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Regulation of intracellular pH in rat uterine smooth muscle, studied by ^{31}P NMR spectroscopy

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Intracellular pH (pH_i) affects smooth muscle function, yet little is known concerning its regulation. I have therefore investigated pH regulation in rat uterus, using ^{31}P-NMR spectroscopy. A change in extracellular pH(pH_e) of 1 pH unit (7.4 to 6.4) elicited a 0.29 change in pH_i; smaller changes in pH_e were accompanied by proportionately smaller changes in pH_i. The pH changes were reversible. There was no fall of uterine ATP or phosphocreatine during the pH changes.

Intracellular pH (pH_i) is known to influence many cellular events. In smooth muscle, pH_i has been shown to affect contractile ability [1]. Interestingly, the affect seems to vary in different smooth muscles. For example, in taenia and some vascular smooth muscles (and striated muscles), there is a decrease in force with a decrease in pH_i, whereas in bladder and other vasculature, force increases as pH_i decreases [1–4]. Thus, changes of pH_i are of functional significance to smooth muscle. However, in smooth muscle there have been relatively few measurements of either pH_i or its regulation. This is partly because the small size of the cells often precludes microelectrode studies. There has only been a single report of pH_i regulation, which was on guinea-pig vas deferens [5]. Furthermore, little is known about the 'energetic cost' of pH regulation. Given the different responses of smooth muscle to changes in pH_i, it is clear that there is a need for more information about pH_i and its regulation in smooth muscle. I, therefore, investigated how well pH is regulated in uterine smooth muscle. Uterine tissue was chosen because pH_i is known to change with the reproductive state of the uterus yet there is nothing known about pH regulation in this tissue [6].

Intracellular pH can be measured by ^{31}P-NMR spectroscopy. The technique is as applicable to small cells as to large ones. In addition, ^{31}P-NMR spectra provide measurements of ATP, phosphocreatine, and P_i. It is, thus, possible to determine whether the pH-regulating mechanisms cause a significant decrease in high-energy phosphorus compounds. I, therefore, used ^{31}P-NMR spectroscopy to investigate the response of pH_i to changes in extracellular pH (pH_e) in uterine smooth muscle cells. (As smooth muscle cells occupy more that 90% of the total cellular volume in the uterus, it can be assumed that the changes described arise from smooth muscle cells [6].

Pregnant Sprague-Dawley rats were killed by cervical dislocation under chloroform anaesthesia. The uterus was dissected and placed into a 15-mm NMR tube and superfused (4 ml/min) with an oxygenated solution of the following composition:

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186 mM Na/161 mM Cl/6 mM K/6 mM glucose/31 mM HCO_3/0.3 mM Ca (pH 7.4). The pH of this solution was varied by altering the [HCO_3] (isosmotically with Cl). The solutions were bubbled with 5% CO_2 (95% O_2) throughout. The ^{31}P-NMR spectra were collected as described in detail elsewhere [6]. Radiofrequency pulses were applied every 2 s and each spectrum took about 10–15 mins to acquire. The pH_i can be calculated from the resonance position of inorganic phosphate (P_i) using the following modification of the Henderson-Hasselbach equation

$$pH = pK + \log\frac{\delta - \delta_1}{\delta_2 - \delta}$$

where pK is 6.65 and δ_1 is the resonance position of $H_2PO_4^-$ (3.14 ppm), δ_2 is the resonance position of HPO_4^{2-} (5.61 ppm) and δ is the experimentally observed resonance of P_i. The temperature inside the spectrometer was 30 °C.

Fig. 1 shows uterine NMR spectra obtained under control conditions (top), with the pH_e changed to 6.4 (middle) and, finally, upon return to control conditions (pH_e 7.4, bottom). The metabolites in the spectra are nucleoside triphosphates (NTP; of which ATP contributes around 75%, [7]), phosphocreatine, P_i and phosphomonoesters. The dotted line indicates the the position of the P_i peak at the start of the experiment. The intracellular acidification (a shift in the P_i peak to the right) upon superfusion of the tissue with solution at pH_e 6.4 can be clearly seen. Upon returning to control bathing solution, the pH_i recovered to its initial position. This recovery occurred at all the pH_e values examined.

It can also be seen from Fig. 1 that there were no changes in the uterine metabolites detected by ^{31}P-NMR spectroscopy, accompanying the acidification. This was true for all external pH values examined.

The mean changes in pH_i from control values at five different values of pH_e were calculated. The results are given in Fig. 2, which is a graph of the mean pH_i against pH_e. The mean pH_i under control conditions was 7.10 ± 0.02 ($n = 11$, external pH 7.4). Fig. 2 shows that the changes in pH_i were considerably less than those in pH_e. Thus a 1 pH unit change of pH_e (from 7.4 to 6.4) elicited a only a 0.29 ± 0.02 ($n = 3$) change in pH_i. Smaller changes in pH_e were accompanied by approximate proportionately smaller changes in pH_i.

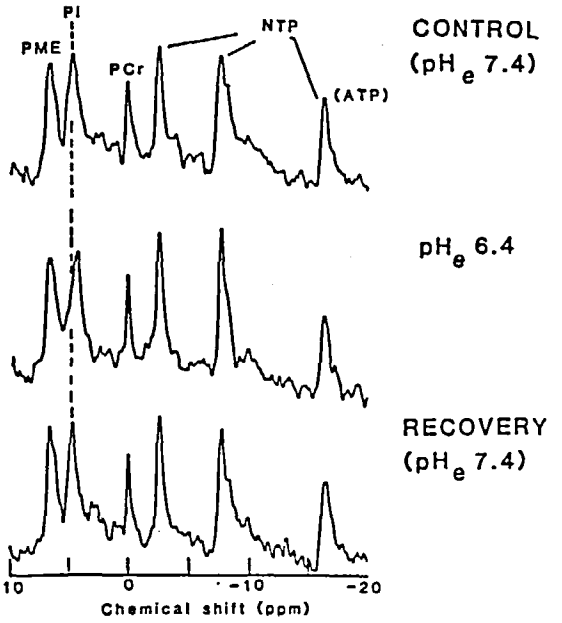

Fig. 1. ^{31}P-NMR spectra from pregnant rat uterus. The spectra were obtained under control conditions (pH_e 7.4, top), after changing to solution of pH_e 6.4 (middle) and upon return to control conditions (bottom). The dotted line indicates the position of the inorganic phosphate (P_i) peak at the start of the experiment. NTP, nucleoside triphosphates (predominantly ATP); PCr, phosphocreatine; PME, phosphomonoesters.

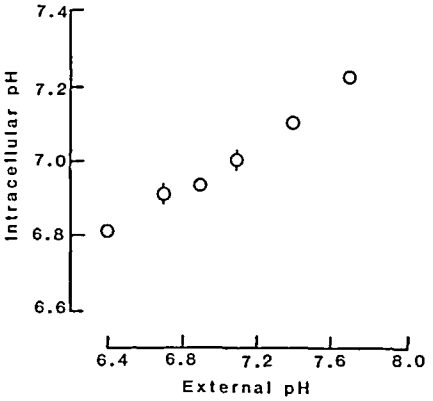

Fig. 2. pH_i against pH_e in the rat uterus. The graph shows the mean intracellular pH found over a range of external pH. All the points are the means of three or more observations. The standard error is shown by the vertical bar, where it is greater than the symbol size.

This study shows quite clearly that pH_i is regulated in the uterus. The resting value of pH_i found, 7.10 at 30°C, is not consistent with a passive distribution of protons across the surface membrane; assuming a resting potential of −60 mV, such a distribution would predict a resting pH value of 6.4. Furthermore, when alterations were made to pH_e, much smaller changes in pH_i than in pH_e followed. This is in agreement with results which have been reported in vas deferens where changes in pH_i were only about 40% of those in pH_e [5]. The changes in uterine pH_i were all reversible. The mechanism of pH regulation in uterine smooth muscle is not known. There is evidence from vascular and *Taeni coli* smooth muscles that Na^+/H^+ exchange is involved in proton extrusion after acid load [8,9] and this may be the mechanism occurring in the uterine cells.

There was no change in uterine high-energy phosphates and P_i with the pH changes. This shows that the uterus was able to extrude the proton load without exceeding its metabolic ability, under these conditions. Interestingly, after parturition in the rat there is a decrease of pH_i which persists for around 48 h [6]. This may indicate that under post-partum conditions when there are large structural changes in the tissue the metabolic demand of proton extrusion may be exceeded. (It may also reflect a difference in metabolism of the pregnant and post-partum uterus.)

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