Changes in frequency and force production of the human myometrium with alteration of pH and metabolism

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Changes in uterine metabolism and intracellular pH occur with uterine activity and these may in turn alter function. We have investigated the effects of changes in intracellular pH and metabolic inhibition on the spontaneous activity of isolated nonpregnant and pregnant human myometrial strips at 37°C. Intracellular alkalinization resulted in a marked increase in the frequency of contractions in both nonpregnant and pregnant preparations. Intracellular acidification reduced force production in pregnant and nonpregnant preparations. The effects of pH were concentration dependent. Metabolic inhibition (2 mmol cyanide l⁻¹) abolished spontaneous contractions in nonpregnant preparations (ten of ten) but pregnant preparations were able to maintain activity, although at a reduced level (eleven of fifteen). Possible mechanisms involved and relevance to uterine dysfunction are discussed.

Introduction

In 2–4% of all labours and up to 8% of primiparous labours, the uterus fails to contract enough to expel the fetus (uterine dystocia; Beischer and Mackay, 1986). Probably the greatest proportion of these abnormal labours is due to uterine muscle dysfunction. O'Driscoll *et al.* (1984) found that approximately 36% of women delivered by emergency Caesarean section, with all its inherent dangers to maternal health, were due to inefficient uterine action. The fact that this is the commonest indication for emergency Caesarean section demands a greater understanding of uterine muscle dysfunction, the causes of which, unfortunately, remain largely unknown.

Myometrial contraction is initiated by an increase in the concentration of intracellular free calcium ([Ca²⁺]_i) and the importance of [Ca²⁺]_i in spontaneous contractility and in contractile responses to hormones has been elucidated with the use of various calcium channel blockers (Poli *et al.*, 1990; Tasaka *et al.*, 1991). Although there has been much work on the effects of hormones and other agonists on uterine contractions, relatively less work has been done to characterize the mechanical and electrical properties of the uterus (Kawarabayashi *et al.*, 1988; Morizaki *et al.*, 1989; Izumi *et al.*, 1990). In particular, nothing appears to be known about the effect of changes in intracellular pH (pH_i) on human uterine contractions.

Changes in uterine metabolism and function may lead to changes in pH_i which in turn may alter function (Wray, 1988). Changes in pH_i may occur for the following reasons. (1) Uterine contractions during labour may be so powerful that they occlude uterine blood vessels (Brinkman, 1990), thereby restricting blood flow and oxygen supply to the tissues. Uterine

pH_i has been shown to fall during hypoxia in animal studies (Wray, 1990) and the same may be true in the human uterus. (2) Increased uterine activity produces acidification (Wray, 1990). (3) Maternal acid–base balance may be altered during labour. Until recently very little was known about pH_i in the uterus of any species. Experiments with isolated rat uteri have shown a profound effect of pH_i on force production (Wray *et al.*, 1992; Heaton *et al.*, 1992). Apart from a recent ³¹P NMR measurement of resting pH in biopsy material (Noyszewski *et al.*, 1989), there appear to have been no studies of pH_i, its relationship to force production and the effect of gestational changes in the human uterus. There is, therefore, a need for a comprehensive study of the relationship between pH_i, metabolic inhibition and force production in the human uterus.

A preliminary account of some of this work has been presented (Phoenix and Wray, 1992).

Materials and Methods

Samples

Myometrial tissues were obtained from 21 pregnant women of mean age 29.6 ± 1.0 years admitted for elective Caesarean section at 38 weeks' gestation owing to previous section, pelvic disproportion, breech presentation or placenta praevia. Informed written consent and local Ethical Committee approval were obtained. Samples were excised from the upper incisional edge of the lower uterine segment before the administration of oxytocics.

Myometrial samples were also taken from the uterine fundus of 19 nonpregnant women of mean age 41.8 ± 2.1 years admitted for hysterectomy for menorrhagia, dysmenorrhoea and fibroids. All samples were immediately placed in cold, modified

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Krebs and transported to the laboratory and were either used within the hour or dissected and stored overnight at 4°C and used for experiments on the next day.

Small strips of myometrium were dissected and mounted in Hepes-buffered salt solution at 37°C, and gassed with 100% oxygen. Force production was measured via a Grass transducer (Model FT03, Quincy, MA). The minimum force detectable was 0.2 mN. All strips were put under a small amount of resting tension, and experiments were started within 90 min, once stable rhythmic spontaneous contractions had developed.

Solutions

A modified Krebs solution was used of composition (mmol l⁻¹): NaCl, 154; KCl, 5.6; MgSO₄, 1.2; Hepes, 10.9; glucose, 11.7; CaCl₂, 3; pH 7.4. The effect of pH_i on force production was investigated by iso-osmotic substitution of NaCl with salts of the weak bases trimethylamine (TMA) or ammonium chloride (NH₄Cl) or the weak acid butyrate (Thomas, 1984). Solutions with high [K⁺] were obtained by iso-osmotic substitution of Na⁺ by K⁺. The effect of metabolic inhibition was investigated by the addition of 2 mmol NaCN l⁻¹ to the salt solution. External pH was maintained at 7.4.

Statistical analysis

Results are given as means ± SEM, with the level of significance taken as $P \leq 0.05$ using an unpaired two tailed Student's *t* test.

Results

Excised myometrial samples continued to contract for many hours in oxygenated, buffered, bathing solution at 37°C. No differences were noted in the contractile properties of samples used immediately compared with those stored overnight. The frequency of contractions was typically 0.1 min⁻¹ and this is in agreement with other workers (Kawarabayashi *et al.*, 1989).

The effect of weak bases on spontaneous human uterine force production

Effect of TMA. The effect of raising pH_i by the addition of 40 mmol TMA l⁻¹ was investigated on twelve preparations of nonpregnant and seven preparations of pregnant myometrium. Typical traces of the results obtained are shown (Fig. 1). TMA increased the frequency of contractions in all preparations. In eleven out of twelve nonpregnant preparations and five out of seven pregnant preparations, an increase in the frequency of contraction was observed with varying degrees of relaxation between contractions during the treatment period. The mean increase in the frequency was 152 ± 25.6% ($n = 10$) compared with control conditions, with no significant difference found in the response of nonpregnant (164.5 ± 39.8%, $n = 6$) compared with pregnant tissues (133.4 ± 27.6%, $n = 4$). Treatment with 40 mmol TMA l⁻¹ produced a sustained contraction in the remaining preparations. There was no significant difference in the treatment period

Fig. 1. The effect of 40 mmol trimethylamine l⁻¹ (TMA) on the spontaneous contractions of (a) nonpregnant and (b) pregnant human myometrial preparations. The solid bar indicates the treatment period.

used (nonpregnant, 15.8 ± 2.2 min; pregnant, 12.0 ± 2.0 min). Upon return to control solution, a delay to recovery of spontaneous activity was seen in eleven out of twelve nonpregnant and all pregnant preparations. Although the delays observed were longer in the pregnant than the nonpregnant uterus, this difference was not significant (28.3 ± 3.6 min and 39.4 ± 5.0 min, respectively).

Dose-dependent effects of TMA. In both gestational states, increasing the dose of TMA along the series 20, 40 and 60 mmol l⁻¹ resulted in a progressive increase in the frequency of contraction during the treatment period compared with control contractions (20 mmol l⁻¹, 63.4 ± 16.9%; 40 mmol l⁻¹, 150.5 ± 45.9%; 60 mmol l⁻¹, 184.9 ± 7.25%, $n = 5$). The delay to recovery of spontaneous contractions upon return to control solution was also increased with increasing dose.

Effect of NH₄Cl. As with TMA, 40 mmol NH₄Cl l⁻¹ increased the frequency of contractions in all preparations by 149.11 ± 24.0% ($n = 12$) and there was no significant difference in the response of nonpregnant (147.0 ± 40.8%, $n = 5$) compared with pregnant tissues (150.6 ± 31.8%, $n = 7$) (Fig. 2). In five of nine nonpregnant and six of eight pregnant preparations, there was an increase in frequency with various degrees of relaxation between contractions during the treatment period. In the remaining preparations, sustained contractions were observed. On return to control conditions, as with TMA, a delay was noted to recovery of spontaneous contractions, with pregnant preparations taking significantly longer (53.5 ± 3.1 min) to recover than nonpregnant preparations (32.2 ± 4.1 min, $P < 0.05$). There was no significant difference in the treatment period used (11.3 ± 0.5 min for nonpregnant; 12.5 ± 1.4 min for pregnant preparations).

The effect of weak acid on spontaneous human uterine force production

Butyrate was applied to ten nonpregnant preparations and resulted in a gradual decrease in the amplitude of contractions

Fig. 2. The effect of 40 mmol ammonium chloride l^{-1} (NH_4Cl) on the spontaneous contractions of (a) nonpregnant and (b) pregnant human myometrial preparations. The solid bar indicates the treatment period.

Fig. 3. The effect of 40 mmol butyric acid l^{-1} on the spontaneous contractions of (a) nonpregnant and (b) pregnant human myometrial preparations. The solid bar indicates the treatment period.

to $52.3 \pm 8\%$ of pretreatment values (Fig. 3). This was often accompanied by an initial increase in the frequency of the contraction. In seven out of ten preparations, recovery of spontaneous force production was seen during the treatment period.

In the nine pregnant preparations tested, butyrate eventually abolished contractions in all preparations. An initial brief increase in frequency was seen in six preparations. Remaining preparations showed an immediate abolition of contractions. A drop in baseline tension was noted in six of nine preparations which only recovered on return to control solution. There was a delay to recovery of spontaneous contractions in eight of nine preparations (12.7 ± 1.2 min). This was not significantly different from the delay in nonpregnant preparations (13.5 ± 3.5 min). It was noted that recovery of spontaneous contractions in pregnant preparations was often associated with

Fig. 4. Contractile responses in 2 mmol cyanide l^{-1} for (a) nonpregnant and (b) pregnant human myometrial preparations. The solid bar indicates the treatment period.

a transient increase in frequency (seven of nine). This was not observed in nonpregnant preparations.

Dose-dependent effects of butyric acid. The dose-dependent effects of butyric acid were investigated in five nonpregnant preparations. Increasing the dose along the series 20, 40 and 60 mmol l^{-1} appeared to cause a greater reduction in the amplitude of contraction before recovery, during the treatment period; however, this did not reach significance. Recovery times were also affected, with an increase in dose apparently causing an increase in time to recovery, during the treatment period and on return to control solution, but again this did not achieve significance. The initial increase in the frequency of contractions was not dose dependent.

Effect of metabolic inhibition on spontaneous force production

Cyanide was used to investigate the effect of inhibition of oxidative phosphorylation (and thus mimic anoxia) and typical results are shown (Fig. 4). Treatment with cyanide completely abolished spontaneous contractions in all ten nonpregnant preparations tested. Recovery of contractions to full amplitude on return to control solution was either gradual (four out of ten), or immediate.

Continued, but decreased, activity during treatment with 2 mmol cyanide l^{-1} was seen in eleven of fifteen pregnant preparations and, of these, four showed an increase in frequency and two showed sustained contractions with gradually decreasing amplitude. The remaining preparations showed an abolition of spontaneous activity.

Effect of high potassium in the presence and absence of cyanide

The previous results had shown that cyanide reduced contractile activity. To determine whether its effects were beyond the surface membrane (e.g. on the contractile proteins), high K^+ solution was used to depolarize the membrane. Treatment of human myometrial samples with a 40 mmol l^{-1} potassium salt solution produced a sustained contraction in both gestational

Fig. 5. The effect of 40 mmol K^+ l^{-1} and 40 mmol K^+ l^{-1} in the presence of 2 mmol cyanide l^{-1} on the spontaneous contractions of (a) nonpregnant and (b) pregnant myometrial preparations (typical continuous recordings). Solid bar indicates the treatment period.

states (Fig. 5). A delay to recovery of spontaneous contractions upon return to control solution was sometimes seen in both gestational states.

When preparations were exposed to metabolic inhibition with 2 mmol cyanide l^{-1} in the presence of 40 mmol K^+ l^{-1} , similar responses were seen in both gestational states and the results shown in Fig. 5 are typical traces from six experimental procedures on nonpregnant and four on pregnant myometrial samples. The initial contraction, produced by the high $[K^+]$, gradually relaxes under the influence of the cyanide, with the rate of relaxation progressively slowing and relaxation to baseline tension on return to control solution.

Discussion

The experiments reported here are the first, as far as we are aware, to investigate the effects of changing pH_i and metabolic inhibition on force production in human myometrial preparations. They show that metabolic inhibition and changes in pH_i can greatly affect the spontaneous force production and that some of these changes depend on the gestational state of the tissue.

The difference in the mean age of the women in the two gestational groups was only 12 years and did not appear to influence the results obtained. An example of this may be seen in Fig. 4, where the results illustrated are from a nonpregnant woman aged 39 years and a pregnant woman aged 37 years. In addition, the results obtained in pregnant and nonpregnant tissue showed no correlation with age. Thus, clearly, the gestational differences observed are real and not related to age differences.

In this study, pregnant lower segment uterine samples have been compared with nonpregnant fundal samples. Considering the expansion of the uterus with gestation, it would be impossible to locate a region of nonpregnant uterus which would correlate with the lower segment samples taken at Caesarean

section. Differences in contractility patterns have been noted (Daels, 1974), but as the samples used in this study all showed comparable contractile activity, regional differences are not thought to account for the results obtained.

The effect of TMA and NH_4Cl on pregnant and nonpregnant human myometrial preparations was similar and this indicates that the results may be attributed to a change in pH_i rather than to any other action. The changes in uterine pH_i produced by weak bases are rapid (Taggart and Wray, 1992). Intracellular alkalinization had no consistent effect on the amplitude of contractions seen in nonpregnant preparations, whereas pregnant preparations showed either contractions of the same or slightly increased amplitude. In all preparations, treatment with either base significantly increased frequency. The lack of any great differences with gestational state in response to internal alkalinization is in agreement with recent work on isolated rat uteri (Heaton *et al.*, 1992). Spontaneous uterine contractions are attributed to pacemaker activity and, although the mechanism of this activity has not been fully elucidated, it is thought to involve changes in K^+ and Na^+ permeability and possibly Cl^- conductance (Parkington and Coleman, 1990). Changes in the pattern of action potentials may also influence contractions (Kawarabayashi *et al.*, 1986). It may be surmised that an intracellular alkalinization acts to influence these ionic mechanisms, thus affecting the frequency of contractions. For example, alkalinization increases membrane Ca^{2+} currents (Mironov and Lux, 1991) and could, therefore, result in the membrane being more depolarized, and, hence, more easily excited. Further studies are needed to investigate this possibility.

The delay to recovery seen in both pregnant and nonpregnant myometrial preparations on return to control solution is presumably due to the transient acidification which will occur (Thomas, 1984, see below) and this has been demonstrated in the rat uterus *in vitro* (Taggart and Wray, 1992). It is interesting that acidification *per se* is normally associated with an immediate transient increase in frequency. The difference in contractile properties observed in these two situations may be due to differences in the absolute pH_i produced by the two manoeuvres.

Intracellular acidification significantly decreased the amplitude of contraction in all preparations. However, in contrast to the response to intracellular alkalinization, gestational state had an effect on the response to intracellular acidification; nonpregnant myometrial strips recovered well, during butyrate treatment and on return to control solutions, whereas the application of butyrate to pregnant myometrial strips abolished spontaneous contractions and no recovery was seen during the treatment period. The lack of recovery in the continued presence of acid was regardless of the length of the treatment period used (results not shown). These results are in contrast to those found using isolated rat uteri, where no such differences were found (Wray *et al.*, 1992), and are difficult to reconcile with the results of metabolic inhibition using cyanide (see below). It is possible that the main effect of intracellular acidification may be at the surface membrane and calcium entry. The greater depression of force in pregnant tissue with intracellular acidification may be due to the altered membrane excitability in pregnancy.

Although there are differences in the two gestational states, it is clear from these experiments that an intracellular acidification

depresses force production in human myometrial samples. The mechanism by which this occurs is unknown. In rat uteri, Wray *et al.* (1992) found that acidification of depolarized preparations did not affect force production. This suggests that H^+ acts on the surface membrane excitability. Acidification in many excitable cells has been shown to reduce the Ca^{2+} current (Kaibara and Kameyama, 1988). This would be expected to reduce contractile activity. Thus, it is suggested that alkalization increases membrane excitability and so increases frequency, whereas acidification decreases excitability and reduces the frequency to zero, i.e. force is abolished. However, other explanations are possible, and the relationship between pH_i and uterine activity may not be simple. In particular, it is difficult to explain the increase in frequency seen in many preparations with the initial application of weak acid.

The recovery of nonpregnant preparations during the treatment period is probably due to pH regulating mechanisms. Measurement of pH_i in isolated rat myometrial cells has shown that butyrate produces an initial acidification, followed by recovery of pH_i during the treatment period (Eisland *et al.*, 1991). The fact that the return of force in nonpregnant human preparations occurs during the period when pH_i would be expected to be returning to normal supports the assumption that it is acidification which decreases force production rather than butyrate *per se*. A possible explanation for the increased susceptibility to intracellular acidification with pregnancy may be that buffering power in the acidic pH range is less in pregnant than in nonpregnant tissues. There was no evidence of pH_i recovery following alkalization by TMA or NH_4Cl , as judged by the maintenance of the contractile effect (increased frequency). This suggests that pH_i may be less able to recover from alkaline than acidic loads.

Pregnant myometrial preparations were able to sustain spontaneous contractions of reduced force during hypoxia, whereas nonpregnant preparations were not. This difference in response to metabolic inhibition with gestational state was masked when cyanide was added in the presence of high K^+ : pregnant and nonpregnant preparations showed a gradual reduction in force production. This experimental protocol was adopted to investigate the mechanism of action of cyanide to reduce force production. It is possible that the effect of metabolic inhibition may be either at the membrane level, acting to affect electrical activity and excitability, or it may affect calcium fluxes or have an influence on processes dependent on calcium fluxes, e.g. contractile protein interaction. The fact that in depolarized preparations force fell in both gestational states in response to cyanide suggests that part of the action of cyanide must lie beyond an initial surface membrane action. The differences seen with pregnant myometrium in normally polarised preparations treated with cyanide are, however, likely to be due to changes in membrane excitability with pregnancy.

Metabolic inhibition leads to a decrease in uterine concentrations of ATP and phosphocreatine, and pH_i and to an increase in inorganic phosphate concentration (Wray, 1990). A fall in ATP concentrations and a rise in inorganic phosphate concentration have both been shown to modulate force production in muscle. Pregnant rat uterus has been shown to have higher concentrations of ATP and phosphocreatine than nonpregnant uterus (Dawson and Wray, 1985). Thus, it may be that, for a similar degree of metabolic inhibition, the pregnant

uterus, with its increased energy store, is better equipped to sustain force production than the nonpregnant uterus. This was indeed found to be the case.

The results with cyanide and butyric acid on pregnant myometrial preparations produce something of an anomaly. Pregnant tissue continues with reduced spontaneous contraction in the presence of cyanide but contractions are abolished on treatment with butyrate, yet both treatments lead to an intracellular acidification. It is possible that butyrate produced a greater acidification than the lactic acidosis due to cyanide and, hence, affected force more. Clearly measurements of pH_i under these conditions would add to our understanding of the mechanisms involved. In their study with isolated rat uteri, Wray *et al.* (1992) showed that when the intracellular acidification produced by metabolic inhibition with cyanide was nullified, spontaneous contractions were still reduced. It was, therefore, concluded that the effects on force by cyanide could be due solely to alteration of metabolite concentrations. These results show that the interactions between gestational state, change in pH_i and metabolic inhibition are complex and require further study.

Given that pH is likely to change during uterine activity, it is of interest to determine whether these changes may be functionally significant, especially if their role in uterine dysfunction is to be evaluated. Occlusion of arcuate and radial blood vessels in the myometrium during contractions (Brotanek *et al.*, 1969) and frequent forceful contractions would be expected to lead to hypoxia of uterine tissues. Such labours are associated with a high incidence of fetal hypoxia and distress (Brace, 1986). An accumulation of waste metabolites and an intracellular acidification due to a stimulation of glycolysis would be expected with reduced blood flow. The results in this paper suggest that *in vivo*, both of these conditions (hypoxia and intracellular acidification) would result in an eventual failure of contractions. Whereas this would protect both fetus and uterus from hypoxic damage during labour, it would also lead to decreased uterine force and inefficient uterine action and as such may account for cases of uterine dystocia characterized by weak contractions. It may be that, in some women, the uterus is more susceptible to changes in oxygen and pH_i . We would like to investigate the relation between hypoxia and pH with other modulators of uterine force production, such as oxytocin.

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