Modulation of force induced by pH in the guinea-pig uterus examined at two stages of the oestrous cycle

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Changes in pH have a marked influence on uterine contractility. Changes in uterine pH occur during pregnancy and labour, when marked endocrine changes are occurring. As hormonal status can also influence contractility, this study investigated whether pH-induced modulation of uterine force is influenced by hormonal changes. The effects of altering intracellular and extracellular pH on uterine contractions were studied in guinea-pigs on day 7 (high progesterone) and day 15 (low progesterone) of the oestrous cycle. Resting values of pH were significantly more acidic on day 15 compared with day 7, and more force was produced on day 15. Changing external pH produced similar changes in intracellular pH and force on both days. External acidification was associated with a large increase in force. In contrast, intracellular acidification, at constant external pH, reduced force. In conclusion, the stage of the oestrous cycle has a large effect on resting pH in the myometrium but only small effects on the pH-induced modulation of force, and the link between pH and force is complex.

Introduction

Previous studies showed that changes in intracellular pH (pH_i) modulate force in a variety of smooth muscles (Wray, 1988; Austin and Wray, 1994; Burdya *et al.*, 1996). In myometrial smooth muscle obtained from both rats (Taggart and Wray, 1993) and women (Parratt *et al.*, 1994, 1995a), intracellular acidification decreases, and intracellular alkalinization increases, spontaneous force. Extracellular pH (pH_o) change also affects force in myometrium but its effects vary with gestational state (Naderali and Wray, 1996; Taggart *et al.*, 1997). In the human uterus, pH_i increases throughout gestation (Parratt *et al.*, 1995b); an effect that has been attributed to hormonal changes.

Myometrial contractility is affected by various factors including hormones (Neulen and Breckwoldt, 1994). Progesterone reduces myometrial contractility and sensitivity to labour-inducing agents. Treatment of patients with anti-progestins, such as mifepristone, increases the sensitivity of myometrium to labour-inducing agents (Swahn and Bygdeman, 1988).

During the oestrous cycle, myometrial contractility is under continuous influence by the sex steroids. However, it is not known whether these physiological changes in hormone concentrations can alter the contractile response of the uterus to pH alteration, which may be of relevance during labour when a decrease in pH is anticipated. This decrease in pH is due to periods of hypoxia, resulting from the occlusion of blood vessels within the contracting uterus, and may inhibit subsequent contractility. Any hormonal

difference, for example, between term and pre-term labours, may influence the response of the uterus to such changes. Hence, the present study investigated the pH and force at two stages of the oestrous cycle, when marked differences in the hormonal environment may be expected. In guinea-pigs, peripheral plasma progesterone concentration is at its highest on day 7 of the oestrous cycle, and is 4.1 times greater than that on day 15 (Poyser, 1983). Therefore, guinea-pig myometrium was studied on these days to elucidate the effect of pH change on uterine contractions during physiological changes of steroid hormone concentrations.

Materials and Methods

Virgin Dunkin–Hartley guinea-pigs (400–700 g) were examined daily and a vaginal smear was taken when the vagina was open. Day 1 of the oestrous cycle was taken as the day before the post-ovulatory influx of leucocytes, when cornification was at a maximum. All guinea-pigs had exhibited at least three ovarian cycles of normal duration before being killed (by CO_2 inhalation) on day 7 or day 15 of the oestrous cycle. Strips of longitudinal myometrium approximately 100 μm thick were dissected and loaded with the membrane-permeant form of the pH-sensitive indicator carboxy-SNARF ($5 \mu\text{mol l}^{-1}$) at room temperature for 3 h. There were no significant differences in the size or mass of the tissues between day 7 and day 15 preparations. After loading, the tissues were transferred to a small (200 μl) organ bath on an inverted microscope, with one end attached to a force transducer (Grass FT03). The tissues were perfused continuously with oxygenated and Hepes-buffered (11 mmol l^{-1}) Krebs' solution of the following composition:

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154 mmol NaCl l⁻¹; 5.4 mmol KCl l⁻¹; 1.2 mmol MgSO₄ l⁻¹; 12 mmol glucose l⁻¹; and 3 mmol CaCl₂ l⁻¹ at 36 ± 1°C, pH 7.4. Previous work had shown that the responses of the myometrium to pH alterations were the same in Hepes- and HCO₃-CO₂-buffered solutions (Taggart and Wray, 1993). The pH signals were calibrated from the fluorescence signals emitted by SNARF at 590 and 640 nm, using a mock intracellular solution, as described by Taggart and Wray (1993). The 'time-averaged force' was calculated by integrating the contraction record over unit time to take into account the effect of experimental manoeuvres on the amplitude, duration and frequency of contractions. The records were normalized for tissue mass and to control contractions at the start of the experiment. Measurements of intracellular pH and contractions were made for up to 3 h.

In some experiments, 40 mmol KCl l⁻¹ (isosmotically substituted for NaCl) was used to depolarize myometrial tissues and produce maintained force. External pH was altered either by raising it from 7.4 to 8.4 or lowering it to 6.4, by addition of strong acid or base to the superfusing solution. Intracellular pH was altered at constant external pH by addition of weak acid (40 mmol butyrate or propionate l⁻¹) or a weak base (40 mmol NH₄Cl l⁻¹), isosmotically substituted for NaCl.

Statistical analysis

The results were analysed by one-way analysis of variance (ANOVA), or Student's *t* test, as appropriate.

Results

Resting pH and contractile activity

The resting value of intracellular pH was significantly ($P < 0.001$, $n = 6$) more acidic at the end of the oestrous cycle (day 15) than at mid-cycle (day 7); $pH_i = 7.12 \pm 0.02$ and 7.34 ± 0.04 , respectively. The amplitude of the contractions on day 15 was higher, compared with day 7, leading to a significant difference in integrated force between the two days ($P < 0.01$). A record of simultaneous force and pH_i at both stages of the oestrous cycle is shown (Fig. 1).

Fig. 1. Simultaneous recording of spontaneous contractions and intracellular pH from guinea-pig myometrium on day 7 and day 15 of the oestrous cycle. The pH_i record was obtained via the pH-sensitive fluorophore, carboxy-SNARF, and the temperature was 37°C.

Effect of depolarization

Depolarization of the uterus with high-K⁺ solution caused a change in the pattern of uterine activity from phasic to maintained (tonic) contraction at both stages of the oestrous cycle (Fig. 2). As with spontaneous activity, significantly more uterine force was produced on day 15 than on day 7. In agreement with previous studies (Naderali *et al.*, 1997), this contraction was associated with a decrease in pH_i (Fig. 2). However, despite the fact that less force was produced, the acidification was twofold greater on day 7 than on day 15 (0.16 ± 0.03 and 0.07 ± 0.01 , respectively; $n = 4$). Small acidifications were also evident with the spontaneous contractions (Fig. 2).

Effects of altering external pH

Extracellular acidification caused a significant increase in force and an internal acidification in both day 7 and day 15 myometrium (Fig. 3). When extracellular pH was decreased by one pH unit (to 6.4), there was a decrease in pH_i of 0.38 ± 0.03 and 0.41 ± 0.07 pH unit in day 7 and 15 myometrium, respectively ($n = 6$). The half times for these changes were also similar, 2.5 ± 0.5 and 2.2 ± 0.3 min, respectively, for days 7 and 15. The increase in force was $242 \pm 34\%$ on day 7 and $164 \pm 22\%$ on day 15. There were no significant differences between the effects of extracellular acidification on changes in pH_i or force between day 7 and day 15 myometrium.

Extracellular alkalinization (pH 8.4) induced an increase in internal pH in both day 7 and day 15 myometrium (Fig. 4). The magnitude of the changes in internal pH was 0.41 ± 0.02 and 0.40 ± 0.04 pH unit for days 7 and 15, respectively.

Fig. 2. The effects of high K⁺-depolarization on force and pH_i in guinea-pig myometrium on day 7 and day 15 of the oestrous cycle. ■, KCl. The pH_i record was obtained via the pH-sensitive fluorophore, carboxy-SNARF, and the temperature was 37°C.

Fig. 3. The effects of external acidification (from pH 7.4 to 6.4) on force and pH_i in guinea-pig myometrium on day 7 and day 15 of the oestrous cycle. ■, $\text{pH}=6.4$. The pH_i record was obtained via the pH-sensitive fluorophore, carboxy-SNARE, and the temperature was 37°C .

Fig. 4. The effects of external alkalinization (from pH 7.4 to 8.4) on force and pH_i in guinea-pig myometrium on day 7 (a) and day 15 (b,c) of the oestrous cycle. The response on day 15 of the oestrous cycle was either abolition (b) or potentiation (c) of force. ■, $\text{pH}=8.4$. The pH_i record was obtained via the pH-sensitive fluorophore, carboxy-SNARE, and the temperature was 37°C .

Extracellular alkalinization resulted in a profound inhibition of spontaneous contractile activity at day 7, when force fell by $95\% \pm 5\%$ ($n=5$) (Fig. 4a). However, pH had one of two effects on the spontaneous contraction of day 15 uterus; in 4/10 preparations, extracellular alkalinization strongly inhibited the contractions (by $86 \pm 9\%$), in a similar manner to that observed in day 7 preparations (Fig. 4b). However, in 6/10 preparations, extracellular alkalinization potentiated the contractions ($273 \pm 43\%$) (Fig. 4c). These differences were not reflected in any differences in the intracellular pH change. As with acidification, the time for pH_i to change when external pH was altered was not significantly different between days 7 and 15 (2.9 ± 0.3 and 2.8 ± 0.3 , respectively).

Effects of intracellular pH change

Application of a weak acid (butyrate) caused a mean decrease in pH_i of 0.12 ± 0.02 pH units ($n=4$), with a half time to maximal response ($t_{1/2}$) of 0.50 ± 0.05 min, and abolition of the spontaneous contraction on day 7 myometrium (Fig. 5a). Spontaneous contractions remained suppressed for as long as butyrate was applied (up to 10 min). Upon removal of the butyrate, pH recovered, and this was accompanied by the re-appearance of spontaneous contractions (data not shown). Butyrate also acidified the uterus (pH 0.15 ± 0.02 , with $t_{1/2} = 0.40 \pm 0.06$ min) and abolished spontaneous contractions on day 15 (Fig. 5b). However, contractions re-appeared during the 10 min application of butyrate (Fig. 5b). The same effect was also seen with the application of another weak acid, propionate (Fig. 5c). This recovery of force in the continued presence of the weak acid was never seen in the day 7 preparations. Regulation of pH was more apparent in the day 15 preparations (Fig. 5b,c) than in the day 7 preparations (Fig. 5a), but the rate of regulation just failed to reach statistical significance ($P < 0.06$).

Application of a weak base (NH_4Cl) caused a rapid increase in pH_i on both day 7 (0.17 ± 0.02 pH units) and day 15 (0.22 ± 0.02 pH units), with no significant differences between them (Fig. 6). The half-times of these increases in pH_i were 0.53 ± 0.05 min and 0.50 ± 0.04 min for day 7 and day 15 myometrium, respectively, $n=4$. There was a rapid regulation of pH towards resting values on both days. A very marked potentiation of the spontaneous contraction occurred in both day 7 and day 15 myometrium, upon application of the NH_4Cl . This potentiation caused the contraction to be maintained (tonic) (Fig. 6). This increase in force persisted as long as NH_4Cl was present, at both stages of the oestrous cycle, despite the presence of pH regulation. Upon removal of weak base, there was a rebound internal acidification and abolition of contraction in both day 7 and

Fig. 5. The effects of intracellular acidification at constant external pH_i by butyrate (40 mmol l^{-1} , a,b) or propionate (40 mmol l^{-1} , c) on force and pH_i in guinea-pig myometrium on day 7 and day 15 of the oestrous cycle. The pH_i record was obtained via the pH-sensitive fluorophore, carboxy-SNARE, and the temperature was 37°C .

Fig. 6. The effects of intracellular alkalinization at constant external pH, by ammonium chloride (40 mmol l⁻¹), on force and pH_i. ■, NH₄Cl. The pH_i record was obtained via the pH-sensitive fluorophore, carboxy-SNARE, and the temperature was 37°C.

day 15 myometrium, followed by a return to resting pH and spontaneous activity (data not shown).

Discussion

Changes in intracellular and extracellular pH affect uterine contractility in rats and women. The present study showed that guinea-pig uterine contractility is also markedly modulated by pH alterations, indicating that its mechanism of action is on the basic process of excitation-contraction coupling in myometrium, and is independent of species. Both contraction and the effects of pH change on contractions are sensitive to the oestrous cycle. Thus, at the end of the oestrous cycle in guinea-pigs, when progesterone is relatively low, more force is produced despite resting pH_i being low, in comparison with day 7 of the oestrous cycle, when progesterone is high. The effects of altering either external or internal pH on spontaneous activity were qualitatively similar at the two times. There was also no difference in the speed or extent to which pH_o changes were transmitted to the cytoplasm. Presumably, the oestrous cycle affects resting uterine pH_i in guinea-pigs via the changing hormonal concentrations (a result consistent with earlier data obtained in women), but has relatively minor effects on the modulation of force induced by pH.

The lower resting pH_i in the tissues on day 15 than in those on day 7 indicates that hormonal status influences pH_i in the uterus, with progesterone favouring alkaline pH. A significant alkalinization of resting pH_i close to term, compared with non-pregnant uterine samples, has been reported in the human uterus (Parratt *et al.*, 1995b). Again, the higher progesterone concentrations at this time of pregnancy appear to be associated with a more alkaline resting pH_i. The mechanism of this effect is unclear but may involve agonist-induced stimulation of the Na-H exchanger, as has been reported for vascular smooth muscle (Berk, 1996).

The amount of force produced by the uterus, either spontaneously or by high K⁺-induced depolarization, was significantly greater on day 15, presumably due to the removal of the inhibitory influence of progesterone. Spontaneous activity of isthmic and ampullary smooth muscles are also affected by oestrogen and progesterone (Higgs and Moawad, 1974; Lindblom *et al.*, 1980). Stage of the oestrous cycle also affects the frequency of contractions of the infundibulum (Friedman *et al.*, 1994). In the present study, there was no difference in the frequency of the spontaneous contraction *in vitro* at different stages of the oestrous cycle. The significant increase in contractile strength when oestrogen concentrations were higher is consistent with earlier reports (Friedman *et al.*, 1994).

There is a correlation between the degree of force production and the magnitude of the acidification-produced pH_i change (Naderali *et al.*, 1997). Therefore, it was expected that, as force was greater on day 15, the pH_i change in day 15 tissue would be greater than that of day 7 tissues, but this did not prove to be the case. It is unlikely that an alteration of intracellular buffering power β can account for this, as the induced pH_i change after addition of weak acid or weak base was not significantly different at day 7 and day 15, indicating no change in β. The different pH_i response may be related to the mechanisms maintaining the resting pH_i more acidic at day 15. The acidification with contraction is considered to arise from the proton influx linked to Ca²⁺ extrusion on the Ca-ATPase (Naderali *et al.*, 1997). If this mechanism was less active on day 15 and more Ca²⁺ was expelled via other mechanisms, for example, the Na-Ca exchanger (Shmigol *et al.*, 1998), then this would decrease the acidification compared with day 7.

The links between pH_i and force were not straightforward in the guinea-pig uterus. Intracellular acidification, at constant pH_o, abolished spontaneous contractions in all tissues tested. These effects were similar to those reported in rats and women (Taggart and Wray, 1993; Parratt *et al.*, 1994, 1995a). However, in the present study, contractions reappeared after 5–10 min in weak acid in day 15 but not in day 7 tissues. This finding may be related to the faster rate of regulation seen in day 15 tissues. Intracellular alkalinization increased force on both days and the effect was very marked, transforming contractile activity from phasic to tonic. The effects of pH_i on uterine force are due to changes in intracellular Ca²⁺ ([Ca²⁺]_i) in rats (Taggart *et al.*, 1996). Thus, acidification reduced [Ca²⁺]_i and contractions. In turn, this effect in single cells was due to an inhibition of the voltage-gated Ca²⁺ channel current by acidification (Shmigol *et al.*, 1995). Given the similar effects on force of pH_i alteration between rats and guinea-pigs, it is expected that similar mechanisms operate in the guinea-pig uterus to alter force.

Lowering pH_o caused a similar increase in force in both day 7 and day 15 tissues, while increasing pH_o decreased force in day 7 tissues and in many of the day 15 tissues. The inhibitory effect of increased pH_o, found also in rat myometrium (Taggart *et al.*, 1997), is unusual; as alkaline changes are often associated with increased tone (for example, blood vessels; Austin and Wray, 1994). In rats, the increased pH_o caused a decrease in [Ca²⁺]_i which could be reversed by Ca²⁺ channel agonists. In the guinea-pig uterus,

the effects of pH_o on force were not due to the induced change in pH_i , as this was shown to have the opposite functional effect, that is, intracellular alkalinization increased force. The effects of external acidification may be explained by the reversal of these effects. On day 15, some tissues showed an opposite effect to alkalinization, that is, force increased. This non-uniform effect of increasing pH_o may be due to variations in the duration of the oestrous cycle. Since the oestrous cycle may last from 15 to 18 days, day 15 in one animal may not be equivalent to day 15 in another animal. However, such a variation was not observed when pH_o was decreased. What is clear from the present study is that there is no significant difference in the transference of the pH_o change to an intracellular pH change with hormonal status. This finding indicates that the permeability of the membrane to protons does not change, nor do any pH_o -dependent pH regulatory mechanisms.

Overall, the present study indicates that the oestrous cycle influences the mechanisms maintaining resting pH_i in guinea-pig uterus. Furthermore, as anticipated, high oestradiol and low progesterone (day 15) are associated with an increased force-producing ability in the myometrium. However, at both stages of the oestrous cycle, pH changes greatly modified force production. The effects of the oestrous cycle and, hence, of hormonal background, on that ability were slight, indicating that pH exerts its effect on the basic process of excitation-contraction coupling, particularly Ca^{2+} entry.

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References

- Austin C and Wray S (1994) A quantitative study of the relation between pH and force in rat vascular smooth muscle *Pflügers Archiv* **427** 270–276
- Berk BC (1996), Regulation of the Na^+/H^+ exchanger in vascular smooth muscle. In *The Na^+/H^+ Exchanger* pp 47–67 Ed. L Fliegel. Springer, New York
- Burdyga TV, Taggart MJ and Wray S (1996) An investigation into the mechanism whereby pH affects tension in guinea-pig ureteric smooth muscle *Journal of Physiology* **493** 865–876
- Friedman R, Lais T, Weber DW and Stormshak F (1994) Responses of the bovine infundibulum to noradrenaline during the oestrous cycle *Journal of Reproduction and Fertility* **101** 311–315
- Higgs GW and Moawad AH (1974) The effects of ovarian hormones on the contractility of the rabbit oviductal isthmus *Canadian Journal of Physiology and Pharmacology* **52** 74–83
- Lindblom B, Hamberger L and Ljung B (1980) Contractile patterns of isolated oviductal smooth muscle under different hormonal conditions *Fertility and Sterility* **23** 283–287
- Naderali EK and Wray S (1996) External alkalinization influence both internal pH and force in pregnant and non-pregnant rat uterus *Journal of Reproduction and Fertility Abstract Series* **18** Abstract 22
- Naderali EK, Buttell N, Taggart MJ, Bullock AJ, Eisner DA and Wray S (1997) The sarcolemmal Ca^{2+} -ATPase affects both $[\text{Ca}^{2+}]_i$ and pH in smooth muscle: a mechanism for co-ordinated control of contraction and excitation? *Journal of Physiology* **505** 329–336
- Neulen J and Breckwoldt M (1994) Placental progesterone, prostaglandins and mechanisms leading to initiation of parturition in the human *Experimental and Clinical Endocrinology* **102** 195–202
- Parrat JR, Taggart MJ and Wray S (1994) Abolition of contractions in the myometrium by acidification *in vitro*. *Lancet* **344** 717–718
- Parrat JR, Taggart MJ and Wray S (1995a) Functional effects of intracellular pH alteration in the human uterus: simultaneous measurements of pH and force *Journal of Reproduction and Fertility* **105** 71–75
- Parrat JR, Taggart MJ and Wray S (1995b) Changes in intracellular pH close to term and their possible significance to labour *Pflügers Archiv* **430** 1012–1014
- Poyser NL (1983) Differential stimulation of prostaglandin and thromboxane synthesizing capacity in guinea-pig uterus and ovary *Prostaglandins and Leukotrienes in Medicine* **10** 163–177
- Shmigol AV, Smith RD, Taggart MJ, Wray S and Eisner DA (1995) Changes of pH affect calcium currents but not outward potassium currents in rat myometrial cells *Pflügers Archiv* **431** 135–137
- Shmigol A, Eisner DA and Wray S (1998) Carboxyeosin decreases the rate of the Ca^{2+} transient decay in uterine smooth muscle cells isolated from pregnant rats *Pflügers Archiv* **437** 158–160
- Swahn ML and Bygdeman M (1988) The effect of the antiprogesterin RU486 on uterine contractility and sensitivity to prostaglandin and oxytocin *British Journal of Obstetrics and Gynaecology* **95** 126–134
- Taggart MJ and Wray S (1993) Occurrence of intracellular pH transients during spontaneous contractions in rat uterine smooth muscle *Journal of Physiology* **472** 23–31
- Taggart MJ, Burdyga ThV, Heaton RC and Wray S (1996) Stimulus-dependent modulation of smooth muscle intracellular calcium and force by altered intracellular pH *Pflügers Archiv* **432** 803–811
- Taggart MJ, Shearer E, Walker S, Naderali EK, Moore S and Wray S (1997) Increasing external pH decreases intracellular $[\text{Ca}^{2+}]_i$ and spontaneous contractions in pregnant rat myometrium *American Journal of Obstetrics and Gynecology* **117** 959–963
- Wray S (1988) Smooth muscle intracellular pH: measurements, regulation, and function *American Journal of Physiology* **254** C213–C225