Functional effects of intracellular pH alteration in the human uterus: simultaneous measurements of pH and force

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Changes in intracellular pH have differing effects on contraction in different types of muscle, and between species. Because of the large number of women requiring a caesarean section due to failure to progress in labour, it is important to know how the human myometrium responds to the changes in intracellular pH that may occur during labour. The pH-sensitive dye, carboxy-SNARF, was used to measure intracellular pH in small strips of human myometrium. Intracellular pH and tension were simultaneously recorded in pregnant and nonpregnant tissue. Intracellular pH was altered by the addition of weak acids and bases. Intracellular alkalization caused an increase in the frequency and amplitude of contractions. Intracellular acidification led to an initial increase in the frequency and/or the amplitude of the contractions, followed by abolition of contractions. Alterations in intracellular pH had profound effects on contraction in human uterine smooth muscle. Possible mechanisms are discussed whereby pH could influence force production, and changes in contraction are related to the speed and extent of the change in intracellular pH.

Introduction

Intracellular pH (pH_i) influences contraction in skeletal muscle (Fabiato and Fabiato, 1978), and in many types of smooth muscle (Aicken, 1984; Irisawa and Sato, 1986; Taggart and Wray, 1993; Taggart *et al.*, 1994a). The effects of alterations in pH_i on contraction, however, appear to depend on the tissue studied, and it is therefore difficult to predict the effect of alteration of pH_i on force in any given tissue.

During labour, uterine contractions can be so strong that the blood vessels supplying and travelling through the myometrium are occluded (Griess, 1965; Brar *et al.*, 1988), leading to myometrial ischaemia and a fall in pH_i due to the build-up of metabolic waste products (Harrison *et al.*, 1994). In rat myometrium, intracellular acidification has been shown to inhibit contractions (Taggart and Wray, 1993), but there has been little study of the effects of known pH_i alterations on force in human myometrium.

Use of pH-sensitive fluorescent indicators, such as carboxy-SNARF, was developed to allow simultaneous measurement of pH_i and contractions in small strips of smooth muscle (Taggart and Wray, 1993), and this method was used in the present study to make simultaneous measurements of pH_i and force in human myometrium.

The results of the study reported here show that alterations in pH_i have profound effects on contractions in human uterine smooth muscle, with intracellular acidification abolishing contractions. If similar changes occurred *in vivo*, for example acidification due to a prolonged labour, or alkalosis due to panting or hyperventilation, they could contribute to inefficient contractions and failure to progress in labour. An understand-

ing of the mechanism of contraction in the uterus, and the effects of alterations in pH_i on that mechanism, will therefore be useful.

A preliminary report of some of these results has been published (Parratt *et al.*, 1994).

Materials and Methods

Tissue

Myometrium was obtained from women undergoing elective caesarean section or hysterectomy at Liverpool Maternity Hospital or Liverpool Women's Hospital, respectively, with Local Ethical Committee approval, and the patients' informed consent. The mean ($\pm$ SEM) age of the patients undergoing caesarean section was 31 ± 1 years ($n = 57$), and that of the patients undergoing hysterectomy was 40 ± 1 years ($n = 28$). The tissue was taken from pregnant women who were not in labour and no women were on any medication and the tissue from women who were not pregnant was taken from the lower, anterior wall of healthy uteri.

Small strips of myometrium (1–2 mm long, 0.5 mm wide and 0.1–0.2 mm thick) were dissected, and pH_i and tension (via a Grass FT03 transducer) were recorded simultaneously.

Intracellular pH measurements

The strips of myometrium were loaded overnight at 4°C in a solution ($5 \mu\text{mol l}^{-1}$) of the acetoxymethylester form of carboxy-SNARF (seminaphtharhodafleur: Molecular Probes, Eugene, OR). After a further 1 h at room temperature, the tissue was placed in a small bath on the stage of an inverted

microscope, with one end of the tissue fixed to the bath, and the other end attached to the tension transducer. The tissue was excited at 530 nm, and the emission signals at 590 and 650 nm were recorded. The records were then calibrated from the ratio of these signals to obtain a linear pH_i measurement, using an *in vitro* calibration with a mock intracellular solution and the indicator at known pH values. The composition of the mock intracellular solution was (mmol l^{-1}): NaCl, 10; KCl, 146; MgSO_4 , 4; ATP, 4.5; Hepes, 11. Previous work has shown that this method of calibration in smooth muscle produces results not significantly different from calibration using the ionophore, nigericin (Taggart *et al.*, 1994a).

Solutions

The tissue was constantly superfused at $35\text{--}37^\circ\text{C}$ with a solution of the following composition (mmol l^{-1}): NaCl, 154; KCl, 5.4; MgSO_4 , 1.2; glucose, 11.7; CaCl_2 , 3; Hepes, 11. Solutions were gassed with 100% O_2 and the external pH was maintained at 7.4.

There is no difference in contractile activity or pH_i changes obtained when Hepes rather than $\text{HCO}_3\text{--CO}_2$ is used to buffer solutions (unpublished observations on human tissue, J. R. Parratt; and on rat tissue, Taggart and Wray, 1993).

Intracellular pH was changed at constant external pH by using a weak base (ammonium chloride, $10\text{--}30\text{ mmol l}^{-1}$), or a weak acid (sodium butyrate, 30 mmol l^{-1}), isosmotically substituted for sodium chloride.

Statistical analysis

Data are given as means $\pm$ SEM and differences were taken as significant if $P < 0.05$ in an unpaired Student's *t* test.

Results

Loading the myometrium with SNARF did not alter contractile activity, as judged by comparing contractile frequency and amplitude before and after loading.

Of the tissue samples obtained at hysterectomy, 67% contracted spontaneously, compared with 64% of the samples from caesarean section; contractions usually occurred after 30–50 min. The contraction rate of tissue from nonpregnant women was 3.9 ± 0.7 in 10 min ($n = 25$), compared with 3.6 ± 0.6 in 10 min ($n = 42$) in the tissue from pregnant women. These contraction rates were not significantly different.

The mean value of resting pH_i was significantly lower ($P = 0.01$) in the nonpregnant women tissue from (7.06 ± 0.03 ; $n = 39$) than in the tissue from pregnant women (7.14 ± 0.01 ; $n = 53$).

Intracellular alkalinization

Addition of the weak base NH_4Cl produced a rapid change in pH_i (Fig. 1). The mean time to half-maximal pH_i response was 47 ± 4 s ($n = 15$) in the nonpregnant, and 48 ± 5 s ($n = 16$) in the pregnant tissue. The mean increase in pH_i was 0.13 ± 0.02 pH units ($n = 15$) in the nonpregnant, and

Fig. 1. The effect of a weak base (ammonium chloride) on contraction and intracellular pH in myometrium from a pregnant woman.

0.14 ± 0.02 pH units ($n = 16$) in the pregnant tissue. During continued application of NH_4Cl , the pH_i started to recover towards resting values, in both pregnant and nonpregnant preparations (Fig. 1). This was presumed to be due to pH_i regulation within the cell. Removal of the weak base produced an acidic overshoot in pH_i , and this was followed by a return to resting values. The mean size of the acidic overshoot was not significantly different, at 0.11 ± 0.02 pH units in the nonpregnant, and 0.12 ± 0.02 pH units in the pregnant tissue.

The intracellular alkalinization produced by addition of NH_4Cl was associated with an increased frequency and amplitude of contraction (Fig. 1). The amplitude of the contractions was increased by $54 \pm 13\%$ in the nonpregnant, ($n = 16$), and by $39 \pm 12\%$ in the pregnant ($n = 14$) tissue. These changes were not significantly different. In many preparations, there was a brief delay before the increased contractility was seen. Removal of NH_4Cl produced an initial increase in frequency and/or amplitude of contractions in 15 of 16 nonpregnant, and 17 of 17 pregnant preparations (Fig. 1). This period of increased contractile activity was followed by an abolition of contractions in all preparations while pH_i was acidic. Contractions recurred when pH_i was restored to near resting values.

The effects of NH_4Cl on pH_i and contractility were concentration dependent (Fig. 2). The mean values of the maximal pH_i change produced by 10, 20 and $30\text{ mmol NH}_4\text{Cl l}^{-1}$ in the nonpregnant tissue were 0.05 ± 0.02 , 0.08 ± 0.03 and 0.12 ± 0.03 pH units, respectively ($n = 4$). The effects of alkalinization by weak base on contractile activity were also dose dependent (Fig. 2). The mean values of the acidic overshoot upon removal of 10, 20 and $30\text{ mmol NH}_4\text{Cl l}^{-1}$ were 0.06 ± 0.02 , 0.09 ± 0.02 and 0.10 ± 0.03 pH units, respectively. The time for which contractions were abolished after removal of the weak base was also related to the concentration of NH_4Cl added (Fig. 2); it was 7 ± 1 , 17 ± 3 and 23 ± 2 min for 10, 20 and $30\text{ mmol NH}_4\text{Cl l}^{-1}$, respectively, in the nonpregnant tissue ($n = 4$). In the pregnant tissue similar results were obtained. Thus, the mean values of pH_i change produced by 10, 20 and $30\text{ mmol NH}_4\text{Cl l}^{-1}$ were 0.06 ± 0.02 , 0.10 ± 0.03 and 0.11 ± 0.02 , respectively ($n = 8$). The mean values of the acidic overshoot on removal of 10, 20 and $30\text{ mmol NH}_4\text{Cl l}^{-1}$ were 0.06 ± 0.01 , 0.05 ± 0.01 and

Fig. 2. The effect of different concentrations of a weak base (ammonium chloride) on contraction and intracellular pH in myometrium of a nonpregnant woman.

0.07 ± 0.02 . The time for which contractions were abolished after removal of the weak base was 4 ± 2 , 9 ± 3 and 14 ± 3 min for 10, 20 and $30\text{mmol NH}_4\text{Cl l}^{-1}$, respectively ($n = 8$).

Intracellular acidification

Addition of sodium butyrate to the preparations produced a decrease in pH_i . As with addition of a weak base, the change in pH_i was rapid (Fig. 3). The mean time to half-maximal response was $42 \pm 4\text{ s}$ ($n = 9$) in the nonpregnant tissue, and $44 \pm 4\text{ s}$ ($n = 6$) in the pregnant tissue. These rates of change were not significantly different. The mean fall in pH_i on addition of butyrate was not significantly different between the nonpregnant ($0.13 \pm 0.02\text{ pH units}$; $n = 9$), and pregnant preparations ($0.17 \pm 0.02\text{ pH units}$, $n = 6$). During the continued application of butyrate, for periods of up to 5 min, the pH_i was seen to recover towards resting values. Removal of sodium butyrate caused a rapid rise in pH_i to above resting values. The mean extent of the pH_i overshoot was $0.08 \pm 0.02\text{ pH units}$ ($n = 9$) in the nonpregnant, and $0.13 \pm 0.02\text{ pH units}$ ($n = 6$) in the pregnant tissue. The intracellular pH then returned slowly to resting values.

After butyrate was added there was an initial increase in frequency or amplitude of contractions in 5 of 9 nonpregnant, and 5 of 6 pregnant preparations. The amplitude of the contractions was increased by $26 \pm 18\%$ in the nonpregnant ($n = 9$), and by $45 \pm 29\%$ in the pregnant tissue ($n = 6$). There was then an abolition of contractions, which was seen in all preparations, and lasted until the butyrate was removed.

Fig. 3. The effect of a weak acid (butyrate) on contraction and intracellular pH in myometrium from a pregnant woman.

After removal of butyrate, normal spontaneous activity gradually returned. This correlated with the return of pH_i to resting values.

The alkalinization produced by removal of the weak acid was associated only with an increase in contractile activity in 2

of 9 nonpregnant preparations, and in none of the pregnant preparations ($n = 6$). This is in contrast to the direct effect of alkalization produced by addition of a weak base (Figs 1 and 2).

Discussion

Although there were indirect observations suggesting that pH_i changes caused alterations in the contractility of human myometrium (Phoenix and Wray, 1993), this is the first full report where pH_i and tension have been simultaneously measured, following a preliminary report (Parratt *et al.*, 1994), allowing the direct examination of the relationship between pH_i and tension in the human uterus. Profound effects of pH_i on contraction were demonstrated, and these may have consequences for uterine function in labour.

Loading the isolated human myometrial tissue with carboxy-SNARF allowed measurements of pH_i to be recorded for up to 4 h, and did not alter the contractile activity of the tissue. The value of pH_i found (approximately 7.1) was similar to that reported in rat uterus (Taggart and Wray, 1993), and in other smooth muscles (Aicken, 1984; Siskind *et al.*, 1989).

Alteration of pH_i had similar effects in pregnant and nonpregnant preparations, and they will therefore be discussed together. Alteration of pH_i by addition of weak acid or base produced early transient effects that were opposite to those occurring later. Addition of a weak base led to a rapid increase in pH_i , with an initial reduction in frequency and/or amplitude of contractions, followed by an increase in contractions. Addition of a weak acid caused a rapid decrease in pH_i , with an initial increase in frequency and/or amplitude of contractions, followed by abolition of contractions. This initial increase in contractions on acidification was also seen on removal of the weak base, when pH_i overshot to below resting values. However alkalization produced by removal of weak acid did not generally lead to the same effects as direct alkalization by a base. The reason for this is unclear.

Intracellular acidification by butyrate eventually abolished contractions in all preparations. Rebound acidification after removal of NH_4Cl reduced pH_i by a similar amount, and also abolished contractions in all preparations. Hypoxia in the rat uterus, simulated by cyanide, has been shown to cause a fall in pH_i of 0.3 pH units (Wray, 1990), and a study of ischaemia in rat uterus *in vivo* showed that pH_i falls on occlusion of the uterine artery (Harrison *et al.*, 1994). During metabolic inhibition by cyanide, and in uterine ischaemia, contractions were either reduced or abolished. Thus, it may be that the acidification associated with metabolic inhibition or ischaemia contributes to the fall in force seen. As this study has shown, acidification will lead to the abolition of contractions in the human uterus. The implications of this for human labour are discussed below.

There are several possible mechanisms whereby H^+ could influence force production. The first mechanism is alteration in Ca^{2+} entry into the cell. Lowering the pH_i value inhibits Ca^{2+} entry at voltage-gated channels in guinea-pig ventricular myocytes (Irisawa and Sato, 1986; Kaibara and Kameyama, 1988), and in vascular myocytes from bovine pial and porcine coronary arteries (Klößner and Isenberg, 1994) and portal

venous smooth muscle (Iino *et al.*, 1994). If such a mechanism was present in the myometrium, then it may have a profound effect on force. Preliminary evidence from rat myometrium suggests that acidification can suppress the Ca^{2+} transients preceding spontaneous contractions (Heaton and Wray, 1994, J. R. Parratt, M. J. Taggart and S. Wray, unpublished observations). This may be due to inhibition of the L-type Ca^{2+} transients. The second mechanism is alteration of Ca^{2+} release from intracellular stores. Alkalization increases $[Ca^{2+}]_i$ in cultured vascular smooth muscle cells (Siskind *et al.*, 1989). A significant rise in $[Ca^{2+}]_i$ occurred even in the absence of extracellular Ca^{2+} , indicating the presence of pH-sensitive intracellular Ca^{2+} stores, presumed to be the sarcoplasmic reticulum. Acidification may be expected to reduce $[Ca^{2+}]_i$, by decreasing Ca^{2+} release from these intracellular stores. The third possible mechanism is displacement of Ca^{2+} from intracellular binding sites. Protons and Ca^{2+} are known to compete for intracellular binding sites (Wray, 1988); a rapid rise in $[H^+]_i$ may therefore lead to a rise in $[Ca^{2+}]_i$, as they are displaced from common binding sites. Such a mechanism could not explain the fall of force with acidification, or increase with alkalization, but may account for the transient (and opposite) changes in force seen during initial application or withdrawal of weak acids and bases. Alteration of the sensitivity of myofibrils to Ca^{2+} is the other possible mechanism. If protons could alter the relationship between calcium and force (i.e. sensitivity), this could contribute to some of the changes in force observed. However, studies using permeabilized myometrium provide little evidence to support this (Crichton *et al.*, 1993).

The transient early changes in contractile activity on addition of a weak acid or base coincided with the period when the pH_i was changing rapidly. A rapid increase in $[H^+]_i$ may lead to the initial effect of increasing contractility by displacing Ca^{2+} from intracellular binding sites, as described in the third mechanism above. As the cell begins to regulate its intracellular pH_i , H^+ ions have effects at other parts of the excitation-contraction pathway, for example, decreasing Ca^{2+} entry into the cell at the cell membrane, or decreasing the release of Ca^{2+} from intracellular stores, as described in the first and second mechanisms above. This would then reduce or abolish contractions. The mechanisms would be reversed on addition of a weak base. Simultaneous measurement of force and $[Ca^{2+}]_i$ may shed light on these processes. It has been suggested that changes in intracellular $[Ca^{2+}]$ account for the biphasic contractile response of another phasic smooth muscle, rat portal vein, to changes in intracellular pH (Taggart *et al.*, 1994b).

The powerful contractions seen in human labour are known to occlude the uterine arteries (Griess, 1965; Brar *et al.*, 1988), which would lead to myometrial ischaemia and a fall in pH_i (Harrison *et al.*, 1994). Even Braxton-Hicks contractions during pregnancy are known to significantly alter uterine blood flow. The effect of a reduction in pH_i , as reported here, is to reduce contraction. Limiting the duration of contraction would prevent uterine ischaemia and necrosis, limit fetal hypoxia, and allow recovery of the myometrium, so that the next contraction may be strong and effective. However, if the uterus suffers hypoxia for extended periods, as may occur in prolonged labour, myometrial cells may not be fully replenished with metabolites between contractions, pH_i will remain acidic, and the uterine contractions will become less efficient. An inefficient

contractile pattern is one of the causes of failure to progress in labour.

In conclusion, we have shown that pH_i and contraction can be simultaneously measured in human myometrial samples. In addition, pH_i can markedly alter contractions, with acidification often abolishing contractions. If similar changes occurred in prolonged labours, they could contribute to inefficient contractile patterns and deleteriously affect the progress of labour.

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