Title page

The combination tocolytic effect of $MgSO_4$ and an oxytocin receptor antagonist in myometrium from singleton and twin pregnancies.

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Conflict of interest

The authors report no conflict of interest

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Condensation and short version of title

Condensation:

The tocolytic potency of $MgSO_4$ is reduced by oxytocin but is reversible by an oxytocin receptor antagonist and is equal in twin and singleton myometrium.

Short version of the article title:

Combination tocolysis *in vitro* involving $MgSO_4$ and atosiban

Abstract

Background: Preterm birth before 37 weeks' gestation is the most common and costly complication of pregnancy and remains the leading cause of neonatal morbidity, mortality and reduced achievement in surviving infants. Magnesium sulfate is one class of tocolytics for threatened preterm labor however its clinical efficacy has been questioned. Twin pregnancies are at increased risk of preterm delivery compared to singleton gestations, suggesting there is twin-specific risk to preterm delivery in twins. The prevention strategies applied to singleton pregnancies however have not been shown to be effective in twin pregnancies.

Objective(s): To compare the relaxant effect of magnesium sulfate ($MgSO_4$) on spontaneous and oxytocin–augmented contractions of human myometrium from singleton and twin pregnancies and examine whether the effect of oxytocin on $MgSO_4$'s potency could be reversed using the oxytocin receptor antagonist, atosiban.

Study Design: Myometrium was obtained at the time of pre-labor cesarean section (36-40 weeks gestation) from women with singleton (n=23) or twin (n=12) pregnancy. Isometric tension recordings were made on myometrial strips mounted in organ baths superfused with physiological saline. Strips were exposed to rising concentrations of $MgSO_4$ and the effect on spontaneous contractions or stimulated with oxytocin (0.5nmol/L) and in the presence or absence of atosiban (100nmol/L) was recorded. The contractile characteristics after each application of $MgSO_4$, including amplitude of contraction and activity integral, were measured. Concentration-response curves were fitted using non-linear regression and comparison of the -logIC50 values.

Results: $MgSO_4$ exerted an equal concentration-dependent inhibitory effect on spontaneous myometrial contractions from both singleton and twin myometrium ($P>0.05$). The

application of oxytocin produced a significant rightward shift in the concentration-response curves ($P<0.001$) but no differences were found between pregnancy groups ($P>0.05$). The addition of atosiban shifted concentration-response curves significantly back to the left for amplitude of contraction and activity integral in singletons ($P<0.001$). However, only activity integral was significantly reversed in twins ($P<0.0001$).

Conclusion(s): $MgSO_4$ is equipotent in suppressing contractions in singleton and twin myometrium. Oxytocin (0.5nmol/L) significantly reduces the tocolytic potency of $MgSO_4$ which may explain in part, $MgSO_4$'s poor efficacy *in vivo* but this can be partially reversed using an oxytocin receptor antagonist. Combination tocolysis involving oxytocin receptor antagonists requires further investigation.

Keywords: atosiban, contraction, magnesium sulfate, $MgSO_4$, myometrium, oxytocin, oxytocin receptor antagonist, pregnancy, tocolysis, twins, uterus

Introduction

Preterm birth (delivery <37 weeks' gestation) is the leading cause of perinatal morbidity and mortality. The majority of cases of preterm birth result from spontaneous preterm labor onset involving premature uterine contractions or premature rupture of the membranes.[1] A number of pharmacological agents have been advocated as suppressants of uterine contractions (tocolytics) including Magnesium sulfate ($MgSO_4$).

In vitro, magnesium (Mg) is known to relax smooth muscle and studies in myometrium have also found it to significantly decrease both spontaneous and induced (e.g. with oxytocin) uterine contractions.[2-5] However despite these clear laboratory findings, few clinical trials have found $MgSO_4$ tocolysis *in vivo* to be effective in preventing preterm birth or reducing newborn morbidities or mortality when compared with an alternative or no tocolytic (placebo) treatment.[1, 6] This lack of efficacy has led the obstetric community to question its use as a tocolytic.[7] The American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine limit their support for the use of $MgSO_4$ for 'short-term prolongation of pregnancy (up to 48 hours) to allow for the administration of antenatal corticosteroids in pregnant women who are at risk of preterm delivery within 7 days'.[8]

In vitro studies have shown the concentration of Mg required to abolish spontaneous contractions to be around 2-3mmol/L but concentrations required to inhibit oxytocin-augmented contractions were much greater (around 8-10mmol/L) which is much higher than that reported to be safe therapeutically[5, 9]. We therefore questioned: Could we use an oxytocin receptor antagonist to increase the tocolytic potential of $MgSO_4$ by removing the negative influence of oxytocin on Mg's potency?

Multiple pregnancies e.g. twins, are especially confounded by higher rates of preterm birth with ~50–60% of twins reported to deliver preterm compared to ~11% of singletons,[10, 11] suggesting there is a twin-specific risk to preterm delivery. The preterm birth prevention strategies applied to singleton pregnancies have not been found to be effective in twin pregnancies[12] and the evidence for the use of tocolysis in twins is limited.

The aims of this study were therefore to investigate 1) the inhibitory effect of $MgSO_4$ on spontaneous and oxytocin-augmented contractions on strips of human myometrium in our *ex vivo* model, 2) to examine for differences in the potency of $MgSO_4$ between myometrium from singleton and twin pregnancies and 3) whether the effects of oxytocin could be reversed or reduced with the use of the oxytocin-receptor antagonist, atosiban.

Materials and Methods

Sample collection and preparation

Myometrial tissue was obtained with written informed consent from 35 women undergoing elective, pre-labor Caesarean section (CS).[13] All women were between 23 and 43 years of age, with a singleton (n=23) or twin (n=12) pregnancy, between 36–40 weeks of gestation. Full details of the demographics for all women according to pregnancy group (singleton or twin) are given in **Table 1**. As expected the median length of gestation was significantly shorter and birth weight (combined in the case of twins) was significantly higher in the twin group ($P<0.001$). Local Research Ethics committee (Liverpool East, REC Ref 10/H1002/49) and institutional review boards of the Research and Development department, Liverpool Women's Hospital and University of Liverpool approved the study.

At operation, myometrial tissue was excised from the upper lip of the lower uterine incision site following delivery of the baby and placenta.[14] Samples were used immediately or within 12 hours of collection with storage at 4°C. In the laboratory, multiple strips (5mm x 2mm x 1mm) were dissected along the direction of longitudinal fibres, as previously described.[15] Strips were attached to aluminium clips and placed in 1mL organ baths continually superfused with physiological saline (PSS, in mmol/L: 154 NaCl, 5.6 KCl, 1.2 $MgSO_4$, 7.8 glucose, 10.9 HEPES and 2.0 $CaCl_2$) at a rate of 1.5ml/min, pH 7.4 at 36°C. One end was attached to a fixed hook and the other to a FORT 10g force transducer (World Precision Instruments, UK). Each sample was subjected to 2mN stretch and left to equilibrate in PSS until spontaneous contractions were established, typically within 2 hours.[16] Contractions were recorded at a sampling rate of 10Hz via a data acquisition system running the associated software (Labscribe 2, WPI UK).

Experimental approach

The experimental methods were based upon our previous studies of tocolytic potency in myometrium.[13] For spontaneous contractions, strips were left to equilibrate for 1 hour after their onset or until a minimum of 4 consecutive contractions of equal amplitude and regular frequency were achieved. For oxytocin–induced contractions, strips were allowed to equilibrate then contractions were stimulated with oxytocin (0.5nmol/L) for a minimum of 45 minutes to achieve a stable baseline activity as previously described.[13] Strips were then exposed to rising concentrations of either $MgSO_4$ alone or in combination with atosiban (100nmol/L).

Each concentration of $MgSO_4$ was added at 20-25 minute intervals based upon previous data showing that maximal response to $MgSO_4$ is achieved at 20 minutes.[3, 5] Measurement of contractile activity was performed by calculation of the integral area under the tension curve in unit time referred to as 'activity integral' or AUC (arbitrary units) and mean maximum peak amplitude of contraction (expressed in mN) as previously described.[13, 17] We have previously shown that spontaneous contractions remain stable for over 3 hours of recording without significant decrease in amplitude or area under the curve during the time equivalent of experimental manoeuvres.[13] Control activity was taken as the contractile activity in the final 20-25 minutes (preceding the first concentration of $MgSO_4$). The effects of $MgSO_4$ during the final 20 minutes of its 25 minute exposure at each concentration, (allowing for 5 minutes of turn-over in the tissue bath) were calculated and expressed as a percentage of the integral and amplitude during the control period (i.e. control activity is equal to 100%).

Statistical analysis

Concentration-response curves were fitted to the logistic equation using non-linear regression (PRISM 5.0, Graph Pad Software Inc., San Diego, Ca. USA). The mean half-maximal inhibitory concentration of $MgSO_4$ which is the concentration of MgSO4 required to cause a 50% reduction in activity (IC_{50}) was calculated to examine its inhibitory effects on contraction amplitude and activity integral. To compare the effect of $MgSO_4$ between the different experimental conditions or patient groups, the negative logarithm of the IC_{50} (-logIC_{50}) values were taken and compared on the basis of the F test for the extra sum of squares principle. Unless stated otherwise, all values represent the mean ± standard error of the mean (SEM) where ''n'' is the number of samples and each representing a different woman. $P < 0.05$ was taken as level of significance.

Results

The application of $MgSO_4$ resulted in a dose-dependent decrease in both spontaneous contraction amplitude and activity integral in all samples tested (n=14: singleton n=8, twin= 6, **Figure 1**). For most samples (10/14), spontaneous contractions were abolished at a concentration of 3.2mM $MgSO_4$ or less. Upon returning to normal PSS (1.2mM $MgSO_4$), contractions recovered. The IC_{50} for $MgSO_4$ on spontaneous contraction amplitude was 2.18mM (± 0.024) and activity integral was 1.80mM (± 0.014). Concentration-response curves for $MgSO_4$ on spontaneous contractions according to pregnancy group (singletons or twins) are shown in **Figure 1B** and **C** and the associated IC_{50} values are given in **Table 2.** As shown, the potency of $MgSO_4$ on spontaneous contraction amplitude and activity integral was equal in strips from both singleton and twin myometrium ($P>0.05$).

The concentration of $MgSO_4$ required to abolish contractions was significantly increased in the presence of oxytocin compared to spontaneous conditions ($P<0.0001$, amplitude and activity integral). In a number of cases contractions persisted even at 12mM $MgSO_4$ which is 10x the concentration in our PSS i.e. control concentration. Concentration-response curves for $MgSO_4$ in the presence and absence of oxytocin are shown in **Figure 2A and B**. The IC_{50} for $MgSO_4$ under oxytocin was 8.13mM (± 0.066) for amplitude of contraction and 5.77mM ± (0.033) for activity integral (n=14, combined data; n=8 singletons, n=6 twins). When stratified according to pregnancy group, there was no difference in the potency of $MgSO_4$ between singleton and twin myometrium on oxytocin-augmented contractions (**Figure 2C** and **D** and **Table 2**).

In an effort to reverse the effect of oxytocin on $MgSO_4$'s potency, the effect of increasing concentrations of $MgSO_4$ on oxytocin-augmented contraction (as performed above) was examined in paired strips in the presence and absence of atosiban (100nmol/L, based on our

previous study).[13] Representative traces are shown in **Figure 3 (A and B)**, as well as appropriate time controls for atosiban alone and oxytocin alone (**Figure 3C and D**). As shown previously[13] contractions persisted for a number of hours in the presence of oxytocin without significant decrease in in amplitude or AUC. Similarly, the application of atosiban (100nmol/L) alone resulted in an initial decrease in force which the remained stable for the remainder of the experiment (n=12).

The combination of $MgSO_4$ plus atosiban significantly shifted the concentration-response curve to the left compared to $MgSO_4$ alone (paired strips). This was true for both amplitude of contraction and activity integral (**Figure 4A and B**). Consequently, the IC_{50} values for the combination of $MgSO_4$ plus atosiban were significantly lower: The IC_{50} values for amplitude of contraction for $MgSO_4$ plus atosiban being 5.01mM (±0.142) compared to 8.59mM (± 0.226) for $MgSO_4$ alone (P<0.0001) and for AUC, $MgSO_4$ plus atosiban was 2.81mM compared to 4.74 ± 0.011 for $MgSO_4$ alone (P<0.0001).

When stratified according to pregnancy group, the curves for both amplitude of contraction and activity integral were shifted significantly to the left in the singleton pregnancy group, with significantly lower IC_{50} values reported in the presence of atosiban (P<0.0001, **Figure 4C and D and Table 3**). For the twin myometrial group however, only the concentration-response curve for activity integral was significantly shifted to the left (P=0.0045). There was a small but non-significant shift to the concentration-response curve for amplitude of contraction (P=0.1829) (**Figure4 E and F** and **Table 3)**.

Comment

Magnesium sulfate has been one of the commonly used tocolytic agents for nearly 40 years[18] and has been the subject of a number of clinical trials evaluating its efficacy (see[1, 6] for reviews). Our data extends the *in vitro* findings confirming Mg's ability to reduce myometrial contractions. The most recent Cochrane Review of tocolysis using $MgSO_4$ for preterm labor however, found that it '*is ineffective at delaying birth or preventing preterm birth'*.[6] To help understand why this discrepancy between *in vitro* and *in vivo* findings occurs, we examined the effect of $MgSO_4$ on both spontaneous and oxytocin-augmented contractions as well as the combined effect of $MgSO_4$ and the oxytocin receptor antagonist atosiban.

We found that a relatively modest increase in $MgSO_4$ from 1.2 (control) to 3.2mmol/M would abolish spontaneous contractions in 70% of samples. This value is very close to the 3.0mmol/L reported by Tica et al., (2007) despite the differences in sample preparation and experimental conditions which included Mg-free PSS as control. The therapeutic concentration for tocolysis (2-3 mmol/L) is also within this range.[9, 19]

The cation of magnesium (Mg^{2+}) is thought to be responsible for its tocolytic effect as magnesium chloride ($MgCl_2$) also displays similar relaxant effects in myometrium. Mg is suggested to act via cationic competition with Ca^{2+} and blocking (L-type) channel-dependent Ca influx, thereby decreasing intracellular Ca availability for contraction as well as reducing agonist-stimulated Ca release from intracellular stores e.g. the sarcoplasmic reticulum.[4] The ability of Mg's action to be reversed upon washout and with the application of the L-type Ca channel agonist BayK8644 supports this, as does clinical data which shows that calcium supplementation in hypocalcaemic women after receiving $MgSO_4$ causes increased uterine activity.[19]

In the presence of oxytocin the tocolytic effect of $MgSO_4$ was significantly reduced with IC_{50} values being shifted significantly to the right, confirming that a significantly greater (>4

times) concentration of Mg is required to inhibit oxytocin-augmented contractions[3, 5] which is a concentration that would not be considered safe.[9] In an earlier study, Tica et al., saw a 30-40% decrease in activity at ≥8mmol/L $MgSO_4$ in the presence of oxytocin. Here, a 50% reduction in contraction amplitude was achieved around 8mmol/L $MgSO_4$ but abolition of contraction under oxytocin was not achieved until ≥12mmol/L. Thus this study is in agreement with those previously showing that Mg at therapeutic concentrations (2-3mmol/L) is only effective at inhibiting *spontaneous* myometrial contractions and not those where oxytocin is present.[5]

We also compared the effect of $MgSO_4$ in singleton and twin pregnancies as the strategies used to prevent preterm birth in singletons have not been found to be effective in twin pregnancies.[12] We may hypothesise that the additional stretch placed on the uterus at an earlier time point in gestation, owing to two fetuses, as well as endocrine differences (e.g. two placentas/membranes), may lead to an increased drive on the uterus towards labor onset in twins, including changes in gene expression which could ultimately affect its contractility and response to tocolytics. Our earlier *in vitro* data on tocolytic potency in the presence of oxytocin in twins supports this.[13] However, here we found no difference in the potency of $MgSO_4$ between the two tissue types, suggesting that despite the potential for any differences between tissues, $MgSO_4$ acts in a similar manner and is equipotent in both groups.

Oxytocin's actions in myometrium are multiple but are primarily thought to involve increased Ca entry via L-type channels, reduced Ca exit and the release of Ca from the SR.[20] Thus the actions of oxytocin are in opposition to Mg and would therefore explain, in part, the increased concentration of Mg required to inhibit contractions augmented by oxytocin. We used oxytocin in our study to mimic the hormonal situation surrounding labor. That the effects of oxytocin in our assay are evident at a relatively low concentration emphasises the

significant negative effect of oxytocin on Mg's potency and adds insight into why $MgSO_4$'s tocolytic effect *in vivo* has been disappointing.

Our previous studies showed that twin pregnancy myometrium responded more greatly to oxytocin stimulation[17] which may indicate a change in the number of oxytocin receptors expressed. We also noted a reduction in the potency of both indomethacin and progesterone to suppress oxytocin-augmented contractions in twins compared to singletons.[13] Thus it was somewhat surprising that we did not detect any differences in the potency of $MgSO_4$ in inhibiting oxytocin-induced contractions between the two groups.

The addition of the oxytocin-receptor antagonist, atosiban resulted in a partial reversal of the effect of oxytocin on $MgSO_4$'s potency in that the IC_{50} for $MgSO_4$ in the presence of atosiban was shifted significantly back to the left compared to $MgSO_4$ alone. This is an important point of interest as it could mean that a combination approach to tocolysis involving anti-oxytocins may be beneficial for improving tocolytic efficacy *in vivo.* In a recent study, we also found other tocolytic agents and suppressants of uterine activity to be less effective in the presence of oxytocin, including indomethacin and the acute application of progesterone.[13] Thus these findings may be extendable to other tocolytic agents. Indeed, the combination of atosiban with the calcium channel blocker nifedipine *in vitro,* has been shown to exert an additive tocolytic effect on the contractility of myometrial strips in both pre-term and term women [21] whilst a combination of atosiban plus ritodrine was found to cause a synergistic inhibition of myometrial activity in pregnant rat myometrium.[22]

The idea of combination tocolytics or dual agent primary therapy to inhibit preterm labor contractions *in vivo* has been explored previously including trials of $MgSO_4$ administered with beta-mimetic agonists e.g. ritodrine or terbutaline.[23-28] The rationale being that as each

agent has a different mechanism of action, there may be some additive effects that inhibit uterine contractions, thus increase the efficacy of tocolysis and hence prolong gestation. However, whilst combined treatment may improve tocolytic efficacy, it may also produce an increase in serious maternal side effects and so should be addressed with caution. This is particularly the case if combinations of agents used have similar sites of action e.g. both nifedipine and Mg target L-type calcium channels which are distributed throughout other smooth muscles and cardiac muscle. Outcomes from a recent Cochrane review of trials involving combination tocolytic use for inhibition of preterm labor found that there was insufficient evidence to show whether combination tocolytic approaches were more or less effective than a standard single tocolytic drug, or if they have more adverse events.[29] They also reported a lack of combination trials involving the most widely used tocolytics such as atosiban or nifedipine.

It should be noted that the IC_{50} values in the presence of atosiban did not mirror those in oxytocin –free conditions, suggesting that the effect of oxytocin was not completely reversed using this concentration of atosiban. In our study of twin myometrium, whilst amplitude of contraction appeared to be reduced in the presence of atosiban, only activity integral was significantly reduced in the presence of atosiban compared to Mg alone. Reasons for this difference are not known but may reflect the small sample size in our twin group which is a limitation of the study.

An additional limitation is that we did not include samples from women in labor or gestations before 36^{+4} weeks as many women laboring pre-term deliver vaginally. Hence it would be interesting to see if similar results would be obtained in these more clinically relevant tissues, when the uterine environment may be different. [30] The use of a homogenous study population of non laboring women with term gestation however, provided a well-controlled *in vitro* comparison of the effect of $MgSO_4$ between singleton and twin pregnancy myometrium

which is a major advantage of this study. As the frequency of contractions in human myometrium is low we had to analysis data over a 20 minute period to ensure there was sufficient activity to measure. This timeframe may however underestimate the effects of $MgSO_4$ on amplitude compared to measuring it in the last 5-10 minutes of the application when activity had stabilized. Antenatal $MgSO_4$ given to women at risk of preterm birth was shown to substantially reduce the risk of cerebral palsy in their children.[31] Currently, pregnant women experiencing threatened preterm birth (less than 30 weeks gestation) are therefore given $MgSO_4$. So whilst $MgSO_4$ is not used for the indication of preterm labor *per se*, and despite the potential for increased risks for maternal or neonatal side effects from dual therapy, it is being administered in combination with a tocolytic agent which is likely to be atosiban (in UK), indomethacin or nifedipine. Further studies, both basic and clinical, should therefore address these different combinations of agents with $MgSO_4$ on myometrium to establish which is most effective, as well as examining for potential harmful side effects of dual therapy, especially when the same cellular sites or pathways are targeted.

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Table 1 Maternal demographics for the study group and according to pregnancy group

Characteristic	**Combined (n=35)**	**Singletons (n=23)**	**Twins (n=12)**
Maternal age, years	32 (± 5.2)	32 (± 4.3)	31.5 (± 5.6)
Maternal BMI	25 [23-29.7]	27.5 [23.4-33.7]	23.8 [22.4-27.0]
Gestational age, days	273 [264-275]	274 [272-275]	263 [259-264]*****
Birth weight(s), g	3870 [3465-4920]	3690 [3270-3880]	5207 [4388-5817]*****
Parity			
Para 0	11 (31.4%)	6 (26.1%)	5 (41.7%)
Para 1	13 (37.1%)	8 (34.85)	5 (41.7%)
Para >1	11 (31.4%)	9 (39.1%)	2 (16.7%)
Reason for CS			
Previous CS	18 (51.4%)	17 (73.9%)	1 (8.3%)
Previous difficult vaginal delivery	1 (2.9%)	1 (4.3%)	0
Breech	2 (5.7%)	0	2 (16.7%)
Fetal Reason	3 (8.6%)	1 (4.3%)	2 (16.7%)
Maternal Reason	4 (11.4%)	4 (17.4%)	0
Maternal choice	7 (20.0%)	0	7 (58.3%)

Abbreviations: BMI, body mass index; CS, caesarean section; IQR, interquartile range; SD, standard deviation.

Data are presented as mean (+SD), median [IQR], or frequencies (counts, n, and percentages) where appropriate. Gestational age and maternal age are age at delivery. The BMI was recorded at pregnancy booking. Birth weights are combined in the case of twins. Asterisk indicates significantly shorter gestation in twins and significantly greater birth weight in twins, Kruskal Wallis Test, $P<0.05$)

Previous difficult vaginal delivery refers to shoulder dystocia in previous labor. Fetal reason included intra-uterine growth restriction (n=2, twins) and fetal ECG outside normal limits (n=1). Maternal reason included previous ectopic pregnancy (n=1), coeliac disease (n=1), chronic fatigue syndrome (n=1) and placenta previa (n=1).

Table 2 IC_{50} values of $MgSO_4$ on spontaneous and oxytocin-augmented contractions from singleton and twin myometrium.

	Singleton (n=8)	**Twin (n=6)**	**P value**
Spontaneous			
Amplitude	2.15mM ± 0.027	2.23mM ± 0.043	0.4676
AUC	1.84mM ± 0.019	1.79mM ± 0.021	0.5214
OT-augmented			
Amplitude	8.55mM ± 0.208	7.67mM ± 0.305	0.3034
AUC	5.81mM ± 0.230	5.77mM ± 0.236	0.9462

Abbreviations: AUC (activity integral, area under the curve) OT (oxytocin),

Concentrations were compared by taking the $-LogIC_{50}$ and the F test extra sum of squares principal

Table 3 IC_{50}s of $MgSO_4$ in the presence an absence of atosiban (100nM) on oxytocin-augmented myometrial contractions according to pregnancy group

	$MgSO_4$	**$MgSO_4$ + atosiban**
Combined samples (n=21)		
Amplitude	8.59mM ± 0.226	5.01 ± 0.142***
AUC	4.74mM ± 0.011	2.81mM ± 0.053***
Singletons (n=15)		
Amplitude	8.13mM ± 0.0225	4.28mM ± 0.125***
AUC	4.57mM ± 0.114	2.62mM ± 0.050***
Twins (n=6)		
Amplitude	10.42mM ± 0.781	8.13mM ± 0.399
AUC	5.33mM ± 0.261	3.43mM ± 0.150**

Asterisk indicates a significant difference between the -logIC50 values in the presence and absence of atosiban where *** is $P<0.001$, ** is $P<0.01$

Figure legends

Figure 1. The effect of $MgSO_4$ on spontaneous contractions in singleton and twin myometrium. A representative trace showing the inhibitory effect of increasing concentrations of $MgSO_4$ on spontaneous contractions of human myometrium is shown in (**A**). In most cases (10/14), contractions were inhibited by ≤3.2mM $MgSO_4$. Note the effect was reversible upon washout. (**B**) and (**C**) show the concentration response curves for the effect of $MgSO_4$ on singleton (blue circles) and twin (red squares) myometrial amplitude and activity integral (AUC) respectively. Data are presented as mean (± standard error of the mean, SEM) percentage amplitude and AUC before and after the application of $MgSO_4$ (control).

Figure 2. The effect of oxytocin on $MgSO_4$'s potency in human myometrium. (**A**) and (**B**) show the concentration-response curves for the effect of $MgSO_4$ on contraction amplitude and activity integral (AUC) respectively, in the presence (purple squares) and the absence (blue circles) of oxytocin (0.5nM). The concentration-response curves for $MgSO_4$ are significantly shifted to the right in the presence of oxytocin (P<0.0001). (**C**) and (**D**) show the concentration-response curves for $MgSO_4$ on contraction amplitude and activity integral (AUC) in the presence of oxytocin according to pregnancy group (singleton: blue circles, twin: red squares). $MgSO_4$ is as potent on oxytocin-augmented contractions in twins as it is in singletons. Data are presented as mean (± standard error of the mean, SEM) percentage amplitude and AUC before and after the application of $MgSO_4$ (control). Asterisk indicates a significant difference between the -logIC50 values where *** is P<0.001, ** is P<0.01 and * is P<0.05.

Figure 3. The effect of the combination of $MgSO_4$ plus atosiban on oxytocin-augmented contractions. Representative recordings (paired strips) showing (**A**) the effect of increasing concentrations of $MgSO_4$ on oxytocin-augmented contractions, (**B**) the effect of $MgSO_4$ in the presence of atosiban (100nM), (**C**) atosiban alone (time control) and (**D**) oxytocin time control. Arrows indicate where oxytocin (0.5nM) was added and remained throughout the experiment. The addition of atosiban significantly reduced the concentration of $MgSO_4$ at which oxytocin-augmented contractions were inhibited; from ≥12mM (**A**) to ~8mM (**B**).

Figure 4. The concentration-response curves for the combination of $MgSO_4$ plus atosiban on oxytocin-augmented contractions. (**A** and **B**) depict the concentration-response curves for the effect of $MgSO_4$ on oxytocin-augmented myometrial contractility (amplitude of contraction and integral of force, AUC respectively) on all samples (n=21) in the presence (green squares) and absence (purple circles) of atosiban (100nM) as well as atosiban time control (grey triangles). When stratified according to pregnancy group; (**C** and **D**, singleton, n=15, **E** and **F** twins, n=6) the application of atosiban shifted the concentration curves significantly towards the left for both amplitude of contraction and activity integral in singleton myometrium, (**C** and **D,** $P<0.0001$). Only activity integral was significantly shifted to the left in the twin group (**E**, $P=0.1829$ and **F,** $P<0.01$). Data are presented as mean (± SEM) percentage of amplitude and activity integral (AUC) before the application of $MgSO_4$ or atosiban (control). Asterisk indicates a significant difference between the -logIC50 values where *** is $P<0.001$, ** is $P<0.01$ and * is $P<0.05$.

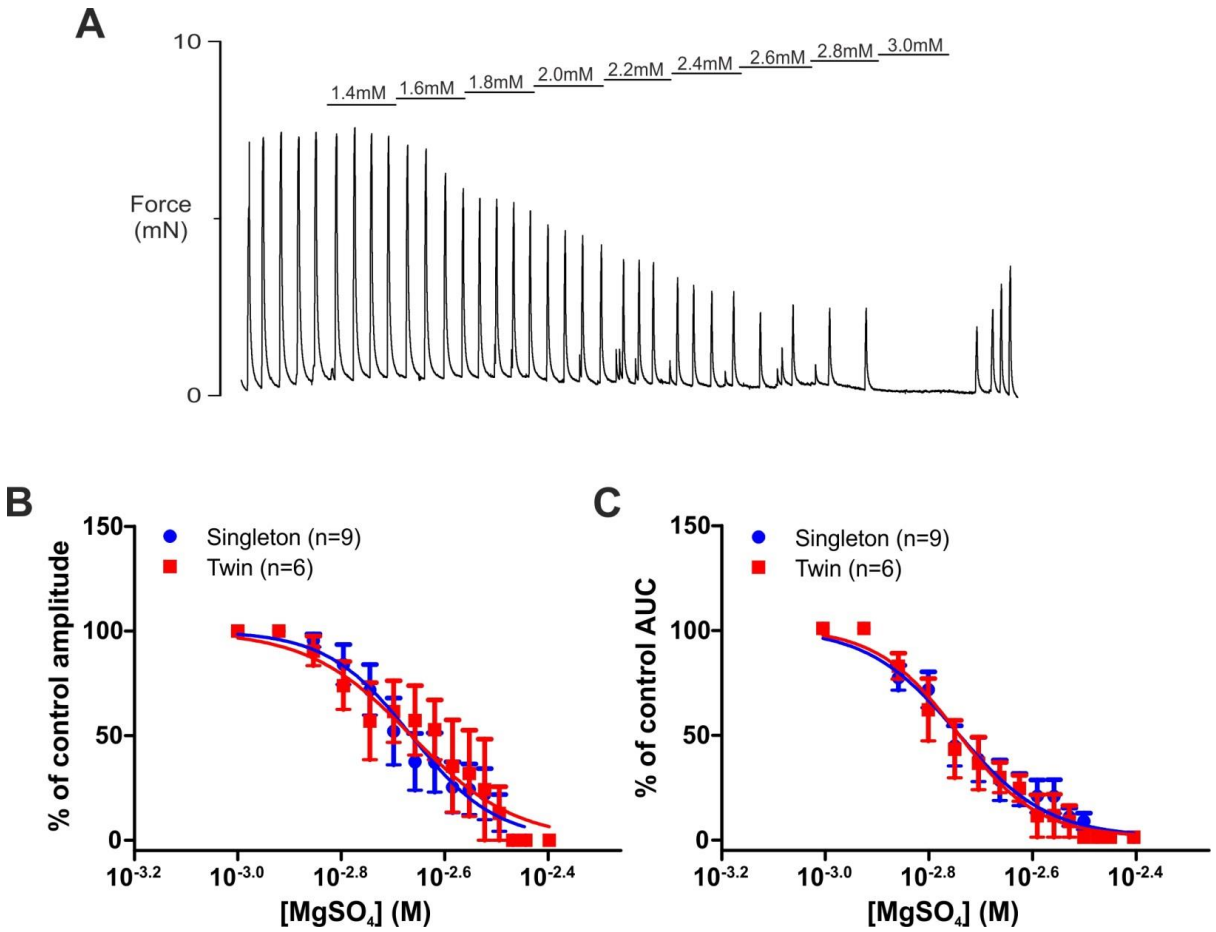
A
10
0
Force
(mN)
1.4mM
1.6mM
1.8mM
2.0mM
2.2mM
2.4mM
2.6mM
2.8mM
3.0mM
B
% of control amplitude
150
100
50
0
Singleton (n=9)
Twin (n=6)
10-3.2
10-3.0
10-2.8
10-2.6
10-2.4
[MgSO4] (M)
C
% of control AUC
150
100
50
0
Singleton (n=9)
Twin (n=6)
10-3.2
10-3.0
10-2.8
10-2.6
10-2.4
[MgSO4] (M)

Figure 1

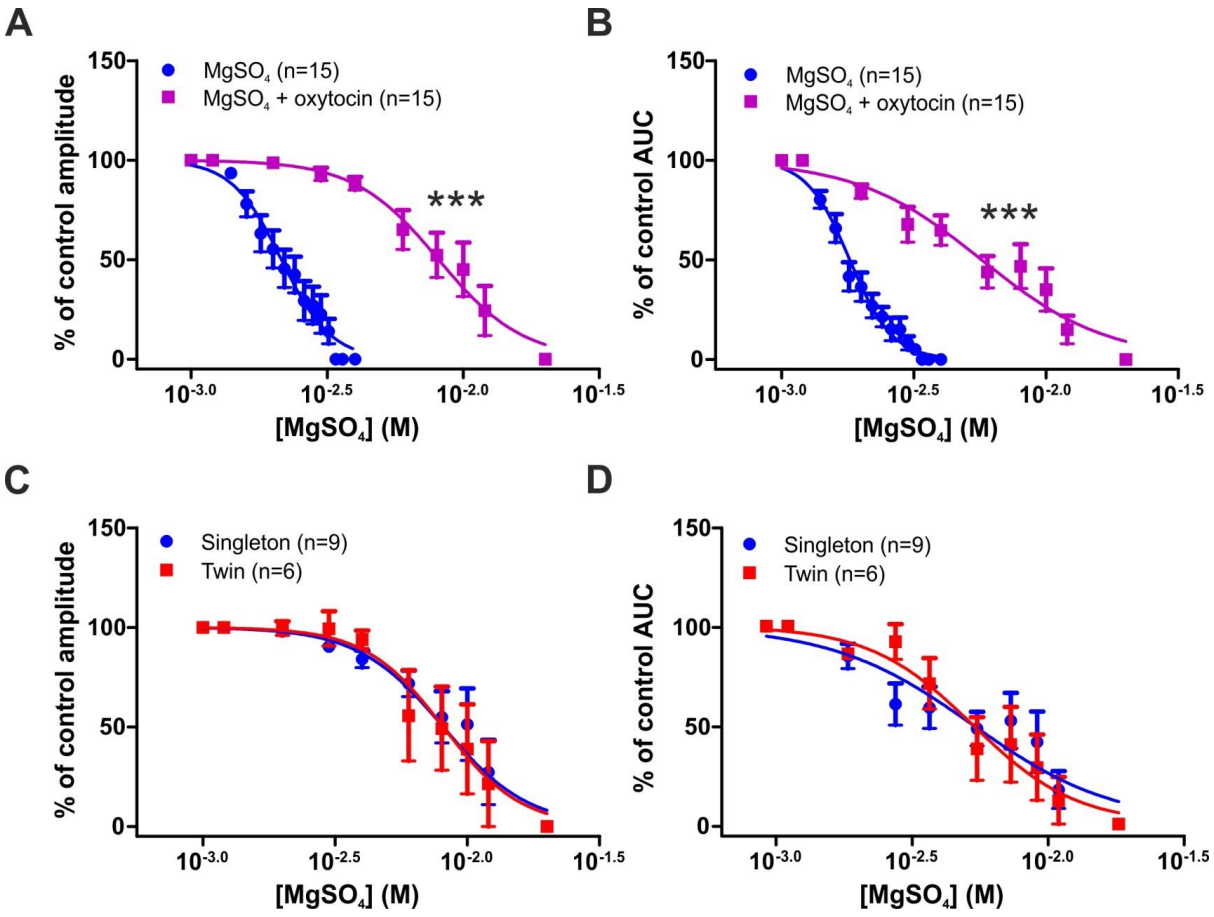
A
B
C
D
MgSO4 (n=15)
MgSO4 + oxytocin (n=15)
Singleton (n=9)
Twin (n=6)
% of control amplitude
% of control AUC
[MgSO4] (M)
***
150
100
50
0
10-3.0
10-2.5
10-2.0
10-1.5

Figure 2

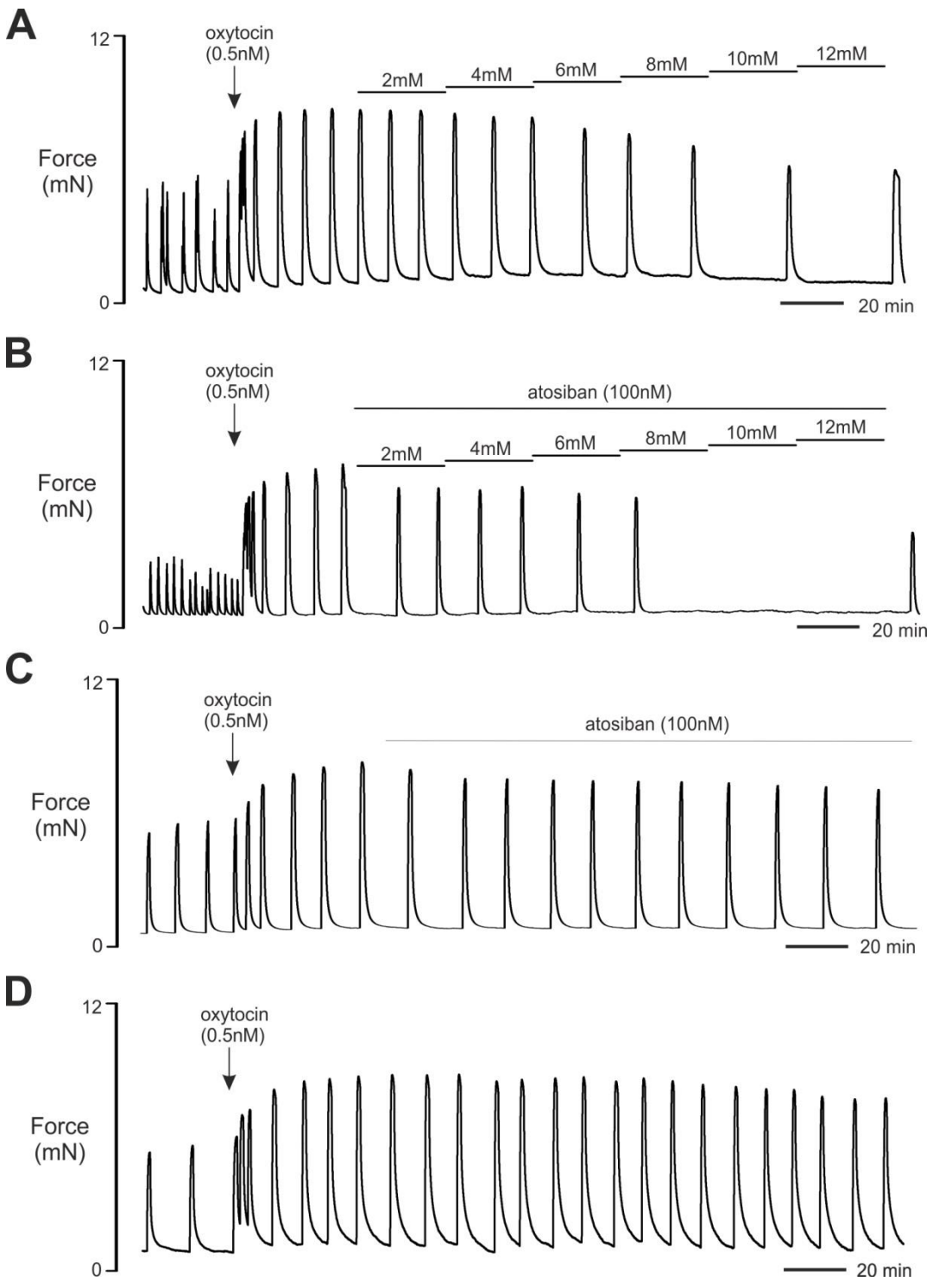
A
12
0
Force
(mN)
oxytocin
(0.5nM)
2mM
4mM
6mM
8mM
10mM
12mM
20 min
B
12
0
Force
(mN)
oxytocin
(0.5nM)
atosiban (100nM)
2mM
4mM
6mM
8mM
10mM
12mM
20 min
C
12
0
Force
(mN)
oxytocin
(0.5nM)
atosiban (100nM)
20 min
D
12
0
Force
(mN)
oxytocin
(0.5nM)
20 min

Figure 3

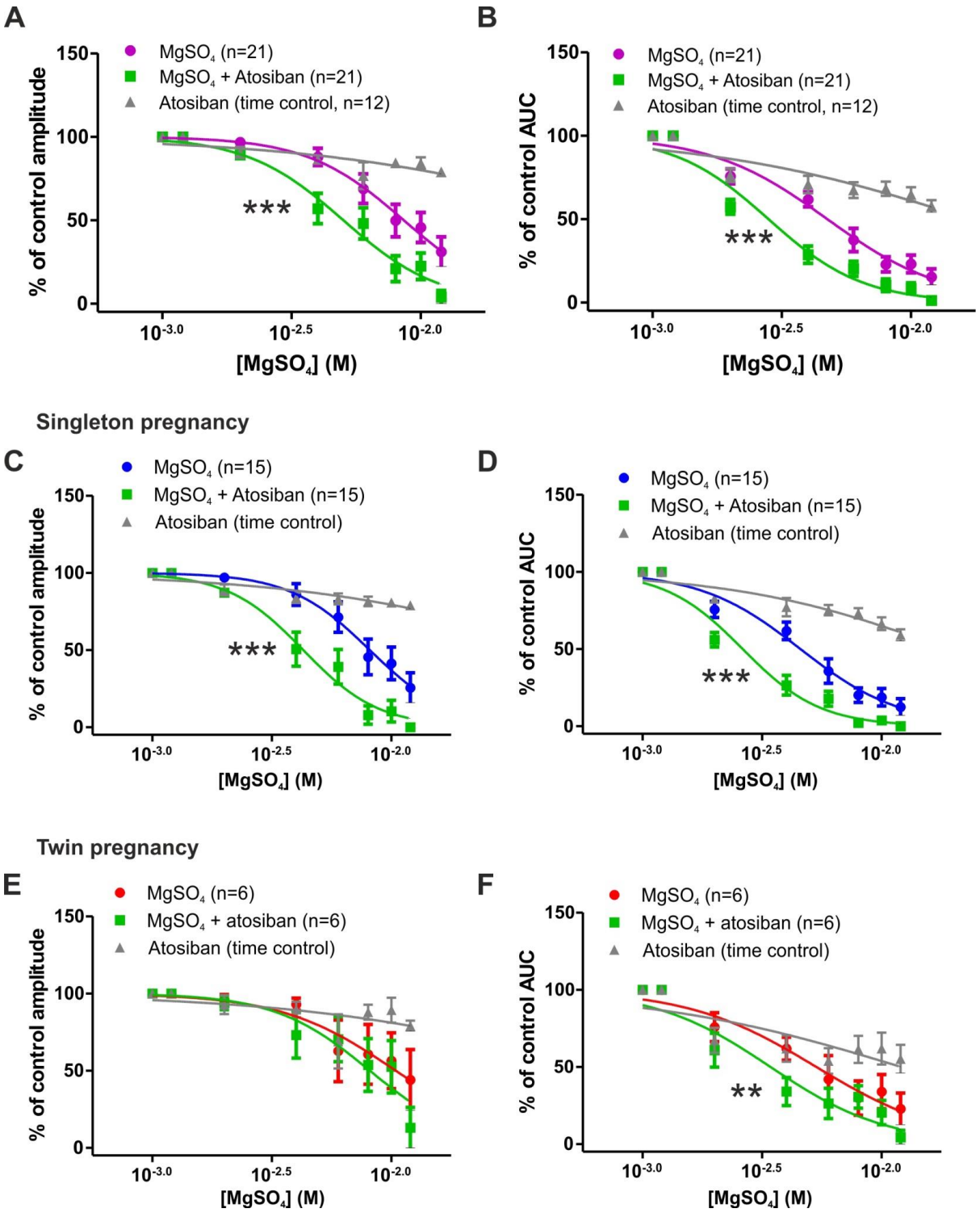
A
B
MgSO4 (n=21)
MgSO4 + Atosiban (n=21)
Atosiban (time control, n=12)
% of control amplitude
% of control AUC
***
[MgSO4] (M)
Singleton pregnancy
C
D
MgSO4 (n=15)
MgSO4 + Atosiban (n=15)
Atosiban (time control)
Twin pregnancy
E
F
MgSO4 (n=6)
MgSO4 + atosiban (n=6)
**

Figure 4