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SHORT COMMUNICATION

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# Changes of pH affect calcium currents but not outward potassium currents in rat myometrial cells

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**Abstract.** Spontaneous contraction of uterine smooth muscle is enhanced by alkalinization and depressed by acidification. We have investigated the ionic currents responsible for this in single myometrial cells. Intracellular acidification (20 mM butyrate) at constant external pH depressed the magnitude of the calcium current to 58±6% of control, but had little effect on outward currents. Similar but slower effects were also observed when the extracellular pH was lowered to 6.9 (56±9% of control). Correspondingly, when the intracellular or extracelluar pH was elevated (20 mM $NH_4Cl$ or pH 7.9 respectively) the calcium current magnitude increased (165±15 % in $NH_4Cl$; 136±2% at pH 7.9) and there was, again, no effect on the outward currents. These observations are consistent with the effects of pH on spontaneous contractile activity being due to an effect on the membrane calcium current.

**Key Words:** pH, uterus, calcium current, potassium current

## Introduction

Previous studies have shown that in both rat and human uterus, alterations of pH can greatly alter the spontaneous contractile activity [7,8]. Specifically, in both pregnant and non-pregnant myometrium, an intracellular acidification will reduce the frequency and amplitude of the contractions. An intracellular alkalinization increases the frequency and/or magnitude of the uterine contractions. The mechanism(s) underlying the actions of pH are not fully understood. A recent report has shown that the calcium transient, which underlies spontaneous activity, can be abolished when the cytoplasm is acidified [3]. It is not known however whether this is due to a direct effect on the calcium channel (as is the case for example in some vascular smooth muscle [5]) or alternatively, via an indirect effect e.g. due to an increased K outward current decreasing the probability of the calcium channels opening. Indeed there has been little or no work on the effects of changes of pH on the electrophysiological properties of spontaneously active smooth muscle, such as the uterus. A further question is whether changes of external pH have direct effects on membrane currents or act via changes induced in $pH_i$ [2].

The aim of the present study was therefore to investigate the effects of intra- and extracelluar pH on inward and outward currents.

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## Methods

Days 19-21 pregnant rats (term, day 22) were killed by a blow to the head and cervical dislocation. The uterus was removed and small strips of longitudinal smooth muscle dissected. Single cells were produced by enzymatic digestion [5] using 0.1% collagenase Type V and 0.04% elastase Type IIA. Voltage clamp control was imposed with the whole cell patch clamp technique. The capacity currents were reduced electronically using the whole cell parameters compensation facility of the Axopatch-200A amplifier. The residual capacity and leakage currents were removed using the P/4 procedure of CLAMPEX (Axon Instruments). Membrane currents were low pass-filtered at 2 kHz and digitized with a sampling frequency of 5 kHz. For measurements of membrane capacitance, we used 10 mV hyperpolarising pulses, 5 kHz filtering, 10 kHz sampling rate and neither capacity compensation nor P/4 protocol were employed. For experiments in which outward currents were measured, the pipettes contained (mM): NaCl, 5; KCl, 120; $MgCl_2$, 5; $KH_2PO_4$, 1; HEPES, 5; EGTA, 0.5; Mg-ATP, 5; creatine phosphate, 5; glucose, 5 (pH 7.2 NaOH). In order to measure calcium currents without contamination from outward currents, KCl was replaced by CsCl. The external solution contained (mM): NaCl, 154; KCl, 5.4; $MgSO_4$, 1.2; $CaCl_2$, 2; HEPES, 11; glucose, 11.7 (pH 7.4 NaOH). Intracellular acidification or alkalinization was produced by isosmotic substitution of 20 mM sodium butyrate or ammonium chloride, respectively, at constant external pH of 7.4. Changes of external pH were produced by addition of strong acid or base. All

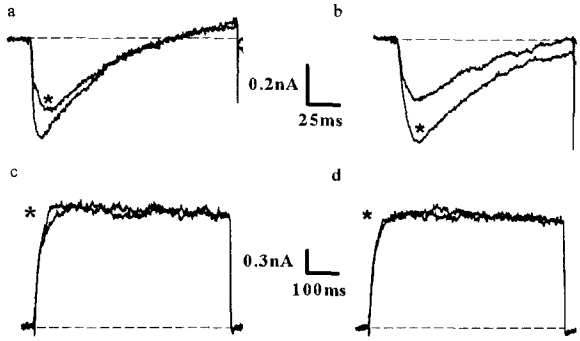


**Fig. 1.** The effects of changes of intracellular pH ($pH_i$) on membrane currents. In all panels the control trace is unmarked and the intervention labelled (*). Panels a and b show the effects on the calcium current of sodium butyrate (20 mM) and $NH_4Cl$ (20 mM) respectively. In both panels 100 ms duration pulses to +10mV were applied from -60 mV. Panels c & d show the effects on the potassium current. In both panels 400 ms duration pulses to +40 mV were applied from -80 mV.

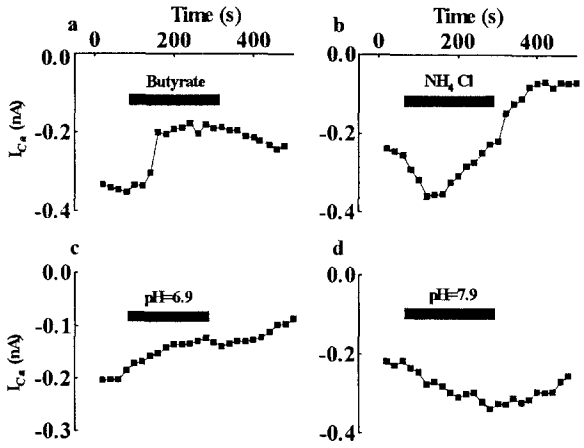


**Fig.2** Time course of the effects of changes of intra- or extracelluar pH on the calcium current. Panels show the effects of adding: a, Butyrate (20 mM); b, $NH_4Cl$ (20 mM) or changing external pH to: c, 6.9; d, 7.9.

experiments were carried out at room temperature (22 °C). All statistics are presented as mean ± s.e.m. The cells were obtained from 9 animals. 'n' values refer to the number of cells.

## Results

The mean capacitance of the cells was 102±45 pF. Depolarization produced an inward calcium current which, when the pipette contained potassium, was followed by an outward current which developed within 20-40 ms and then inactivated. The magnitude of this inactivation was variable (20-50% when stepping to +70 mV for 1 sec). The current records are broadly similar to those reported previously [6]. In the experiments illustrated in this paper the inward currents were studied in isolation using Cs-filled pipettes. Under these conditions, inward Ca currents were observed in 18 out of 19 cells. The peak current was reached with a depolarization to +10 mV and was completely inhibited by 2 $\mu$M nifedipine (not shown).

The mean value of the calcium current was 516±50 pA (n=12). The experiment illustrated in Fig.1a shows the effects of adding butyrate. This produced a clear decrease of the calcium current. On average the addition of butyrate decreased the calcium current to 58±6% of control (n=6). The addition of the weak base $NH_4Cl$ increased the magnitude of the calcium current (Fig 1b) on average to 165±15% of control (n=7). In contrast to the prominent effects on the calcium current, changes of intracellular pH in either direction had no effect on outward currents (Figs 1c & d).

The timecourse of the effects of adding weak acids or bases on the calcium current is shown in Fig. 2 a & b. Some 'run down' of the Ca current is evident. Nevertheless the effects of butyrate and $NH_4Cl$ are at least partly reversible. In addition, removal of $NH_4Cl$ produces a rapid decrease of the calcium current presumably due to the expected acid rebound. This suggests that the effects of $NH_4Cl$ are due to the change of $pH_i$ rather than a direct effect of $NH_4Cl$ itself. The maximum effect is reached within 60 sec, a time which is comparable to that taken for change of the superfusing solution. This rapid onset can be compared with the effects of changing *external* pH. As shown in Fig. 2 c & d, external pH changes produced effects on the calcium current in the same direction as those resulting from changes of internal pH. The mean change produced by pH 6.9 was a decrease to 56 ± 9 % (n=4) and by pH 7.9 an increase to 136 ± 2 % (n=4). However the effects of external pH develop considerably more slowly than those of internal pH. Changes of external pH had no effect on outward current (not shown).

### Discussion

The data clearly shows that changes of pH, whether produced by a change of external or internal pH, affects the membrane Ca current in myometrial cells, but have little or no effect on outward K-currents. Although $pH_i$ was not measured in the cells, much previous work on rat uterus suggests that 20 mM butyrate or $NH_4Cl$ will produce a change in $pH_i$ of around 0.15 pH unit [8]. Such changes in $pH_i$ may be considered to be within the physiological or pathophysiological range, occurring for example during labour contractions or ischaemia [7]. As mentioned earlier, acidification is associated with a decrease in the frequency and amplitude of the spontaneous contractions. However work on permeabilised myometrium, had shown that when $[Ca^{2+}]_i$ was maintained constant, intracellular acidification did not reduce force [1]. This therefore suggested that a reduction in $[Ca^{2+}]_i$ occurred during acidification in intact

preparations and that this reduced contraction. This idea was subsequently supported by the report that acidification could inhibit the calcium transient which underlies contraction in the myometrium [3]. The observation that changes of $pH_i$ affect the frequency of contractions [8] is most consistent with the hypothesis that they work by affecting the membrane currents responsible for spontaneous electrical activity. In principle the change of rate produced by changes of $pH_i$ could result from changes of either inward or outward currents. For example the inhibition of contraction produced by cyanide is associated with an increase of potassium permeability as shown by K efflux [4]. The observations in the present paper suggest, however, that the effects of pH result from changes of inward calcium currents rather than outward currents. In this respect then the effects of $pH_i$ on uterine contraction are similar in mechanism to those in vascular muscle [5]. The observation that the effects of changes of extracelluar pH are slower in onset than those of intracellular pH suggests that much of the effect of extracelluar pH may occur as a result of the induced change of $pH_i$ rather than being a direct effect of external pH.

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## References

1. Crichton CA Taggart MJ Wray S Smith GL (1993) The effects of pH and inorganic phosphate on force production in α-toxin permeabilized isolated rat uterine smooth muscle. J Physiol (Lond) 465:629-645
2. Fry CH Gallegos CRR Montgomery BSI (1994) The actions of extracellular $H^+$ on the electrophysiological properties of isolated human detrusor smooth muscle cells. J Physiol (Lond) 480: 71-80
3. Heaton RC Wray S (1994) Intracellular acidification increases intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) but decreases force in isolated rat uterine smooth muscle. J Physiol (Lond) 477P: 42-43P
4. Heaton RC Wray S Eisner DA (1993) Effects of metabolic inhibition and changes of intracellular pH on potassium permeability and contraction of rat uterus. J Physiol (Lond) 465: 43-56.
5. Klöckner U Isenberg G (199). Intracellular pH modulates the availability of vascular L-type $Ca^{2+}$ channels. J Gen Physiol 193: 647-663.
6. Miyoshi H Urabe T Fujiwara A (1991) Electrophysiological properties of membrane currents in single myometrial cells isolated from pregnant rats. Pflugers Arch 419: 386-393.
7. Parratt JR Taggart MJ Wray S (1994 ) Abolition of contractions in the myometrium by acidification in vitro. Lancet 344: 717-718.
8. Taggart MJ Wray S (1993) Simultaneous measurement of intracellular pH and contraction in uterine smooth muscle. Pflugers Arch. 423: 527-529.