An *in vivo* study of the effects of ischaemia on uterine contraction, intracellular pH and metabolites in the rat

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1. There are no data concerning the functional or metabolic effects of hypoxia *in vivo* in smooth muscle. We have therefore used ^{31}P-NMR spectroscopy and intra-uterine pressure measurements to examine simultaneously, *in vivo*, the effect of ischaemia on uterine metabolites, intracellular pH (pH_i) and force.

2. A 1–2 cm portion of uterus from day 1 postpartum anaesthetized rats was exteriorized and an NMR surface coil placed on it. A balloon catheter in the uterine lumen recorded intra-uterine pressure changes from the same area. Reversible occluders were placed around the uterine artery.

3. Occlusion produced a decrease and then abolition of contractions, within 10 min. In four of five animals contraction was abolished within 2 min. Upon reperfusion force was rapidly restored (1 min), in all preparations. The mean level of force was significantly *above* control (pre-occlusion) 20–30 min after reperfusion.

4. The NMR data showed a significant fall in [ATP] (28 %) and [phosphocreatine] (34 %) during occlusion. Inorganic phosphate doubled in concentration during this period. Metabolites recovered slowly upon reperfusion, taking 20–30 min to return to pre-occlusion levels.

5. The mean pH_i fell from 7·32 to 7·00 upon occlusion and was rapidly reversed upon reperfusion. The changes in pH_i closely correlated with the changes in uterine force. Decreases of pH_i of a similar magnitude *in vitro* have previously been shown to abolish contractions; thus it is suggested that during ischaemia *in vivo* the depression of contraction is caused by the large fall in pH_i.

The effect of hypoxia or ischaemia *in vivo* on smooth muscle function, metabolites and intracellular pH (pH_i) is unknown. Given the important roles played by smooth muscle cells within the body and the fact that tissue blood flow and oxygenation may well alter under different physiological conditions, it is important that such information be obtained. We have therefore investigated the effects of ischaemia on uterine smooth muscle *in vivo*. The uterus was chosen because it is well documented that as it contracts its blood flow decreases, due to occlusion of its blood vessels (Greiss, 1965). This leads to ischaemia and hypoxia within the uterus. Recent *in vitro* studies on rat and human myometrium have indicated that hypoxia and its associated acidification can markedly affect myometrial contraction (Wray, Duggins, Iles, Nyman & Osman, 1992; Taggart & Wray, 1993; Phoenix & Wray, 1993). Contraction is decreased or even abolished by hypoxia or acidification *in vitro*, in both spontaneously active and agonist-stimulated myometrium (Earley & Wray, 1993). Thus if hypoxia has similar effects *in vivo*, i.e. depressing contraction, then it may have a role in cases of uterine dystocia. Dystocia is difficult or abnormal labour, often of unknown aetiology, where contractions can become so weak and unco-ordinated that the baby has to be delivered by emergency Caesarian section. It may be that in these labours hypoxia has caused intracellular changes, e.g. fall in pH or [ATP], which depress the ability of the uterus to contract. It is therefore of interest to determine the effect of hypoxia *in vivo* on uterine contraction.

During hypoxia *in vitro* several mechanisms have been postulated to account for or contribute to the fall in force. As mentioned above, acidification is associated with hypoxia (Wray, 1990), and acidification *per se* can inhibit force production (Wray *et al.* 1992). ATP and phosphocreatine (PCr) have been shown to fall and inorganic phosphate (P_i) rise (Wray, 1990). A rise in [P_i] can depress contraction in permeabilized myometrial preparations (Crichton, Taggart, Wray & Smith, 1993). There is also

evidence that K^+ permeability is increased, which will make the surface membrane more difficult to excite and thus lead to a failure of contraction (Heaton, Wray & Eisner, 1993). No *in vitro* studies, however, have simultaneously measured metabolites, pH_i and contraction. Thus the relative time courses of the changes seen in hypoxia and their interactions are unknown. Hence to help understand the involvement of the above mechanisms it would be useful to obtain simultaneous data on these parameters. We have therefore used ^{31}P-NMR spectroscopy to obtain the first *in vivo* measurements of uterine ATP, PCr, P_i and intracellular pH (pH_i) simultaneously with contraction data, to study the mechanisms of hypoxia on contraction and pH_i and high energy phosphates in smooth muscle.

Part of this work has been presented to the Physiological Society (Harrison, Larcombe-McDouall, Earley & Wray, 1994).

METHODS

Animals

Day 1 postpartum Wistar rats were anaesthetized with urethane (I.P., 1 ml (200 g body weight)$^{-1}$, of a 36 % (w/v) solution). A small mid-portion of a uterine horn and associated mesometrium was exteriorized through a ventral incision in the skin and body wall. A 1–2 mm incision was made at the distal end of the exposed uterus and a balloon catheter placed within the uterus and fed along to the mid-region of the exposed uterus, to allow recording of the intra-uterine pressure (Fig. 1). The integral of the contraction data was calculated over 5 or 10 min periods to take into account variation in frequency and amplitude of uterine force; this is referred to as 'time-averaged force'. The uterus was covered in cling film to keep it warm and moist and a 1 cm diameter, 3-turn NMR surface coil placed on top of the mid-uterine portion. The pressure-detecting balloon was inside the uterus directly under the coil so that contractile and metabolic data were being obtained from the same region of tissue (see Fig. 1). The uterine artery was located in the mesometrium proximal and distal to the exposed uterus. Adhering fat was gently removed and then the artery slipped over a small (2 mm) U-shaped plastic former. Running at 90 deg to the artery, a catheter with an inflatable balloon was inserted into the plastic former, so that its inflation would occlude the uterine artery (see Fig. 1). By positioning two such non-metallic occluders the blood supply to the portion of uterus under the NMR coil could be occluded while the anaesthetized animal was inside the NMR spectrometer without disturbing the positioning of the coil. After preparation the vessel and associated tissue were replaced within the body cavity, leaving only the small area of uterus containing the surface coil outside. At the end of the experiment the animal was killed, while still anaesthetized, by cervical dislocation.

NMR spectroscopy

The anaesthetized rat was placed in a plastic cradle on a heating mat and positioned in the bore of a 4·7 T, 15 cm horizontal bore Biospec I NMR spectrometer. Radiofrequency (RF) pulses of 4 μs duration (45 deg) were repeated every 1·4 s (acquisition time 0·205 s) and spectra accumulated over 5 or 10 min, depending upon the signal:noise ratio. Such long acquisition periods are needed because of the low level of metabolites in smooth muscle, and the small amount of tissue available. A total of 2048 data points were acquired and zero filled to 4096 data points. Line broadening of 20 Hz was used

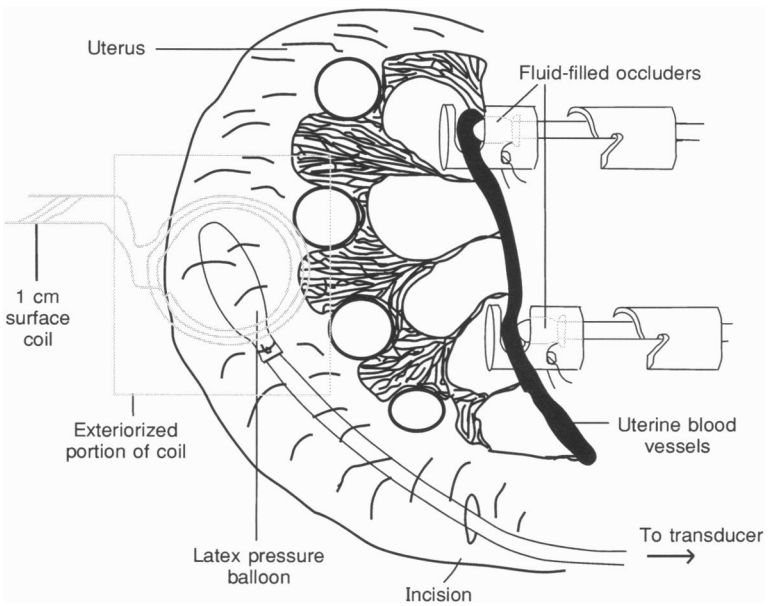

Figure 1. A schematic drawing of part of a rat uterine horn and blood supply
The positions of the NMR surface coil, pressure balloon and arterial occluders are indicated. The portion of the uterus exteriorized for the study is that bounded by the box.

before Fourier transforming the data. Resonance positions of the spectral peaks were measured relative to PCr in parts per million (p.p.m.). Peaks were identified on the basis of their resonance positions. Relative metabolite concentrations were obtained from integration of the peaks and corrected for effects of signal saturation using previously obtained factors (Dawson & Wray, 1985). To quantify the metabolite concentrations, a value for ATP under control conditions (1·8 mM) obtained in a previous study (Dawson & Wray, 1985) was used. The other metabolite concentrations were then calculated relative to this value. Intracellular pH was calculated from the resonance position of P_i using the following modification of the Henderson–Hasselbalch equation:

$$pH = pK + \log_{10}\frac{\delta - \delta_1}{\delta_2 - \delta},$$

where δ is the observed P_i position and δ_1 and δ_2 are the chemical shifts of $H_2PO_4^-$ and HPO_4^{2-} (3·28 and 5·69) respectively. The pK used was 6·73.

Statistics

Figures given throughout are mean values of n observations with S.E.M. Significance of differences was tested using the appropriate t test. Differences at the 5 % level or below were taken as being significant.

RESULTS

General

Intra-uterine pressure could be recorded for many hours from the animals and usually started either immediately or within 30 min of surgery. The values obtained (10–30 mmHg) were comparable to those obtained by other workers (Abel & Hollingsworth, 1985) indicating no adverse effect of the procedures or the constraints of the NMR spectrometer. The uterus was of healthy appearance at the end of the experiment: warm, pink, contractile and non-oedematous. During control data collection, little or no change occurred in the spectra over the period examined, usually 60–90 min, indicating that the preparation was stable. The method of occluding the uterine artery gave reproducible effects and flow measurements using a laser Doppler flowmeter in parallel experiments showed little or no blood flow within the portion of uterus under study during occlusion (N. Harrison & S. Wray, unpublished observations).

The effect of occlusion on uterine contraction

In five of five animals uterine contractions were decreased in amplitude and/or frequency and were eventually abolished. In four of five animals abolition occurred within 2 min and in the remaining animal there was a marked drop in the first 5 min and abolition within 10 min. An intra-uterine pressure recording is shown in Fig. 2. It can be seen that upon release of the occlusion, contractions recovered quickly (within 1 min), increasing in both strength and frequency towards control levels. In the other preparations (four of five) contractile strength then increased beyond pre-occlusion levels. This can be seen more clearly with the averaged data from the five animals shown in the top part of Fig. 4. Control contractile activity was collected for 40 min and the maximum activity normalized to 100 % for each preparation. It can be seen that there is a large decrease in time-averaged force in the first 10 min period (to 28 % of control) and that force has fallen to around 0 at the end of the second 10 min period. Restoration of blood supply restores force to about 75 % of control within 10 min. The activity then increases beyond control (130–140 %) for the next 30 min.

NMR spectra

Figure 3 shows representative ^{31}P-NMR spectra from the uterus, during control (top), occluded (middle) and recovery (bottom trace) conditions. The resonance peaks arise from ATP, PCr, P_i and a phosphomonoester (PME) peak, which is predominantly phosphoethanolamine (J. Phoenix & S. Wray, unpublished observations). Although the ATP peaks contain a small contribution from nucleotides other than adenosine (Oliver & Kellie, 1970) we refer to them as ATP throughout. The concentrations of metabolites were expressed relative to the β-ATP peak (the ATP peak at

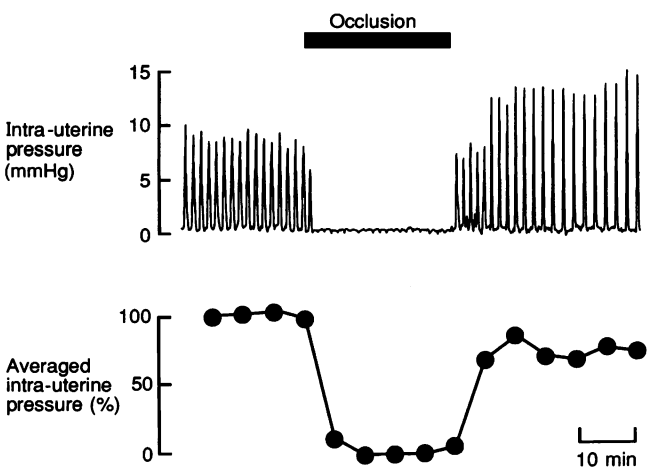

Figure 2. Spontaneous uterine contractions recorded in the anaesthetized postpartum rat
Changes in intra-uterine pressure (mmHg) were recorded via a latex pressure balloon in the uterine lumen. Below the original record is plotted the averaged pressure record. This is the integral of the contraction data over 5 min periods and reflects changes in both frequency and amplitude of contractions.

around −16·5 p.p.m. which does not contain a contribution from ADP), such that under control conditions ATP = 1·8 mM; PCr = 1·08 ± 0·08 mM and P_i = 0·86 ± 0·10 mM. When the uterine artery was occluded changes occurred in the relative amounts of ATP, PCr, and P_i. These are shown as mean data in Fig. 4. The amount of ATP and PCr fell significantly from control values during occlusion: 27 ± 8 and 43 ± 8 %, respectively ($n = 5$). Upon release of the occlusion, their levels increased and were back to control levels by 20 min. The concentration of P_i rose markedly throughout the occlusion to 177 ± 9 %. This value fell slowly during reperfusion and had returned to control levels after 30 min (Fig. 4).

The effect of occlusion on pH_i

The mean resting value of pH_i in the rat uterus was found to be 7·32 ± 0·03, $n = 20$. It can be seen in Fig. 4 that this value is well maintained during the control period. A marked reduction in pH_i occurred with occlusion, with the majority of the fall occurring within the first 10 min. Thus the mean value was 7·02 ± 0·05 after 10 min and 7·00 ± 0·06 after 20 min. In four of the five preparations a fall in pH_i was the first metabolic change seen during occlusion. A rapid recovery of pH_i occurred upon release from occlusion; pH_i was not significantly different from control values at the first reading made. Again pH_i was well maintained during the rest of the recovery period.

The bottom panel in Fig. 4 shows the P_i (inverted), pH_i and contraction data normalized and superimposed. It can be seen that the changes in pH_i correlate more closely with the fall in force during occlusion than those in P_i. Furthermore the recovery of pH_i is also rapid and correlates with the restoration of force upon release of the occlusion. This is in contrast to the effects of P_i which, although marked, do not mirror the effects on force. These data suggest that pH_i may be responsible for the changes in force which occur with occlusion.

DISCUSSION

We have obtained the first *in vivo* NMR spectra of smooth muscle and studied the effects of ischaemia on rat uterus. The information obtained shows significant changes in uterine contractile activity (decreased) and metabolites and pH_i upon occlusion of the uterine blood vessels. As ischaemia and hypoxia occur in human myometrium at labour, the relevance of these findings to uterine dystocia is discussed. The results suggest a causal effect of acidic pH_i in the depression of uterine force in ischaemia.

NMR and smooth muscle

Smooth muscle is difficult to study by NMR because of (i) the low level of high energy phosphates, e.g. PCr at 2–5 mM compared to 20–30 mM in cardiac muscle, and (ii) only small amounts of tissue can ordinarily be obtained. This means that NMR signals have to be averaged from hundreds of RF pulses and thus the time resolution is

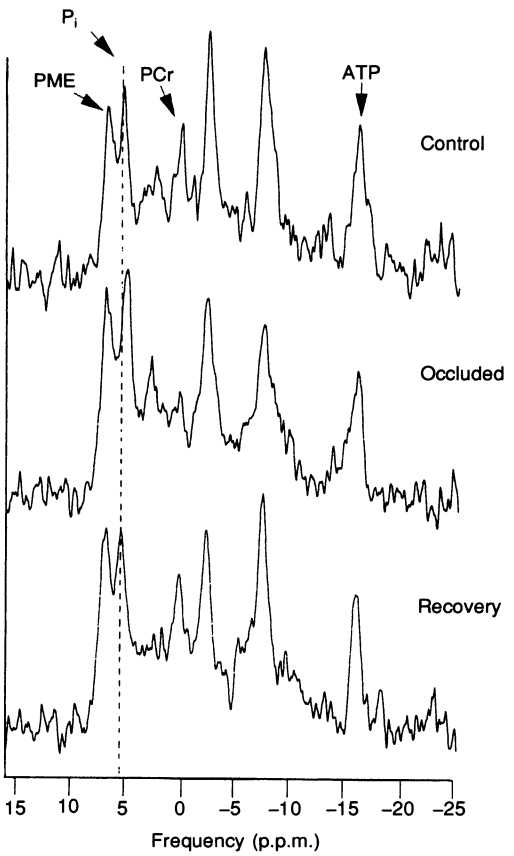

Figure 3. ^{31}P-NMR spectra from *in vivo* rat uterus
The top panel shows metabolites under control conditions, the middle panel during occlusion of the uterine blood supply and the bottom spectrum was obtained following reperfusion upon release of the occlusion. The metabolite peaks arise from ATP, PCr (phosphocreatine), P_i (inorganic phosphate) and phosphomonoesters (PME). The x-axis is frequency in parts per million (p.p.m.) relative to the PCr resonance. The P_i resonance position is sensitive to intracellular pH. The dotted vertical line indicates its position under control conditions. Its movement to the right during the occlusion indicates a fall in pH.

around 5–10 min, compared to say 30–60 s in cardiac muscle. The postpartum uterus was chosen because sufficient material could be obtained for the NMR study to enable spectra to be collected in a reasonable period. In addition, the hormonal status immediately postpartum is well documented (Wynn, 1977). To ensure that the NMR signals are detected from uterus without contamination from overlying tissues a small piece of uterus was exteriorized. The uterus studied was intact and undamaged and still connected to its blood supply. The tissue remained healthy throughout and was clearly functionally viable. Values of intra-uterine pressure were comparable to those obtained in other studies when the uterus was not exteriorized (Abel & Hollingsworth, 1985). Thus this appears to be a good model for studying the effects of ischaemia.

The metabolic and contractile data show significant effects of occlusion. Thus the uterus is not resistant to ischaemic periods. The changes in metabolites show that normal levels of ATP and PCr cannot be maintained by anaerobic glycolysis. This is in agreement with *in vitro* findings (Wray, 1990). ATP and PCr both fell by around a third. This is reflected in the large increase of $[P_i]$. Although the rise in P_i is considerable, from around 0·8 to 1·6 mM, this is not considered to be the cause of the contractile depression observed, since (i) its time course does not correspond (as shown in the bottom part of Fig. 4, when force has recovered to control levels, P_i is still elevated (128 %) and shows a much slower recovery time), and (ii) *in vivo* studies of permeabilized myometrium have shown that the levels of P_i found in this study, during occlusion, would have very little effect on Ca^{2+}-activated force, and certainly would not abolish force (Crichton *et al.* 1993). The fall in [ATP] from 1·8 to 1·4 mM likewise would not be expected to have much effect on contraction. It is still above the Michaelis–Menten constant (K_m) for myosin ATPase and other ATPases involved in ionic homeostasis (Wray, 1993).

The resting pH_i value found *in vivo* was more alkaline than the values reported in previous *in vitro* studies of postpartum uterus, (6·8, Dawson & Wray, 1985) or non-pregnant uterus (7·2, Taggart & Wray, 1993). This may be due to the presence of other components *in vivo*, e.g. HCO_3^-/CO_2 elevating pH_i, or to some stimulation of lactic acid production in *in vitro* studies.

There was a significant fall in pH_i during the occlusions (0·34 ± 0·08 pH units). This is a similar value to the fall in pH_i in the *in vitro* uterus reported during metabolic inhibition with cyanide (Wray, 1990). The present results suggest that the fall of force during occlusion is due to the fall in pH_i, since in four of five preparations pH_i was the parameter to change first and correlated with force falling. The recovery of force also correlated with the recovery of pH_i. As seen in the bottom part of Fig. 4, pH_i falls with or before force and recovers before or with force upon reperfusion. Finally, in *in vitro* experiments, when only

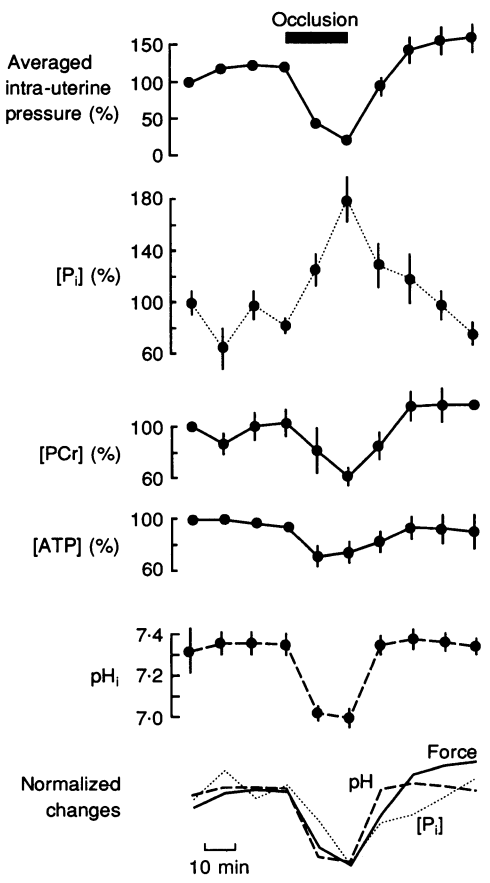

Figure 4. Mean changes in intra-uterine pressure (top) metabolites and intracellular pH (middle) before, during and after occlusion of the uterine blood supply
Vertical lines indicate S.E.M. The bottom panel shows the normalized changes in force (continuous line), P_i (dotted line) and pH (dashed line). Note that the P_i record has been inverted to facilitate comparison of the time course of the changes.

pH_i is altered by acidification to the level found *in vivo*, contractions are abolished (Taggart & Wray, 1993).

Regardless of the mechanism, the changes in uterine contraction with occlusion were profound. In four of five preparations all spontaneous activity ceased within 2 min of occlusion. During labour, contractions last for 1–3 min and at their peak will occlude blood flow within the uterus. The data presented here suggest that these occluded periods will change metabolites and pH_i. These changes may then inhibit the contractile process and force will fall or even fail. It may be that in some labours where contractions are particularly powerful and/or frequent these metabolite changes during occlusion build up and begin to limit contractile force. This may present as dystocic labour, i.e. ineffectual or unco-ordinated contractions. It may be that correction of pH_i or a period of relaxation (to restore metabolite levels) may lead to restoration of useful, strong contractions, rather than administration of contractile agonists such as oxytocin. These results may also be of relevance to other smooth muscles which may be affected by changes of blood flow or oxygenation, e.g. bladder, vascular and gastrointestinal.

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