# A role for adrenergic receptors in the uterotonic effects of ergometrine in isolated human term non-laboring myometrium

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## ABSTRACT

**Background:** Ergometrine is a uterotonic agent that is recommended in the prevention and management of post partum hemorrhage. Despite its long-standing use the mechanism by which it acts in humans has never been fully elucidated. The objective of this study was to investigate the role of adrenoreceptors in ergometrine's mechanism of action in human myometrium. The study examined the hypothesis that alpha adrenoreceptor antagonism would result in the reversal of the uterotonic effects of ergometrine.

**Methods:** Myometrial samples were obtained from women undergoing elective cesarean delivery. The samples were then dissected into strips and mounted in organ bath chambers. Following generation of an ergometrine concentration-response curve ($10^{-15}$ to $10^{-5}$M), strips were treated with increasing concentrations of ergometrine ($10^{-15}$ to $10^{-7}$M) alone and ergometrine ($10^{-7}$ to $10^{-5}$M) in the presence of phentolamine ($10^{-7}$ M), prazosin ($10^{-7}$ M), propranolol ($10^{-6}$ M) or yohimbine ($10^{-6}$ M). The effects of adding ergometrine and the effect of drug combinations were analysed using linear mixed effects models with measures of amplitude (g), frequency (contractions/10min) and motility index (g*contractions/10min).

**Results:** A total of 157 experiments were completed on samples obtained from 33 women. There was a significant increase in the motility index (adding 0.342 g*counts/10min/μM; 95% CI from 0.253 to 0.431, P<0.001), amplitude (0.078 g/μM; 95% CI, from 0.0344 to 0.121, P=5e-04) and frequency (0.051 counts/10min/μM; 95% CI, 0.038 to 0.063, P<0.001) in the presence of ergometrine. The α adrenergic antagonist phentolamine and the more selective $\alpha_1$ adrenergic antagonist prazosin, inhibited the ergometrine mediated increase in motility index, amplitude and frequency (-1.63 g*counts/10mins/μM and -16.70 g*counts/10mins/μM for motility index, respectively).

**Conclusions:** These results provide novel evidence for a role for α adrenergic signaling mechanisms in the action of ergometrine on human myometrial smooth muscle in the *in vitro* setting. Information that sheds light on the mechanism of action of ergometrine may have implications for the development of further uterotonic agents.

**Key Words**: ergometrine, human myometrium, phentolamine, prazosin, propranolol, alpha adrenergic receptors.

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## INTRODUCTION

Powerful and efficient myometrial contractions and retractions are essential to compress the uterine vasculature arresting blood flow from the placental bed after delivery of the foetus and placenta[1]. Failure of the uterus to contract leads to uterine atony and postpartum haemorrhage (PPH). PPH remains a common cause of both maternal morbidity and mortality[2-4]. Evidence is emerging of increasing rates of PPH worldwide[2, 5-8] which has largely been attributed to an increased incidence of uterine atony[2, 7, 9, 10]. Using prophylactic uterotonic agents compared to conservative management lowers maternal blood loss and reduces the risk of PPH[11]. Oxytocin is commonly used as the first line utertonic agent but if the uterus fails to contract adequately current practice guidelines recommend the use of additional utertonic agents to prevent and treat PPH such as ergometrine, misporostol and carboprost[12-14]. Availability of pharmacological agents that exert their effects through alternative receptor mechanisms is extremely relevant in light of emergencing evidence of decreased responsiveness to oxytocin in myometrium previously exposed to oxytocin[15, 16]. Despite the long standing use of ergometrine, the exact mechanism by which it exerts its uterotonic effect in human tissue has never been fully elucidated. Its administration has been linked with an increase in frequency of myometrial contractions corresponding with increased basal tone. The increase in basal tone is thought to be due to an effect on the inner layer of the myometrium, as this layer is rich in adrenoceptors, and reacts to epinephrine administration with an increase in tonus and frequency of contractions[17,18]. The mechanism of action of ergometrine has been linked to stimulation of human $\alpha_1$ adrenoreceptors[19, 20]. In this study we have investigated the effects of a number of adrenoceptor ligands on the action of ergometrine on isolated human myometrial smooth muscle.

## METHODS

### Subjects

The study was conducted as a prospective laboratory investigation. Biopsy specimens of human pregnant myometrial tissue were obtained from non-laboring women undergoing elective lower segment cesarean delivery. All patients were ASA physical status 2 pregnant women and gave written informed consent. Ethical approval for the study was obtained from the Research Ethics Committee of the Coombe Women and Infants University Hospital (2006-22). All samples were collected during the four months from September to December in each of 2011, 2012, and 2013. Women with a singleton gestation at 38-40 weeks who were not in labor prior to their cesarean delivery were considered for inclusion. Exclusion criteria included ultra-sound findings consistent with fetal IUGR, polyhydramnios or oligohydramnios, history of a chronic medical condition in pregnancy or pregnancy related condition requiring the use of medication, history of ruptured membranes, a diagnosis of human immunodeficiency virus, hepatitis B or C, suspected abnormal placentation and a booking BMI >30 kg/m$^2$. Indications for cesarean delivery included breech presentation and prior cesarean delivery. All patients received antacid prophylaxis with 30 ml of 0.3 M sodium citrate and 400 mg cimetidine orally preoperatively and a spinal anesthetic with 2.0 to 2.4 ml 0.5% hyperbaric bupivacaine, with 20 to 25 microgram (mcg) of intrathecal fentanyl and 100 to 150 mcg of intrathecal morphine. Oxytocin (Novartis Pharmaceuticals UK Ltd., Horsham, West Sussex, UK) 5IU was administered by slow intravenous bolus following delivery of the baby and cord clamping.

### Tissue preparation

The myometrial biopsy was excised from the midline of the upper margin of the lower uterine segment incision (inner myometrial layer) following delivery of the baby and placenta. All specimens were thoroughly rinsed in Ringer's lactate solution ensuring all traces of blood were removed and that the specimen was free of placental tissue. For tissue bath experiments the biopsies were placed in a sterile container and refrigerated at 4ºC until used, which was within 2 to 20 h of collection.

### Contractility Analysis

Biopsies were dissected into longitudinal muscle strips of approximately 12 by 5 by 2 mm. Isometric tension recordings were obtained from an eight-chamber organ bath (10 ml, water jacketed) system (Myobath, World Precision Instruments Inc. Sarasota, Florida). The organ baths contained Krebs-Henseleit physiological salt solution (PSS; NaCl 118 mmol/l, D-glucose 11.1 mmol/l, $NaHCO_3$ 24.9 mmol/l, $MgSO_4$ 1.2 mmol/l, KCl 4.7 mmol/l, $KH_2PO_4$ 1.2 mmol/l, and $CaCl_2$ 2.5 mmol/l, pH 7.4) and were aerated with a gas mixture of 95% $O_2$ and 5% $CO_2$ and maintained at 37 ºC. Myometrial strips were then allowed to equilibrate at 1 to 2 grams (g) tension until a steady tension was achieved as previously described[21]. During this equilibration period, the Krebs solution was changed every 10 min. When spontaneous contractions became regular (within 60-90 min) the amplitude (g) and frequency (contractions/10min) were recorded as follows: (1) a 30 min control period followed by cumulatively increasing concentrations of ergometrine every 30 min from $10^{-15}$ M to $10^{-8}$ M (n=14) or $10^{-7}$ M to $10^{-5}$ M (n=22) and (2) a 30 min control period followed by the addition of antagonist and then 30

min later by cumulatively increasing concentrations of ergometrine from $10^{-7}$ M to $10^{-5}$ M. A time matched control strip from each patient, exposed only to Krebs solution was run in parallel with each separate experiment to ensure tissue viability for the duration of the experiment. In addition a time-matched control for antagonists alone (over 120 min) was also carried out. The motility index (amplitude x frequency; g*contractions/10min) was calculated to determine the uterine activity and the strength of contractions with the use of the Powerlab software, Chart (version 5.0; AD Instruments Pty Ltd, Bella Vista, NSW, Australia). The primary outcome was the motility index of myometrial contractions induced by ergometrine, epinephrine or antagonists. Secondary outcomes included amplitude and frequency parameters.

**Reagents**

All stock solutions of drugs were prepared according to the supplier's instructions. All stock solutions were stored at -20˚C. Drugs were diluted further immediately before each experiment from the stock solution using Krebs solution. Following completion of the experiment the weight of each muscle strip was recorded to ensure weight and size equality. Ergometrine (Hameln pharmaceuticals ltd, Gloucester, United Kingdom), oxytocin (5 IU/mL, Sigma-Tau Industrie Farmaceutiche Riunite, Spain) and norepinephrine were prepared using Krebs-Henseleit physiological salt solution (PSS). Phentolamine hydrochloride, prazosin hydrochloride and yohimbine hydrochloride were dissolved in distilled water, (S)-(−)-propranolol hydrochloride was dissolved in ethanol. All products (unless otherwise indicated) were purchased from Sigma Aldrich, UK. All drugs and vehicle solutions were prepared freshly each day. Table 1 summarises the pharmacological properties of the agents used.

**Table 1.** Patient characteristics. Values presented as mean (standard deviation). Only data from the patients included in the final analysis are presented here

| | |
|---|---|
| Age (years | 31.6 (2.7) |
| Weight (Kg) | 70.8 (8.3) |
| Body Mass Index (Kg/m$^2$) | 26.1 (2.4) |
| Gestational age (weeks) | 38.6 (0.1) |
| Indications (%) Repeat | 72 |
| Breech | 15 |
| History of genital tract trauma | 13 |

**Statistical Methods**

**Effect of ergometrine alone on myometrial contractility**

In order to study if ergometrine had a statistically significant effect on contractions we used a linear mixed effect (LME) model[22]. In LME the effects of variables are considered either fixed or random. The fixed effects are the variables that act equally on all observations. The random effects refer to patient specific effects[23]. We chose a random intercept per patient, and to adjust for within patient correlation, a compound symmetry covariance structure (CSS) was used. A CSS assumes that observations from the same patient are equally correlated[24]. We compared two LMEs to determine what independent effect if any, ergometrine had on each contraction measurement. The first LME, the null model, had two variables, a random and a fixed intercept. This LME assumes that all the variance in contractions can be explained by patient specific effects and does not account for any effect that ergometrine may have. The second LME, the full model, had three variables, a random and fixed intercept, and the concentration of ergometrine. The second LME assumes that the variance of contractions can be explained by patient specific effects and the action of ergometrine. We used an ANOVA test to compare the two models. The analysis was performed using the NLME package in R[25].

**Sample size calculation for the effects of ergometrine on myometrial contractility**

In order to calculate the number of observations needed to detect an effect of ergometrine, we set a statistical power of 80% to detect an effect size of f-squared = 0.05. This effect size refers to the lower limit of detection that the test statistic (in our case, the ANOVA) can detect, while preserving the statistical power selected. An effect size of 0.05 is equivalent to an increase of 5% of the explained variance in contraction measurements by ergometrine in comparison to the variance in the data that is unexplained by neither ergometrine nor patient specific effects[24,25]. Given samples available from 23 patients, *post hoc* power calculation indicated that the available data was sufficient to identify an effect size of f-squared = 0.05 with 83% power. This N was chosen to cover concentrations of ergometrine from $10^{-15}$ M to $10^{-10}$ M and in a separate experiment, 15 samples from $10^{-9}$ M to $10^{-5}$ M. No individual experiment was duplicated in any one patient tissue. The analysis was performed using the PWR package in R.

**Effect of ergometrine combined with the other drugs**

As in the previous section, for Figures 4 and 5 we compared two LMEs to determine the effect of drugs in combination with ergometrine on contraction measurements independently. In this case, the null model had a random and fixed intercept, and the concentration of ergometrine was the fixed effect. The full model included the same variables as the null model plus a binary variable that represents the presence or absence of any of the drugs assayed. We used an ANOVA test and the Benjamini-Hochberg procedure to correct for the multiple tests. The effects of drugs whose full model had an adjusted P-value < 0.05 were accepted as statistically significant. The analysis was performed using the NLME package in R.

**Sample Size Calculation of ergometrine combined with the other drugs**

As previously described, we set a statistical power of 80%. Since a multiple comparisons procedure was in use we set a significance level of 0.0125 estimated using Boole's inequality to take into consideration multiple comparisons adjustment. This time we selected a moderate to high effect size of 0.23. The analysis indicated a statistical power of 80%, p-value of 0.0125, effect size of 0.23 required of N=60 observations from 8 patients. The package PWR in R was used to calculate the sample sizes. As in the previous section, no individual experiment was duplicated in any patient tissue.

**RESULTS**

A total of 33 myometrial samples were obtained from 33 patients yielding 157 strips in total. The characteristics of the myometrial tissue donors are shown in Table 2. Figure 1 summarizes patient recruitment and sample distribution for all of the experiments. Those strips which failed to establish a regular frequency (contractions/10min) within 90 min were excluded (15 strips). There were no incidents of PPH in any of the patients who consented to participate.

**Table 2.** Pharmacological properties of the antagonists used in the study

| **Agent** | **Receptor affinity** |
|---|---|
| Propranolol | Non-specific β adrenoreceptor |
| Phentolamine | Non-specific α adrenoreceptor |
| Prazosin | $\alpha_1$ adrenoreceptor |
| Yohimbine | $\alpha_2$ adrenoreceptor high affinity; $\alpha_1$ adrenoreceptor moderate affinity; some affinity dopamine and 5-HT receptor |

**Generation of spontaneous contractions**

In all experiments during the equilibrium period, the basal amplitude tension generated was 4.1±1.2 g; n=36), the frequency (contractions/10min) was 1.39±0.21 and motility index (g*contractions/10 min) was 5.5±1.9. Myometrial contractions were characterized by a slow rising phase, followed by a plateau, after which there is a phase of relaxation which occurs at a rate that is almost identical to that of the rising phase. Figure 2A shows

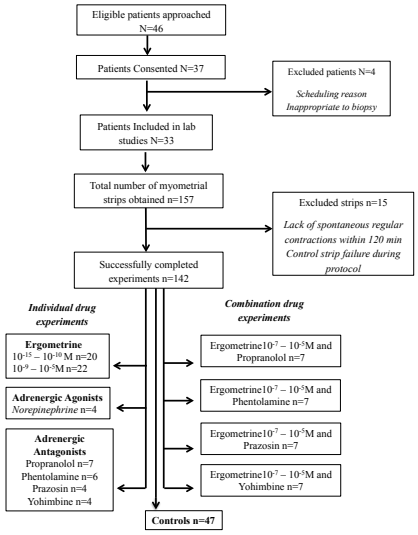


**Figure 1**
*Flowchart showing patients and myometrial samples in the various groups. N = number of patients and n = number of experiments. At least one control strip from each myometrial sample was used for each experiment.*

representative traces of the spontaneous contractions observed in the absence and presence of increasing concentrations of ergometrine.

Fig 2

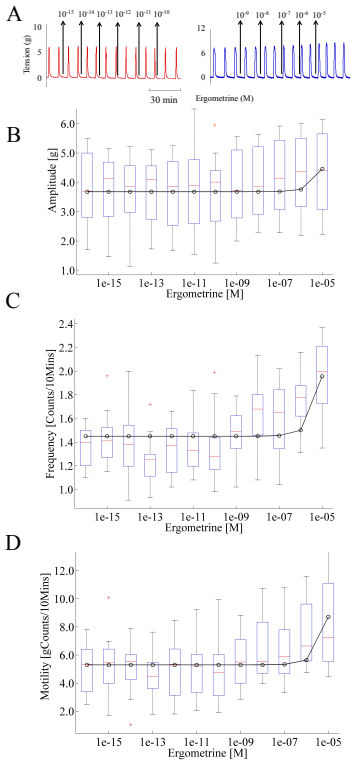


**Figure 2**
*Effects of increasing concentrations of ergometrine from $10^{-15}$ M to $10^{-5}$ M on spontaneous contractions in human myometrium. **A**. Sample traces showing spontaneous contractions over a 3 hr period in the presence of increasing concentrations of ergometrine, $10^{-15}$ M to $10^{-10}$ M, left and $10^{-9}$ M to $10^{-5}$ M, right. B-D. Concentration-response curves of (B) amplitude (g), (C) frequency (contractions/10 min) and (D) motility index (g*contractions/10min) for ergometrine treated samples. The curves are from regression models, whereas the box plots are from the actual data.*

**Effects of ergometrine on spontaneous myometrial contractions**
We analysed the effect of ergometrine on myometrial contraction using an LME and quantified it with a model coefficient obtained after taking into consideration the within patient correlation. The model coefficient is equivalent to how much the contraction measurement would change per µm of ergometrine added. It was found that there was a significant increase in the motility index of contractions in the presence of ergometrine (0.342 g*counts/10min/µM; 95% CI, from 0.253 to 0.431, P<0.001; n=15; Figure 2D). Similarly, ergometrine also increased the amplitude of spontaneous contractions adding 0.078 g/µM (95% CI, from 0.034 to 0.121, P=5e-04; n=15; Figure 2B) and frequency (adding 0.051 counts/10min/µM; 95% CI, 0.038 to 0.063, P<0.001; n=15; Figure 2C).

**Effects of noradrenergic ligands on myometrial contractions**
Norepinephrine applied at increasing concentrations from $10^{-7}$ to $10^{-5}$M had an increasing effect on the motility index of spontaneous contractions based on visual inspection of the data (Figure 3A). Since tissue was only available from 5 patients we did not carry out a full statistical assessment of this data. The effect of prazosin (0.1µM), phentolamine (1µM), propranolol (0.1µM) and yohimbine (1µM) applied alone on uterine contractions were measured in strips from 4 different patients. These preliminary data indicated little effect of prazosin, propranolol or yohimbine on myometrial contractility. Application of phentolamine in all four tissues gave rise a small reduction in myometrial contractions. Again since tissue was only available from 4 patients in this case we did not carry out a full statistical assessment of this data (Figure 3B-E).

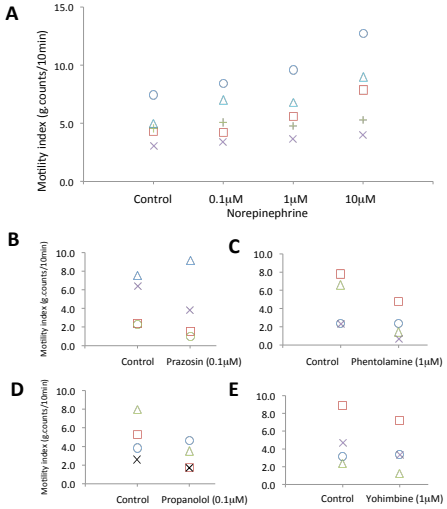


**Figure 3**
Effects of noradrenergic drugs alone on myometrial contractility. **A.** Effects of increasing concentrations of norepinephrine from $10^{-7}$ M to $10^{-5}$ M on the motility index (g*contractions/10min) of spontaneous contractions in human myometrium. The data from 5 tissues from separate patients at each concentration is represented in a scatter plot. **B-E.** The effects of prazosin (0.1µM; B), phentolamine (1µM; C), propranolol (0.1µM; D) and yohimbine (1µM; E) alone on the motility index of myometrial contractions. The data from 4 tissues from separate patients in the presence of drug is represented in a scatter plot and compared to control tissue.

**Effect of ergometrine in the presence of propranolol, phentolamine, prazosin and yohimbine on myometrial contractions**
The effects of ergometrine on motility index and frequency in the presence of both prazosin (Figure 4A-C) and phentolamine (Figure

4D-F) were inhibited when compared to ergometrine alone. The LME for prazosin and phentolamine in frequency yielded negative coefficients, namely -1.93 counts/10min/μM (95% CI, from -3.381 to -0.725, FDR adjusted P=0.0018) and -0.205 counts/10min/μM (95% CI, from -0.338 to -0.060, FDR adjusted P=0.004) respectively. The effect was similar for the motility index where prazosin and phentolamine had coefficients of -16.70 g*counts/10min/μM (95% CI, from -26.704 to -6.609, FDR adjusted P=0.002) and for phentolamine, -1.63g*counts/10min/μM (95% CI, from -2.637 to -0.628, FDR adjusted P=0.007); all data n=7-9. In contrast,

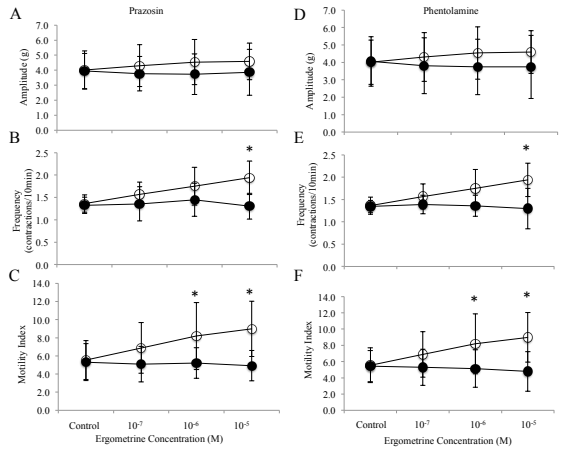


**Figure 4**
Concentration-response curves for ergometrine ($10^{-7}$ M to $10^{-5}$ M) in the absence (O) and presence (•) of prazosin (**(**$10^{-7}$ M; **A-C**) and phentolamine ($10^{-6}$ M; **D-F).** The effect of ergometrine in the presence of prazosin on myometrial contractions is shown for amplitude (g) (A), frequency (contractions/10 min) (**B**) and motility index (g*contractions/10min) (**C**). The effect of ergometrine in the presence of phentolamine on myometrial contractions is shown for amplitude (g) (D), frequency (contractions/10 min) (**E**) and motility index (g*contractions/10min) (**F**).

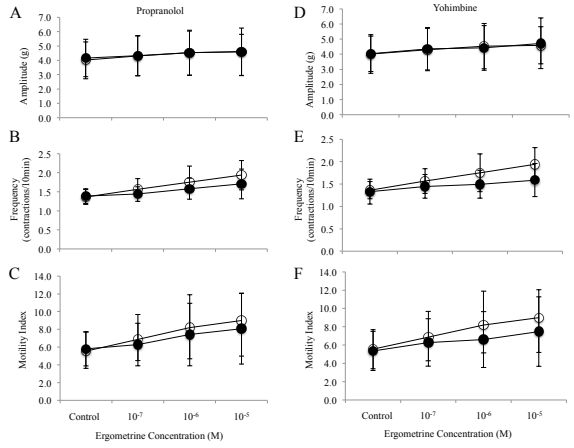


**Figure 5**
Concentration-response curves for ergometrine ($10^{-7}$ M to $10^{-5}$ M) in the absence (O) and presence (•) of propranolol (**(**$10^{-7}$ M; **A-C**) and yohimbine ($10^{-6}$ M; **D-F).** The effect of ergometrine in the presence of propranolol on myometrial contractions is shown for amplitude (g) (A), frequency (contractions/10 min) (**B**) and motility index (g*contractions/10min) (**C**). The effect of ergometrine in the presence of yohimbine on myometrial contractions is shown for amplitude (g) (D), frequency (contractions/10 min) (**E**) and motility index (g*contractions/10min) (**F**).

ergometrine in the presence of both propanolol and yohimbine had a similar effect on spontaneous contractions to that observed for ergometrine alone (Figure 5A-C and Figure 5D-F).

## DISCUSSION

In this study we analysed the effects of ergometrine on spontaneous human myometrial contraction *in vitro* using the parameters of amplitude, frequency and motility index. Previous human work has shown that ergometrine increases the frequency of uterine contractions, possibly by increasing the basal tone such that the time taken to reach maximal contraction was reduced[19]. Our results show that concentrations above $10^{-9}$M have a significant effect on frequency of contractions and above $10^{-8}$M on amplitude and motility index. Furthermore we have shown that these effects are mediated through alpha adrenoceptors. These findings are novel as the mechanism of action of ergometrine in human myometrium has not been described using this methodology before.

Unlike oxytocin a specific ergometrine receptor has never been described. In general ergot alkaloids act as partial agonists at alpha-adrenoreceptors[26]. Furthermore the oxytocic effect of ergometrine has been attributed to the inner as opposed to the outer muscle layer in human[27] and animal myometrium[28] where it is believed to cause sustained tonic uterine contraction in both the upper and lower uterine segments. This study has found strong evidence implicating $\alpha_1$, but not $\alpha_2$, adrenoceptors in the mechanism of action of ergometrine. Phentolamine (a non-selective α adrenergic antagonist) alone appeared to have a small reducing effect on the motility index of contractions (Figure 3C) although further experiments will be required to see if this effect is significant. Previous work investigating the effects of phentolamine with different methodologies has produced conflicting results[18,29,30]. However, in our experiments phentolamine was observed to significantly reduce the uterotonic effect of ergometrine on the frequency and motility index of contractions (Figure 4E, 4F). Likewise, while the specific $\alpha_1$ adrenoceptor antagonist, prazosin, had little effect on the parameters of contraction measured when exposed to tissue on its own (Figure 3B), we also observed prazosin to significantly attenuate the effects of ergometrine on frequency and motility index (Figure 4B, C). It was interesting to note that the effects of the non-specific phentolamine and the more specific prazosin had similar effects on the actions of ergometrine over the differenct concentrations. In contrast, yohimbine (a $\alpha_2$-adrenoceptor antagonist) did not alter the effects of ergometrine on any of the contractile parameters measured (Figure 5 D-F). Moreover, propanolol (a non-selective β adrenoceptor antagonist) had no significant effect on the uterotonic effect of ergometrine (Figure 5A-C). The effects of selective β adrenoceptor antagonists were not investigated. The results combined give strong support to the hypothesis that ergometrine functions at least partially through the stimulation of α, adrenoceptors in the human myometrium.

Previous work has drawn a direct link between ergometrine and the activation of postsynaptic $\alpha_1$ receptors in mice anococcygeus muscle[31]. There is no published research looking at adrenoreceptor number and distribution in the myometrium at term or the exact mechanisms by which stimulation of α adrenoceptors results in uterotonic effects. It is also notable that phenylephrine, which is being increasingly used for the management of spinal anesthesia induced maternal hypotension, does not appear to result in any significant consequential uterotonic effect in the range of phenylephrine doses used clinically. The reasons for this can only be postulated - variation in the alpha-receptor subtype, expression and density present on various smooth muscle types, the possibility that an additional receptor type such as 5HT receptors are involved in the action of ergometrine resulting in a synergistic response, or perhaps because local concentrations of phenylephrine are insufficient to activate the relevant signalling pathways. There are no phenylephrine concentration effect studies published in this tissue type and this certainly warrants further investigation.

With the incidence of PPH (in some cases requiring blood transfusion and/or hysterectomy) having increased significantly over the past

decade[2-4, 6] uterotonics have become increasingly important in clinical practice. Additionally there is evidence of attenuation of the contractile response to oxytocin alone in myometrium previously exposed to oxytocin with a superior contractile response recorded with the combination of oxytocin and ergonovine or carboprost[16, 32]. This highlights the role of these uterotonics either prophylactically or as treatment in cases of poor uterine tone in response to oxytocin treatment alone, especially when the uterus has been pre-exposed to oxytocin during labor - a relatively commonly encountered clinical scenario. The need to optimize preventative and therapeutic clinical practices in order to reduce rates of atonic PPH has recently been highlighted[33].

This study is subject to limitations inherent in the *in vitro* environment, which fails to encompass the complexity of chemical and mechanical interactions in the *in vivo* environment. It is however an accepted method of studying the effects of agents that modulate contractility in the myometrium and each strip had a control strip, harvested from the same individual, for the duration of the experimental protocol[34]. Drug concentrations that were chosen were based on standard dose-response studies previously published[16, 32] but may not reflect either the plasma or local myometrial concentrations, which have not previously been measured in the term parturient.[19]

**Conclusion**

In summary, we have demonstrated the role of adrenergic receptors in mediating the uterotonic effects of ergometrine most likely via the $\alpha_1$ adrenoceptor. These findings are of clinical importance, with the rates of uterine atony and PPH increasing worldwide, there is a need to optimise preventative and therapeutic strategies for uterine atony[1]. Elucidating the mechanism of action may lead to the development of novel agents on the same therapeutic targets that may have a more favourable side effect profile.

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