. Effect of erythromycin on the contractile activity of non-pregnant the rat uterine smooth muscle strips

nificantly increased the tension of the rat uterine smooth muscle *in vitro* ($p < 0.001$, Tab. 1).

Effect of hexamethonium, indomethacin, phentolamine, atropine, metoclopramide on responses to erythromycin

Hexamethonium (10^{-5} mol/l), indomethacin (10^{-5} mol/l), phentolamine (10^{-5} mol/l), atropine (10^{-5} mol/l) and metoclopramide (10^{-5} mol/l) did not affect the spontaneous contractile activity of the rat uterine smooth muscle *in vitro* (Tab. 2). After 5 min of joint incubation with erythromycin (1.55×10^{-3} mol/l), these agents also did not affect the increased contractile frequency and tension induced by erythromycin ($p > 0.05$, Tab. 2).

Effect of diphenhydramine and verapamil on responses to erythromycin

Diphenhydramine (10^{-6} mol/l) and verapamil (10^{-7} mol/l) did not affect the spontaneous contractile activity of the rat uterine smooth muscle *in vitro* (Tab. 2). When erythromycin was added after 5 min, diphenhydramine (10^{-6} mol/l) reduced the increased tension and the increased contractile frequency induced by erythromycin (1.55×10^{-3} mol/l, $p < 0.05$, Tab. 2), and verapamil (10^{-7} mol/l) inhibited the increased tension induced by erythromycin (1.55×10^{-3} mol/l, $p < 0.001$, Tab. 2). Moreover, it reduced the increased contractile frequency induced by erythromycin (1.55×10^{-3} mol/l, $p < 0.05$, Tab. 2).

Table 1. Effect of different concentration of erythromycin on the tension and contractile frequency of the rat uterine smooth muscle strips

	Erythromycin			
	0 (mol/l) Control	5×10^{-5} (mol/l)	5.5×10^{-4} (mol/l)	1.55×10^{-3} (mol/l)
Tension	0 (n = 12)	0 (n = 12)	0 (n = 12)	$14.7 \pm 5.8^{***}$ (n = 12)
Frequency (cycle/min)	0.80 ± 0.26 (n = 12)	0.85 ± 0.23 (n = 12)	$1.33 \pm 0.37^{**}$ (n = 12)	$2.53 \pm 0.99^{***}$ (n = 12)

The control group was designated as 0 mol/l. The effects of different concentrations of erythromycin were compared with the control (Student's t -test), ** $p < 0.01$ vs control; *** $p < 0.001$ vs control. The data are presented as mean increase \pm SEM. For frequency, correlation coefficient (r) was calculated, $r = 0.7609$, $p < 0.001$

Table 2. Effect of erythromycin (1.55×10^{-3} mol/l) on the tension and contractile frequency of the rat uterine smooth muscle strips after pretreatment with antagonists

	Ery (con)	Ery+ Hex	Ery+ Ind	Ery+ Phe	Ery+ Dip	Ery+ Atr	Ery+ Met	Ery+ Ver
Tension	14.7 ± 5.8 (n = 12)	16.8 ± 9.7 (n = 12)	11.8 ± 6.1 (n = 12)	11.5 ± 5.8 (n = 12)	9.8 ± 1.6* (n = 12)	12.5 ± 7.6 (n = 12)	16.2 ± 9.3 (n = 12)	0*** (n = 12)
Frequency	2.53 ± 0.99 (n = 12)	2.52 ± 0.71 (n = 12)	2.23 ± 0.80 (n = 12)	2.28 ± 0.70 (n = 12)	1.72 ± 0.36* (n = 12)	2.32 ± 0.75 (n = 12)	2.42 ± 0.45 (n = 12)	1.78 ± 0.56* (n = 12)

The effect of erythromycin (1.55×10^{-3} mol/l) without pretreatment with antagonists was considered the control group, and the effect of erythromycin (1.55×10^{-3} mol/l) with pretreatment with each antagonist was compared with the control (Student's *t*-test), * $p < 0.05$ vs erythromycin, *** $p < 0.001$ vs erythromycin; n – the rats number; Ery – erythromycin (1.55×10^{-3} mol/l); Hex – hexamethonium (10^{-5} mol/l); Ind – indomethacin (10^{-5} mol/l); Phe – phentolamine (10^{-6} mol/l); Dip – diphenhydramine (10^{-6} mol/l); Atr – atropine (10^{-5} mol/l); Met – metoclopramide (10^{-5} mol/l); Ver – verapamil (10^{-7} mol/l)

DISCUSSION

The uterus is spontaneously active, which means that, without any nervous or hormonal stimulation, a piece of isolated, pregnant or non-pregnant, uterus will produce regular spontaneous contractions [27]. Our results show that erythromycin dose-dependently increased the contractile frequency of uterine smooth muscle in non-pregnant rats. Based upon data derived from our study, the frequency-increasing effects of erythromycin were not affected by pretreatment with hexamethonium (the nicotinic cholinergic antagonist), indomethacin (prostaglandin inhibitor), phentolamine (α -adrenergic receptor antagonist), atropine (muscarinic cholinergic antagonist) and metoclopramide (dopamine D2 receptor antagonist). Therefore, the mechanism of erythromycin-induced increase in the contractile frequency would not involve these pathways. Diphenhydramine (histamine H1 receptor antagonist) and verapamil (calcium channel blocker), however, reduced the increased contractile frequency induced by erythromycin (1.55×10^{-3} mol/l). Both H1 and H2 histamine receptors exist in mature rat uterus [15] and human uterus [11], the former mediating contraction and the latter relaxation. In recent research, activation of the H1 receptor led to the stimulation of proteoglycan synthesis, enhanced the activity of protein kinase C (PKC) and evoked increases in the levels of intracellular Ca^{2+} [22]. The frequency of contractions was increased by ionic changes that 1) increase pacemaker activity and 2) shorten the action potential [27]. Because verapamil, a standard calcium channel blocker [4], inhibited this effect too, we can demonstrate

that erythromycin would change intracellular calcium concentration that consequently increased the contractile frequency.

Our results also show that erythromycin (1.55×10^{-3} mol/l) significantly increased the contractile tension of uterine smooth muscle *in vitro* in non-pregnant rats. Conventional antagonists such as hexamethonium, indomethacin, phentolamine, atropine and metoclopramide did not affect this tension increased by erythromycin. These results indicate that the erythromycin-effected spontaneous contractile tension increases in the rat uterine muscle strips are not generated by prostaglandin, α -adrenergic receptor, dopamine D2 receptor, or nicotinic and muscarinic cholinergic input. But similarly as with the effect on frequency, histamine H1 receptor blocker diphenhydramine and calcium channel blocker verapamil, inhibited the tension increase induced by erythromycin, suggesting that the action might be mediated through H1 receptor and calcium channels. Since verapamil blocked the tension increase completely, so calcium channel would contribute more to this response induced by erythromycin. In addition, verapamil also has α -adrenoceptor antagonistic activity [14], so we used phentolamine also in our study, and phentolamine had no effect on the changes produced by erythromycin, suggesting that α -adrenoceptor receptor was not involved in this process.

It has been known for long time that in the myometrium, calcium is the major intracellular second messenger, and an increase (from 10^{-7} to 10^{-5} molar concentration) in intracellular Ca^{2+} is necessary for myofilament interaction to produce a contraction [9]. Certain agonists that bind to

smooth muscle receptors stimulate these cells to contract by increasing the intracellular Ca^{2+} concentration. The elevated amount of Ca^{2+} binds to calmodulin, resulting in the formation of a Ca^{2+} -calmodulin complex, which subsequently binds to the myosin light-chain kinase, thereby activating the enzyme. This activation results in phosphorylation of the myosin light chain, which subsequently interacts with an actin-activated magnesium adenosine triphosphatase, then producing contraction [24]. The rise in intracellular Ca^{2+} could be due to 1) increased entry *via* voltage or receptor-operated channels, 2) increased release from and/or decreased uptake into internal stores including surface membrane binding sites, 3) decreased or reversed Na-Ca exchange, or 4) decreased surface membrane Ca extrusion *via* the Ca-ATPase pump [27]. Since in our study, a Ca^{2+} -containing and isoosmotic KCl solution was applied, the erythromycin-primed contractile tension increase would be the effect of Ca^{2+} influx. Calcium-induced calcium release plays little role in sarcoplasmic reticular Ca^{2+} release from the myometrium [25], so extracellular Ca^{2+} influx through the H1 receptor-mediated signal transduction pathway and calcium channel leading to the intercellular Ca^{2+} increase would be the cause of erythromycin-induced frequency and tension increase.

In pregnant rat uterus, Granovsky-Grisaru et al. [5] found that erythromycin produced a decrease in the pregnant rat myometrial activity *in vitro*, independent of the stimulant. In their research, exposure to erythromycin caused a sustained decrease in phasic contractions induced by oxytocin or carbachol. This effect started at 0.01 mmol/l. At 1 mmol/l, erythromycin reduced the contractions amplitude to 22% of the control and the frequency was reduced to 38% of control [5]. But in the non-pregnant rat, we found that erythromycin (1.55×10^{-3} mol/l) produced an increase in the uterine smooth muscle spontaneous activity *in vitro*, and the action on frequency and tension increase would be *via* histamine H1 receptor and calcium channel to increase the intercellular Ca^{2+} . The discrepant results could well demonstrate the manifold interesting changes that emerge in uterus during pregnancy. In pregnant rats, maintaining the relatively quiescent uterus throughout gestation for the development of the fetus is important [27], but in the non-pregnant uterus, different patterns of contractility were shown during the menstrual cycle [3]. Many impor-

tant factors involved in the process of producing contraction are differentially expressed in the pregnant and non-pregnant myometrium, such as G-protein-coupled receptor kinases [1], ryanodine-sensitive Ca^{2+} release channels [10]. So the mechanism of erythromycin-primed contractions in pregnant and non-pregnant uterine smooth muscle is worth to study further.

In summary, the findings of our study indicate that erythromycin increased the contractile frequency and tension in uterine smooth muscle in non-pregnant rats. The frequency and tension increase could be induced through histamine H1 receptor and calcium channel. Further *in vivo* studies of erythromycin influence on uterine activity in rats and *in vitro* research on human myometrium will allow us to gain new insights into the effect of erythromycin on uterus.

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