European Review for Medical and Pharmacological Sciences 2017; 21: 5028-5033

Effects of erythropoietin on spontaneous and oxytocin induced myometrial contractions in the nonpregnant rat

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Abstract. – OBJECTIVE: Erythropoietin (EPO) is a glycoprotein hormone that regulates erythropoiesis. EPO activity has also been detected in a variety of tissue including the nervous system, and female and male reproductive organs. It has been shown that EPO causes relaxation in vascular smooth muscle. In the present study, we have investigated effects of EPO on spontaneous and oxytocin-induced contractions of non-pregnant rat myometrium.

MATERIALS AND METHODS: Myometrial stripes were obtained from adult Wistar rats at the oestrous stage. The samples were placed in an isolated organ chamber under physiological conditions and 1 g passive tension. Epoetin beta (rEPO) was added cumulatively at 0.1, 1 and 10 IU/ml concentrations to the myometrial samples showing regular spontaneous contractions for periods of 30 min. Frequency and amplitude of contractions were electrophysiologically recorded and analyzed by using a BIOPAC data acquisition system.

RESULTS: rEPO inhibited both area under curve and frequency of spontaneous contractions (ANOVA, n1, 2 = 9, f1 = 20.938, f2 = 20.492, *p*1,2 = 0.000). The inhibitory effect was insignificant at 0.1 mIU/ml rEPO level (Tukey HSD, *p*1 = 0.051, *p*2 = 0.581). In the oxytocin treated myometrial samples, a single dose of 1 IU/ml rEPO was studied. The area under curve and frequency values of these samples were inhibited by rEPO (Student's *t*-test, n = 9, t1 = 4.776, *p*1 = 0.000; t2 = 2.835, *p*2 = 0.012, respectively).

CONCLUSIONS: rEPO inhibited spontaneous and oxytocin-induced rat myometrial contractions at 1 and 10 IU/ml concentrations. It appeared that the effect was dose-dependent.

Key Words:
Erythropoietin, EPO, Epoetin, Myometrium, Contractility.

Introduction

Erythropoietin (EPO) is a glycoprotein hormone, which is the principle regulator of erythropoiesis. It is released in response to hypoxia and, in turn, stimulates proliferation and differentiation of the erythroid progenitor cells and prevents apoptosis of proliferating blood cells[1,2]. EPO is synthesized and secreted principally by the kidneys. However, EPO activity has also been shown in extra-renal tissues including nervous tissues, cardiovascular system, male and female reproductive organs[3-10].

Recombinant human EPO (rEPO) molecules are synthetic analogues of the original molecule, which differ in carbohydrate moiety. rEPO has long been used in chronic renal failure associated anemia cases where *de novo* synthesis of this hormone is not sufficient[11]. Demonstration of anti-ischemic effects of EPO on nervous tissue has allowed development of new protocols for the treatment of ischemic events of central nervous system with rEPO[3,12].

Myometrial contractility is of clinical importance including dysmenorrhea, chronic pelvic pain and delivery of the fetus. Contractile pattern of myometrium changes throughout the men-

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strual cycle. During the follicular phase estrogen dominates and myometrial contractility increases while opposite is true for the luteal phase when effect of estrogen is overridden by progesterone[5]. Although the mechanism is currently unknown, EPO has also been shown to cause smooth muscle relaxation in vascular smooth muscle and in cardiovascular system[13,14]. Receptor of EPO has been shown to exist in skeletal muscle as well as in myoma uteri[15,16].

EPO binds to a specific receptor on plasma membrane (EPO rec.) and causes a conformational change and activation of intracellular pathways. Modulators of EPO synthesis have been reported including cytosolic calcium, cAMP and protein kinase C that are important factors involved in muscle contractility[17]. Oxytocin is released from the neurohypophysis and physiologically plays an important role in myometrial contractility. It acts on its own membrane receptor and then triggers a series of mechanisms resulting in myometrial contractions[18]. In the present study, effects of rEPO on spontaneous or oxytocin-induced contractions of rat myometrium have been investigated in an isolated organ bath system.

Material and Methods

The protocol of the present work was reviewed and approved by the Yeditepe University Experimental Ethics Committee in accordance with the Institute of Laboratory Animals Research (ILAR) Guide for Care and Use of Laboratory Animals[19].

Tissue Preparation

Adult virgin female Wistar rats were obtained from the Yeditepe University Medical School Experimental Research Center (YÜDETAM). They were housed under standard conditions with free access to standard rat pellets and tap water. Oestrous cycle of the animals was monitored by vaginal smear. Rats were decapitated at estrous stage before noon. The uteri were immediately removed and placed in a flask containing Krebs' solution of the following composition (in mmol/L): NaCl 154; KCl 5.4; CaCl2 2; MgSO4 1.2; NaHCO323.8; glucose 11.5. Experimental procedure has previously been reported by our laboratory[20].

Experiments were commenced within 5 min of removal of tissue samples. The excised uteri were carefully freed from the adjacent tissues. Muscle strips, approximately 2.0 cm in length, were prepared as tubes, which were then suspended in a 30 ml organ bath containing Krebs' solution continuously bubbled with a mixture of 95% O_2-5% CO_2 to provide pH 7.4at 37°C. The lower ends of the strips were fixed to a metal hook and the upper end was attached to an isometric force-displacement transducer (MAY 10BS99; Commat, Ankara, Turkey). The transducer signals were amplified through an interface (BIOPAC MP35 Data Acquisition System, Biopac, Ankara, Turkey) and data were analyzed on a personal computer.

Recording of Isometric Tension

Strips were allowed to equilibrate under a passive resting tension of 1 g for 30 min. After manifestation of spontaneous contractions during this equilibration period, contractions of myometrial strips were recorded either spontaneously or evoked by 10 Mm oxytocin (Postiutrin, Ibrahim Ethem, Istanbul, Turkey). Effects of cumulative concentrations of rEPO on the isometric tension of each myometrial strip were examined.

During the pre-drug period, spontaneous contractions of myometrial strips were recorded or contractions evoked by oxytocin, so that each strip served as its own control. Additionally, 8 strips (4 spontaneous, 4 oxytocin induced) were studied for four periods without addition of drug in order to serve as time controls. The effects of rEPO on the spontaneous and oxytocin-induced contractions were quantified by changes in mean amplitude (g), area under the contractile curve (AUC), and frequency. These values were analyzed and given as per cent of those of control periods. Contractile activity (mean amplitude, frequency and AUC) during the control period was taken as 100%.

Statistical Analysis

Data were analyzed statistically using SPSS 11.5 for Windows program (SPSS Inc., Chicago, IL, USA). Statistical analyses were performed by Student's *t*-test, ANOVA, analysis of variance for repeated measurements and Tukey HSD tests, as appropriate. Level of significance was set at $p<0.05$.

Results

Measurements of spontaneous and induced myometrial contractions in the control periods following 30 min of accommodation are summa-

Table I.

	Spontaneous (n=4)	Oxytocin induced (n=4)
Control (g)	920 (238%)	3699 (239.50%)
2nd (% of control)	115 (11.35%)	91 (1.25%)
3rd (% of control)	120 (14.15%)	100 (6.12%)
4th (% of control)	122 (11.78%)	102 (8.05%)

rized in Table I. Untreated 8 myometrial strips were randomized and either spontaneous (n=4) or oxytocin-induced (n=4) contractions were measured for 4 periods of 30 min. AUC values did not significantly differ by time (Table II, analysis of variance for repeated measures, f1=139.464, f2=0.606; p1-2>0.05).

For the spontaneous myometrial contractions, AUC measurements were 100±0, 78±3.139, 51±8.058 and 42±7.637% for levels of rEPO 0,0.1, 1 and 10 mIU/ml doses, respectively. AUC measurements were lowered by rEPO (ANOVA, n=9, F=20.938, p=0.001) (Figure 1). This inhibitory effect was not statistically significant at 0.1 mIU/ml rEPO level (Tukey HSD, p=0.051), but there was a significant inhibition at rEPO doses of 1 and 10 mIU/ml (Tukey HSD, p=0.001). There was no significant difference between those measures at 1 and 10 mIU/ml rEPO (Tukey HSD, p=0.660).

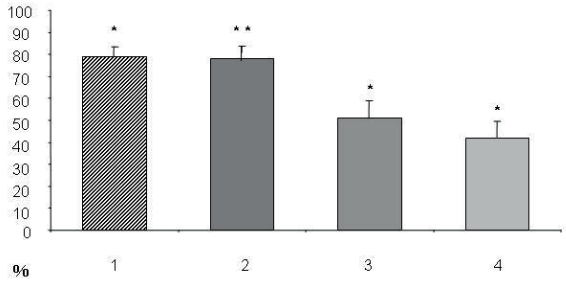

Figure 1. Effects of epoetin beta (rEPO) on area under contractile curve measurements (given as pixel) of spontaneous and oxytocin (pitocin 1 mIU/ml) induced contractions of nonpregnant rat myometrial strips at 0.1, 1 and 10 IU/ml concentrations for 30 min periods. Measures were given as per cent inhibition of the control period. 1 = oxytocin induced at 1 IU/ml rEPO; 2, 3, 4 = spontaneous contractions at 0.1, 1, 10 mIU/ml rEPO; *: p=0.000; **: p=0.051

Frequency of spontaneous contractions at rEPO concentrations of 0, 0.1, 1 and 10 mIU/ml were 100 ± 0.00 85±9.41, 61±9.24 and 17±9.11%, respectively. rEPO significantly inhibited the frequency of spontaneous contractions (ANOVA, n=9, F=20.492, p=0.001). No significant difference was observed between frequencies at 0.1 IU/ ml rEPO and control (Tukey HSD, p=0.581), whereas frequency of the contractions were found to be lower at 1 and 10 IU/ml dose of rEPO compared to the control group values (Tukey HSD, n=9, p1=0.010, p2=0.001) (Figure 2). Numbers of contractions were also lower at 10 IU/ml than 1 IU/mlr EPO (Tukey HSD, n=9, p=0.005).

No significant difference was observed between frequencies at 0.1 IU/ml rEPO and control (Tukey HSD, p=0.581), whereas frequency of the contractions were found to be lower at 1 and 10 IU/ml dose of rEPO compared to the control group values (Tukey HSD, n=9, p1=0.010, p2=0.001) (Figure 2). AUC and frequency values of these strips were 79±4.397% and 82±6.376% of the controls, respectively. rEPO decreased area under curve and frequency values of oxytocin induced myometrial contraction compared to their controls (Student's t-test, n=9, t1=4.776, p1=0.000; t2=2.835, p2=0.012, respectively).

Table II.

	Spontaneous (n=9)	Oxytocin induced (n=9)
Mean amplitude of contractions (g.)	0.330±0.038	1.964±0.498
Area under contractile curve (pixel)	590±66	3537±898
Amplitude of maximal c ontractions(g)	2.157±0.315	4.018±0.805
Frequency (in 30 min.)	14.889±1.148	39.111±4.303

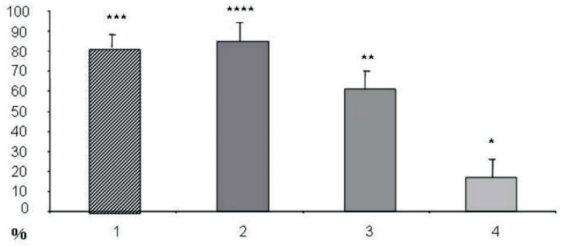

Figure 2. Effects of epoetin beta (rEPO) on area frequency of spontaneous and oxytocin (pitocin 1 mIU/ml) induced contractions of nonpregnant rat myometrial strips at 0.1, 1 and 10 IU/ml concentrations for 30 min periods. Measurements were given as per cent inhibition of the control period. 1=oxitocin induced at 1 IU/ml rEPO, 2, 3, 4=spontaneous contractions at 0.1, 1, 10 mIU/ml rEPO; *: $p = 0.000$; **: $p = 0.010$; ***: $p = 0.012$; ****: $p = 0.581$

Discussion

The present study has demonstrated that rEPO inhibited both frequency and amplitude of the spontaneous and oxytocin-induced rat myometrial contractions in isolated organ bath system in a dose-dependent manner. To the best of our knowledge, this is the first study in the literature reporting the effects of EPO on myometrial contractions.

Plasma EPO activity in a healthy human ranges between 6-32 IU/ml; these values do not differ between men and women[21,22]. Originally, 1 IU of EPO has been defined as the amount needed for the same activity on erythropoiesis with 5 μM cobalt[10,17]. In our work, the treatment doses of rEPO in the experiments correspond to the normal plasma EPO levels, and it is concluded that EPO inhibits the myometrial contractility in physiological concentrations. Myometrial contractility is critically important during pregnancy. Normal pattern of myometrial contractility is hindered in cases such as preterm labor, post term pregnancy and postpartum hemorrhage. Serum EPO levels increase early in the pregnancy to a plateau in the second trimester when physiological anemia secondary to hemodilution is maximal, and decreases towards the term when myometrium becomes more contractile[23,24]. These changes appear to coincide well with plasma EPO activity[16]. Another interesting event is the promotion of the spontaneous contractions in labor, which occur more often at night. The cause of this event is currently notknown[23,25]. Renal and extra-renal synthesis of EPO also follows a circadian rhythm[26].

Synthesis of EPO is abolished after 6 pm, so the plasma activity of EPO decreases to almost zero at night[17]. Together with the finding of

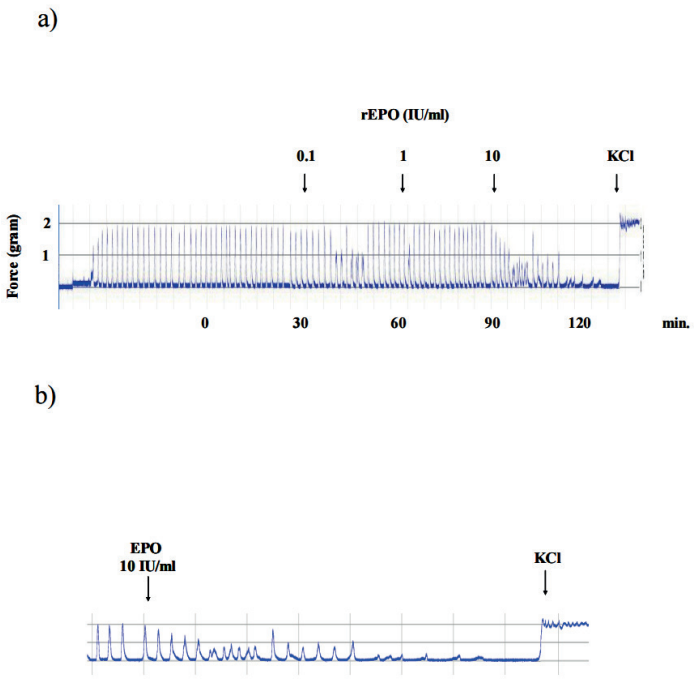

Figure 3. ***(A-B)*** Illustration of spontaneous contractions of rat myometrium in organ bath and the effect of epoetin beta (rEPO) at 0.1, 1 and 10 IU/ml concentrations for 30 min periods.

inhibitory effect of EPO on myometrium, it is suggested that coupling of the onset of the term and preterm labor contractions and cessation of EPO activity at night are among the cocktail of factors involved in decreasing myometrial quiescence near the term.

Half-life of EPO in human plasma is about 5 h and mechanism of its clearance is unknown[19]. In the experimental protocol of our study, myometrial contractions at different concentrations of EPO were measured by adding EPO cumulatively to the organ bath chamber every 30 min while contractions were continuously recorded. Serial measurement of myometrial contractility of 30 min periods for each concentration might fail to demonstrate the whole effect of this molecule on contractility, which in part could occur in a longer period of exposure. So, the effect of EPO in different concentrations might be repeated by using a different protocol that each experiment consisting of measurement of a single EPO concentration and/or for a longer period, in order to elucidate the confounding effect of time on the results. This could also be attributed to a possible effect of non-muscular tissues in the uterus such as unmyelinated afferent nerve fibers, which are known to serve also as effectors by releasing neuromediators stimulating or inhibiting myometrial contractions. This possible effect might be examined simply by incubating the muscle strips within a high concentration of capsaicin solution for a short period.

Minimal and maximal effective concentrations of EPO should be further examined. Two cytosolic events have been shown to be changed by EPO in a variety of tissues *in vivo* (i.e. calcium ions), which also affect contractility may contribute or terminate the effect of EPO on contractility[17]. Long-term effects of EPO on myometrial tissue including hyperplasia, hypertrophy and angiogenesis would be another issue to be investigated *in vivo* to clarify the net effect of this hormone on myometrium.

Conclusions

Spontaneous and oxytocin induced non-pregnant rat myometrial contractions in isolated organ bath chamber were inhibited by rEPO at 1 and 10 IU/ml significantly. Further studies are needed to clarify the mechanism of this inhibition as well as the possible effect of EPO on pregnant myometrium.

Funding
This study was supported by Yeditepe University Research Foundation.

Authorship
All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval to the version to be published.

Conflict of interest
Niyazi Tug, Habibe Ayvaci, Mehmet Akif Sargin, Bilge Dogan Taymur, Ahmet Ayar, Ertugrul Kilic and Bayram Yilmaz declare that they have no conflict of interest.

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