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EFFECTS OF METABOLIC INHIBITION AND CHANGES OF INTRACELLULAR pH ON POTASSIUM PERMEABILITY AND CONTRACTION OF RAT UTERUS

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SUMMARY

1. We have investigated the role of changes of potassium efflux in the inhibition of uterine force produced by cyanide. K^+ efflux (^{86}Rb) was measured from pregnant and non-pregnant rat myometrial strips during metabolic inhibition with cyanide and following manoeuvres to displace intracellular pH (pH_i).

2. Cyanide greatly reduced or abolished spontaneous contractions. If the membrane was depolarized directly at this stage (by elevating external K^+) then contraction redeveloped. This suggests that the initial depression of force is due to a failure of membrane excitation.

3. Cyanide reversibly increased ^{86}Rb efflux (30–35%) in both pregnant and non-pregnant uteri and contraction was reduced. The increase in ^{86}Rb efflux with cyanide was not secondary to changes of membrane potential as it also occurred in both high-K^+ and Ca^{2+}-free solutions.

4. Glibenclamide (20 μM), an antagonist of K^+_{ATP} channels, reduced the cyanide-evoked increase of ^{86}Rb efflux by about 50%. The glibenclamide-*insensitive* component of efflux persisted in a Ca^{2+}-free solution. Despite its action on ^{86}Rb efflux, glibenclamide did not restore contraction.

5. Intracellular pH falls during metabolic inhibition. We therefore investigated whether reducing pH_i (in the absence of cyanide) had an effect on ^{86}Rb efflux. Application of the weak acid butyrate (60 mM, at constant external pH, 7·4) had no significant effect on ^{86}Rb efflux. Thus it is unlikely that the acidification in hypoxia contributes to the increased K^+ efflux.

6. Intracellular alkalinization produced by the weak base trimethylamine (60 mM) increased the frequency of uterine contraction and the ^{86}Rb efflux. However, there was no effect on the ^{86}Rb efflux in a Ca^{2+}-free solution. The increased efflux is therefore presumably a consequence of the increased frequency.

7. It is concluded that metabolic inhibition produced by cyanide, produces an increase in K^+ efflux from the myometrium. Part of this efflux is glibenclamide sensitive. This increased K^+ efflux will lead to hyperpolarization of the myometrial membrane and thus decrease excitation. Thus reduced surface membrane excitability will contribute to the fall of force in hypoxia; specifically it may cause the initial loss of spontaneous contractions in the uterus.

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INTRODUCTION

During labour the forceful contractions of the uterus occlude its blood supply (Greiss, 1965; Brinkman, 1990), which in turn will cause hypoxia. It has recently been shown that cyanide and anoxia can reduce and even abolish uterine force in isolated rat and human uterus (Heaton & Wray, 1991; Wray, Duggins, Iles, Nyman & Osman, 1992; Phoenix & Wray, 1993). Thus hypoxia may be a contributing cause to uterine dystocia, the term given to inadequate uterine contractile activity during labour. The causes of uterine dystocia remain largely unknown and it frequently results in emergency Caesarian delivery. A recent survey has cited dystocia as the commonest cause of emergency Caesarian delivery (O'Driscoll, Foley & MacDonald, 1984).

The mechanism underlying the fall of uterine force in cyanide is unresolved. Recent studies have shown that the associated intracellular acidification (due to stimulation of anaerobic glycolysis and hence lactic acid production), along with the fall in [ATP], [PCr] (phosphocreatine) and rise in inorganic phosphate (P_i) (Wray, 1990; Wray *et al.* 1992) will contribute to the fall of force. These studies have emphasized effects on the contractile machinery. In this paper we have examined the role of surface membrane effects on the fall in force. In cardiac muscle it is known that metabolic inhibition abolishes contraction and that this is due to an increase of potassium conductance rendering the surface membrane inexcitable (Lederer, Nichols & Smith, 1989). If such a mechanism existed in the uterus it would explain the decrease of both frequency and magnitude of the contractions (Wray *et al.* 1992). We have therefore investigated the effects of cyanide on the K^+ efflux by using ^{86}Rb as a marker. The effects of cyanide on contraction depend on the gestational state of the uterus – the non-pregnant uterus is affected more than the pregnant (Wray *et al.* 1992) – we have therefore also investigated whether there are differences in the K^+ efflux between the pregnant and non-pregnant uterus.

Cyanide produces an intracellular acidification which has been linked to an increased lactic acid production (Wray, 1990). Changes in intracellular pH (pH_i) have been shown to alter contraction in the uterus (Wray *et al.* 1992; Heaton, Taggart & Wray, 1992). However, little is known of the mechanisms underlying these effects of pH_i and it seems reasonable to examine whether changes in K^+ efflux could form the basis of the action on frequency. Such an examination would also establish what role, if any, the acidification in cyanide plays in any changes in K^+ efflux under these conditions.

It was found that cyanide produced a large increase in K^+ efflux from both pregnant and non-pregnant uterine preparations. This increase in K^+ efflux was partly blocked by glibenclamide, suggesting that the K^+ efflux was partly via K^+_{ATP} channels. Thus decreased membrane excitability probably contributes to the fall of force in cyanide. Consistent with this was the finding that after cyanide has abolished spontaneous contractions, force could be produced by depolarizing the uterus (in the continued presence of cyanide). Changes in intracellular pH had no significant effect upon K^+ efflux. The relevance of these results to the fall in force in hypoxia is discussed.

A preliminary account of some of these results has been published (Heaton, Wray & Eisner, 1992).

METHODS

Tissues

Wistar rats were used either virgin (200–250 g) or pregnant (day 20–21); parturition occurs on day 22. Animals had access to food (Bantam & King) and water *ad libitum*. Under terminal chloroform anaesthesia the uterus was removed via a large abdominal incision. Small strips of myometrium were dissected, loaded with ^{86}Rb (see below) and mounted in an organ bath with one end attached to a force transducer (Grass FT03). The tissue was continually superfused with solution at 37 °C. The bathing solution was of the following composition (mM): NaCl, 154; KCl, 5·6; glucose, 11·7; $CaCl_2$, 3; $MgSO_4$, 1·2. The solution was buffered with 10 mM Hepes, pH adjusted to 7·4 and gassed with 100% oxygen.

To simulate anoxia, oxidative phosphorylation was inhibited by adding 2 mM sodium cyanide to the bathing solution (external pH maintained at 7·4). In some experiments [K^+] was raised to 60 mM by the isosmotic replacement of Na^+ in the bathing solution. For Ca^{2+}-free solutions, $CaCl_2$ was omitted from the bathing solution. Changes in intracellular pH at constant external pH were made by isosmotically substituting salts of a weak acid (butyrate), or base (trimethylamine) for NaCl. Glibenclamide (20 μM) was used to inhibit K^+_{ATP} channels and was a gift from Hoechst.

^{86}Rb efflux. Tissue was loaded with ^{86}RbCl (20 μCi ml^{-1}) in the bathing solution (see above) for 90 min at 20 °C, before being transferred to the organ bath. The tissue effluent was collected over 2 min periods and the β emission counted. At the end of the experiment the tissue was dissolved in 1% Triton-X 100 (Sigma, UK) to assay remaining counts. After subtraction of background counts the ^{86}Rb efflux rate constant was calculated. The efflux data points shown in the figures are the mid-points of each 2 min collection period.

Statistics

Figures given throughout are mean values ± standard errors of the means. Statistical differences were tested using the appropriate *t* test, as indicated in the text. *n*, number of animals.

RESULTS

Effects of cyanide on spontaneous and high-K^+ contractions

Cyanide application reduced both the amplitude and frequency of spontaneous uterine contractions. As previously observed (Wray *et al.* 1992), the effects of cyanide were more pronounced in the non-pregnant than the pregnant uterus. Thus in six out of ten non-pregnant preparations contractions were abolished, compared to only two out of ten pregnant preparations. An example of the effects of cyanide on spontaneous contraction from non-pregnant uterus is shown in Fig. 1*A*. This shows a rapid abolition of spontaneous contraction. If reduced membrane excitability is playing a role in depressing these spontaneous contractions, then it should be possible to override this depression by applying high-K^+ solution to depolarize the uterus. We therefore investigated whether high-K^+ solution could elicit uterine force after it had been abolished by cyanide. As shown in Fig. 1*A* force can be produced by the uterus, if the membrane is depolarized. The amount of force which could be produced depended upon the interval between the last contraction and application of the high-K^+ solution; the longer the interval the less force was produced. This is shown graphically in Fig. 1*B*. Figure 1*C* shows the effects of cyanide directly on the contraction produced by high K^+. In the absence of cyanide (shown in Fig. 3) the high-K^+ contraction is maintained. When cyanide is added with high K^+ the contraction relaxes. However, this relaxation is considerably slower than the abolition of the spontaneous activity. The mean time for cyanide to abolish spontaneous contractions was 2·04 ± 0·22 min (n = 16). As can be seen from the graph

of mean data obtained for depolarized preparations (Fig. 1*D*), cyanide had only reduced force by one-third when spontaneous contractions would have been abolished.

The above results suggest that cyanide may produce two inhibitory effects on contraction: (i) a rapid abolition of spontaneous activity which is presumably due to

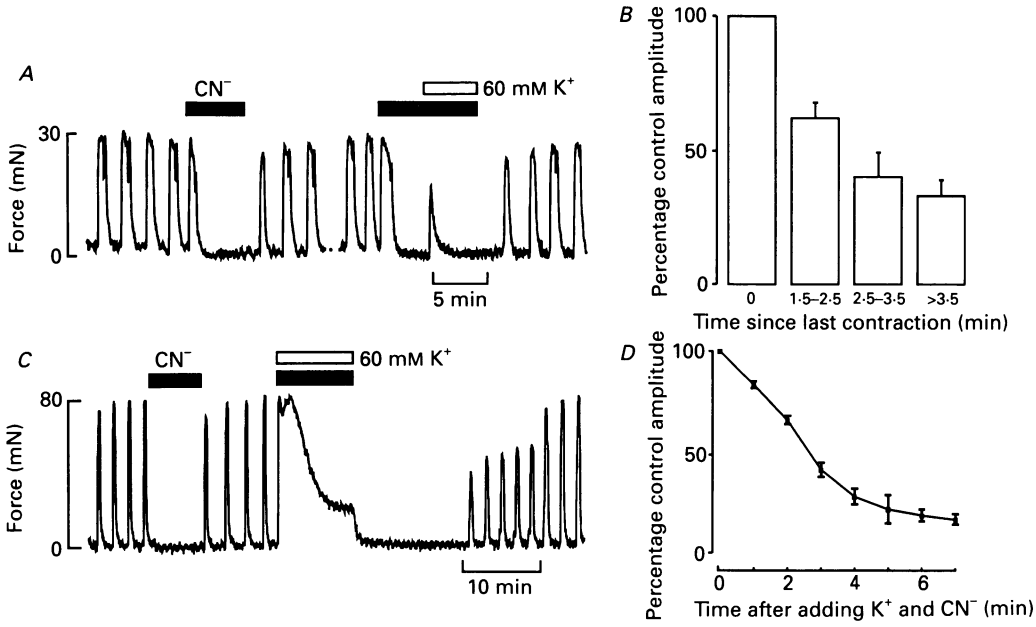

Fig. 1. The effects of cyanide (CN^-) on spontaneous and depolarization-evoked contractions of non-pregnant rat uterus. The times of addition of cyanide and elevation of $[K^+]_o$ are shown by the bars above the traces. *A*, the effects of cyanide on spontaneous contractions and then depolarization by high-K^+ solution following abolition of spontaneous force. *B*, the response to depolarization (amplitude of contraction), in the continued presence of cyanide, at increasing times since the last contraction in cyanide. The data were summed in three intervals; 1·5–2·5 min, 2·5–3·5 min and greater than 3·5 min and these points are shown as the bars in the histogram. The data in each range were from at least four preparations, and the vertical bars show the size of the S.E.M. *C*, the effect of cyanide on spontaneous and high-K^+ contractions. *D*, the mean time course of force decline (amplitude of contraction compared to control) in high-K^+ plus cyanide ($n = 7$).

a surface membrane effect; (ii) a more slowly developing decrease of the response to depolarization which may reflect intracellular mechanisms. In the rest of this paper we have therefore investigated the role of K^+ permeability in the former effects.

Effects of cyanide on ^{86}Rb efflux

To examine K^+ efflux in cyanide, simultaneous measurements of ^{86}Rb efflux (rate constant) and force were made. In the control bathing solution there was a rapid initial loss of ^{86}Rb from the myometrium for the first 10–15 min, following which a steady efflux was seen. Figure 2 shows typical records of the effect of cyanide on ^{86}Rb

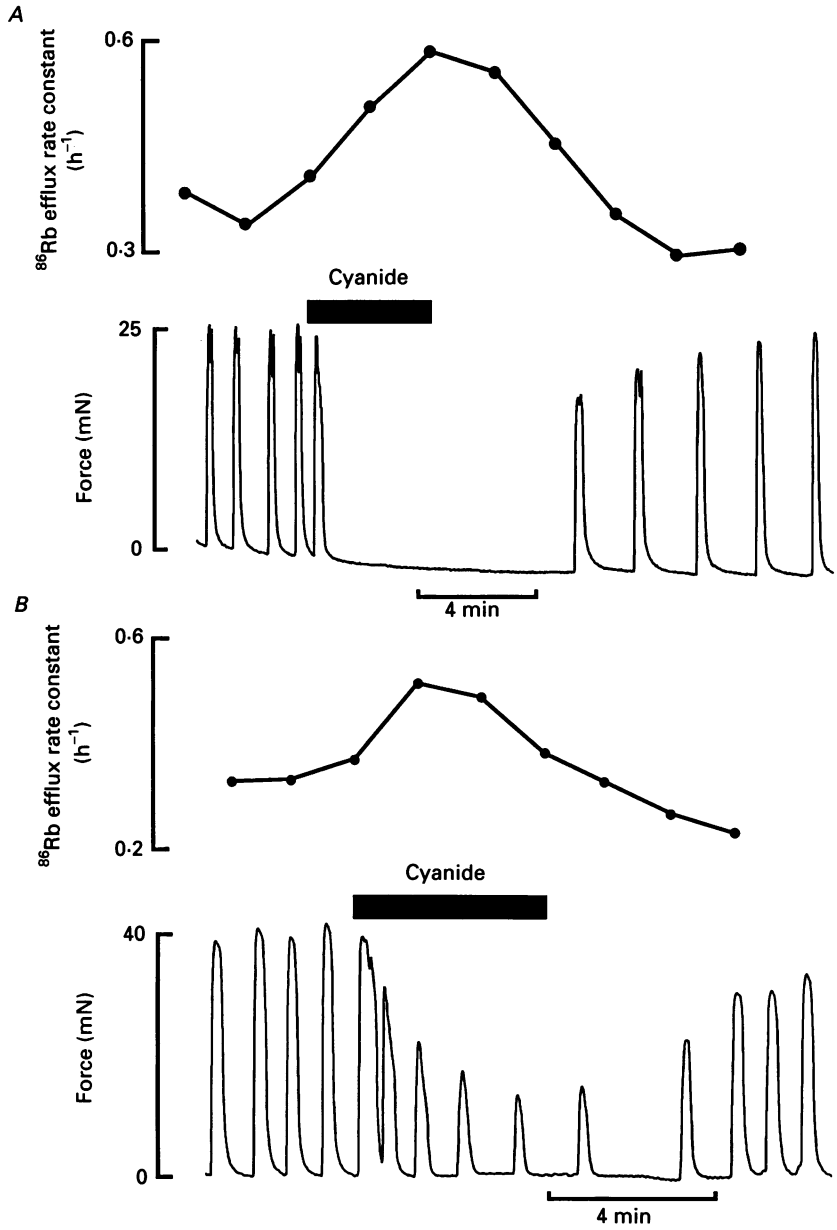

Fig. 2. The effects of cyanide on ^{86}Rb efflux and contraction. In both panels traces show: top, ^{86}Rb efflux; bottom, contraction. Cyanide (2 mM) was added for the period indicated. *A*, non-pregnant uterus; *B*, pregnant uterus. Note the persistence of spontaneous contractions (at decreased frequency and amplitude) in the pregnant but not in the non-pregnant uterus. The ^{86}Rb efflux was collected over a 2 min period. In this and all subsequent figures the data points plotted are the mid-points for each period.

efflux. It can be seen that cyanide produced a large increase in the efflux of ^{86}Rb, from both pregnant and non-pregnant myometrium. In non-pregnant uteri the mean increase in rate constant produced by cyanide was $33 \pm 7\%$ ($n = 10$) and in pregnant

uteri it was $30 \pm 5\%$ ($n = 10$). The increase in rate constant was not maintained in some preparations, as shown in Fig. 2*B*. Upon removal of cyanide, contractions gradually returned to control levels, as did the rate constant of ^{86}Rb efflux.

As cyanide clearly has a large effect upon contraction, the movement of ^{86}Rb may be influenced not only by the effects of cyanide on the membrane excitability but also

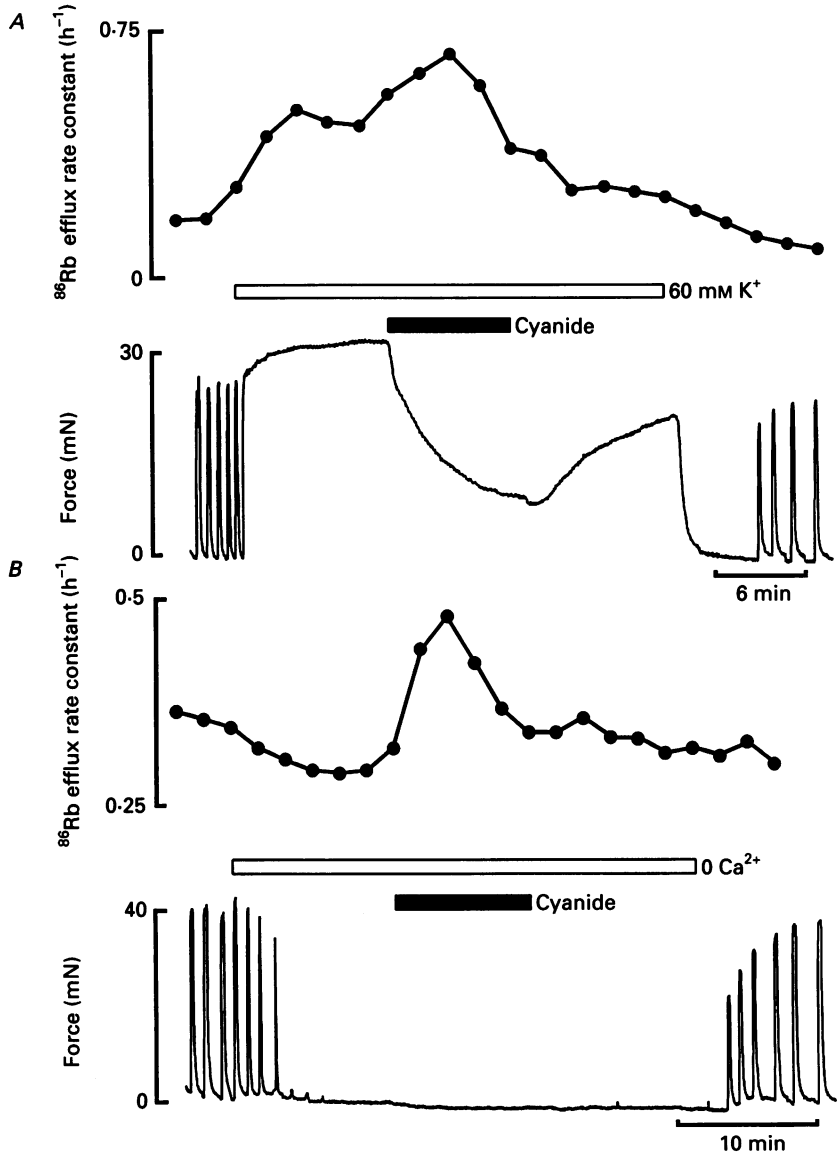

Fig. 3. The effects of cyanide on ^{86}Rb efflux and contraction in the absence of spontaneous activity. The experiment was performed on non-pregnant uterus. In both panels traces show: top, ^{86}Rb efflux; bottom, contraction. *A*, the effects of high-K^+ solution. $[K^+]_o$ was elevated to 60 mM and cyanide (2 mM) added for the periods indicated. *B*, the effects of Ca^{2+}-free solution. Ca^{2+} was removed and cyanide was added for the periods indicated.

by changes in electrical activity secondary to altered contractile activity. Therefore to determine whether in cyanide the changes in ^{86}Rb efflux were secondary to alteration in electrical activity associated with contraction, experiments were performed in either high-K^+ (60 mM) or Ca^{2+}-free solutions. High-K^+ solution depolarizes the uterus hence removing any changes in electrical activity, and causes a maintained contraction (Fig. 3*A*). This contraction is not maintained in cyanide, and slowly declines. The ^{86}Rb efflux is increased by depolarization ($82 \pm 32\%$) as shown in Fig. 3*A*. Application of cyanide causes a further significant increase in ^{86}Rb efflux, $35 \pm 9\%$ ($n = 3$), compared to high K^+.

When the uterus is placed in Ca^{2+}-free solutions, spontaneous contractions are abolished, as shown in Fig. 3*B*. When cyanide is added (still in Ca^{2+}-free solution) there is still a significant increase in the efflux of ^{86}Rb ($37 \pm 6\%$, $n = 3$), shown in Fig. 3*B*.

Thus cyanide produces a significant increase in the rate constant of ^{86}Rb efflux which was not significantly different in control, high-K^+ and Ca^{2+}-free solutions.

Effect of glibenclamide on ^{86}Rb efflux and contractions in cyanide

A variety of K^+ channels have been described in the uterus (Toro, Stefani & Erulkar 1990), which could be involved in the increased K^+ efflux seen in cyanide. However, under conditions of metabolic inhibition it seemed reasonable to consider the role of K^+_{ATP} channels, which open when [ATP] falls, and Ca^{2+}-activated K^+ channels, which open when $[Ca^{2+}]_i$ rises. Glibenclamide is an antagonist of K^+_{ATP} channels in smooth muscles (Standen, Quayle, Davies, Brayden, Huang & Nelson, 1989; Piper, Minshall, Downing, Hollingsworth & Sadraei, 1990). The effects of glibenclamide (20 μM) on the rate constant of ^{86}Rb efflux in cyanide were therefore examined. The effects of glibenclamide and cyanide were investigated, and compared with the effect of cyanide alone on the same preparation. The protocol was varied so that in some experiments cyanide alone preceded cyanide and glibenclamide, while in others cyanide alone followed cyanide and glibenclamide. The rate constant of ^{86}Rb efflux was increased $36 \pm 4\%$ by cyanide ($n = 10$), but only by $16 \pm 8\%$ with cyanide and glibenclamide in non-pregnant animals and $17 \pm 5\%$ compared to $30 \pm 5\%$ in cyanide alone, in pregnant animals ($n = 10$). These differences produced by glibenclamide were statistically significantly ($P < 0{\cdot}05$, paired t test). A typical record is shown in Fig. 4.

It can be seen that although glibenclamide reduced the ^{86}Rb efflux by about 50%, no improvement in contractile activity resulted. This was typical of all experiments, for both non-pregnant and pregnant animals.

The above experiments have shown that glibenclamide does not abolish all the cyanide-evoked increase of ^{86}Rb efflux. There are two possible explanations for the glibenclamide-insensitive ^{86}Rb efflux: (i) it could result from incomplete inhibition of K^+_{ATP} channels by glibenclamide; or (ii) some other K^+ channel may be activated. We have therefore investigated a possible role for Ca^{2+}-activated K^+ channels. If these channels are activated in cyanide due to a rise in $[Ca^{2+}]_i$ which may occur under these conditions, then the reduction of K^+ efflux produced by glibenclamide should be further increased if the experiments are performed in the absence of external calcium. In four experiments on non-pregnant uteri, Ca^{2+}-free solution was used throughout

and glibenclamide and cyanide applied either before or after cyanide alone, as previously described. However, the glibenclamide-insensitive component was unaffected. No further reduction in K^+ efflux additional to that produced by glibenclamide was seen. This is consistent with the data described above, where

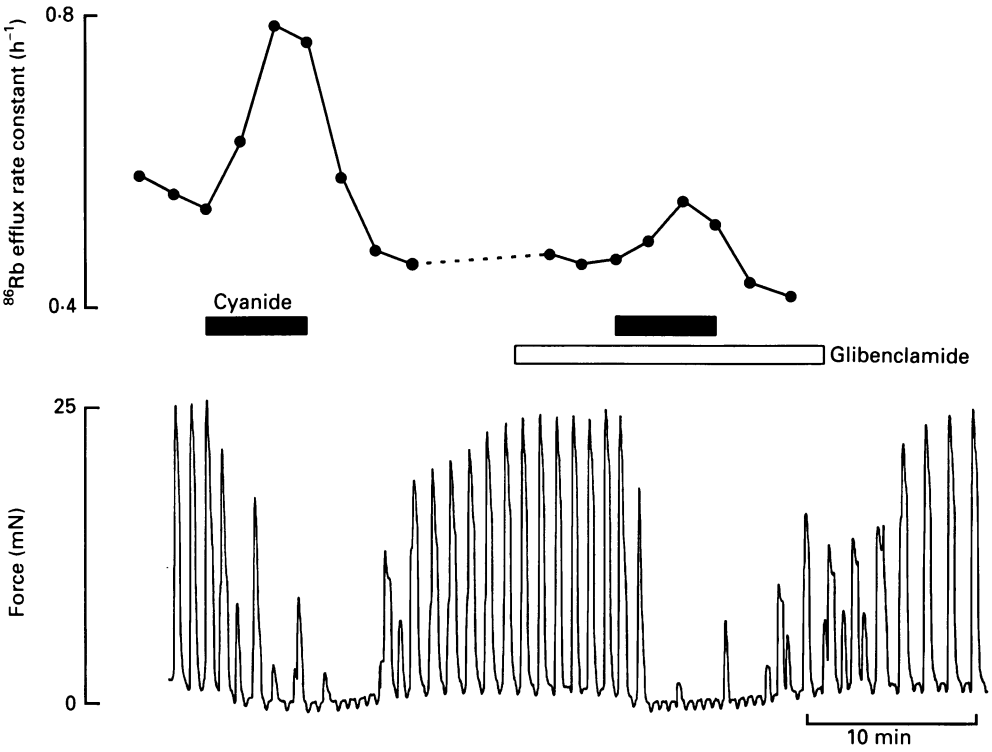

Fig. 4. The effect of glibenclamide on ^{86}Rb efflux (top) and contraction of the isolated rat uterus (non-pregnant). Cyanide (2 mM) and glibenclamide (20 μM) were added for the periods indicated.

cyanide elevated the rate constant of Rb^+ efflux by a similar amount in the presence and absence of calcium from the bathing solution.

Effects of changes of intracellular pH on ^{86}Rb efflux

Intracellular alkalinization greatly increases the frequency of uterine contractions and acidification depresses contraction (Heaton *et al.* 1992; Wray *et al.* 1992). We have therefore examined whether these changes may arise because of changes in surface membrane excitation. Figure 5 shows the effects of the weak base trimethylamine (TMA) on contraction, in non-pregnant uteri. (The base will cause an intracellular alkalinization at unchanged external pH.) There was a large increase in the frequency of contraction upon the addition of base (Fig. 5*A*). Upon return to control bathing solution spontaneous contractions reappear after a brief period of no

contractile activity (see below). The simultaneous ^{86}Rb efflux record shows a large increase in the rate constant (mean increase $32 \pm 2\%$, $n = 3$), which returned to control levels upon return to normal bathing solution. However, the increase in ^{86}Rb efflux appears to be secondary to changes in electrical activity since it was abolished

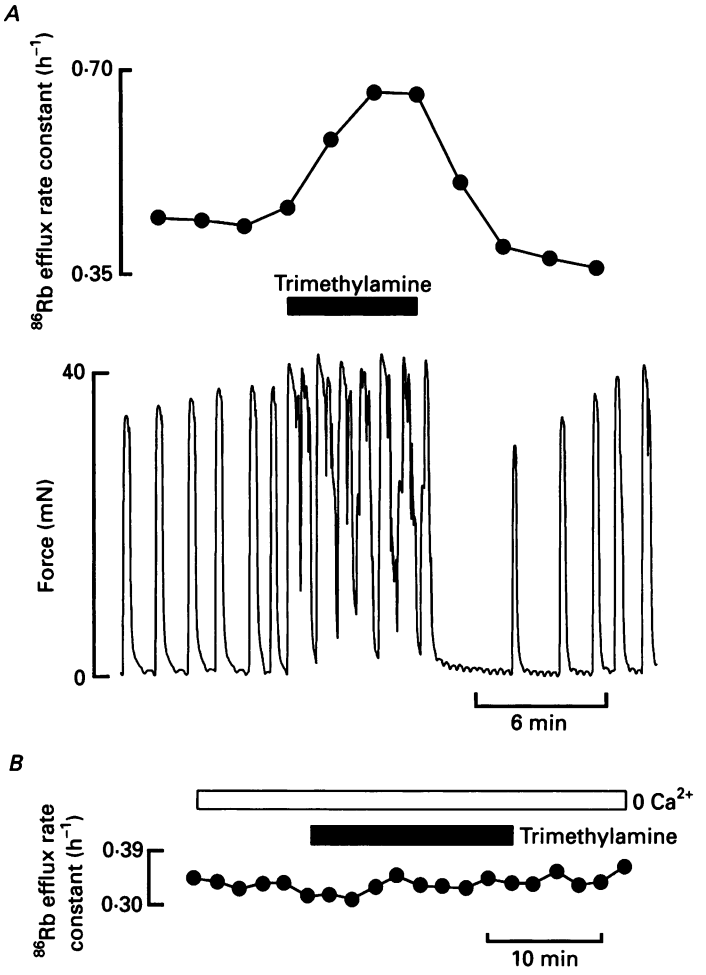

Fig. 5. The effect of intracellular alkalinization on ^{86}Rb efflux and contraction. *A*, effects on a spontaneously contracting tissue. Trimethylamine (60 mM) was added for the period indicated. *B*, effects in a Ca^{2+}-free solution.

by working in Ca^{2+}-free solution (Fig. 5*B*, mean $0{\cdot}95 \pm 0{\cdot}03$ compared to Ca^{2+}-free control, $n = 3$).

Application of weak acid (butyrate) abolished or reduced contractions ($n = 11$) but had no significant effect on the rate constant of ^{86}Rb efflux either in control or Ca^{2+}-free solution. The removal of weak *base* produces a rebound acidification and consequently a reduction in force, as seen in Fig. 5*A*. In experiments performed in normal bathing solution removal of base had no effect on flux. However, in some

experiments (9/14) performed either in high-K^+ or 0 Ca^{2+} solution, removal of the base gave a small (5–10%) and transient increase in the efflux (see later).

DISCUSSION

The results in this paper show that cyanide increases K^+ (^{86}Rb) efflux from the myometrium, partly via K^+_{ATP} channels, and that this is not secondary to electrical or contractile changes. In contrast, changes in pH_i did not produce significant changes in K^+ efflux, other than those related to changes in contractile and electrical activity. No significant differences in the effects of cyanide or pH on K^+ efflux were found between pregnant and non-pregnant rat uteri.

Cyanide and K^+ efflux

^{86}Rb was used as a convenient marker for K^+, because of its longer half-life than ^{42}K. It has been validated as a marker for K^+ in other smooth muscles (Imaizumi & Watanabe, 1981), and recently in the uterus (Hollingsworth *et al.* 1987; Hollingsworth, Edwards, Miller, Rankin & Weston, 1989). Thus for the remainder of the discussion we will take ^{86}Rb efflux to be indicative of K^+ efflux. Following loading with ^{86}Rb, there was an initial rapid wash-out from the tissue followed by a steady efflux. Efflux was then expressed as the rate constant to examine changes produced by experimental manoeuvres. The rate constant remained reasonably stable under control conditions in the majority of preparations.

Cyanide was used to simulate anoxia as it blocks oxidative phosphorylation. Previous work has shown that similar effects on force are produced when oxygen is replaced by nitrogen, as when cyanide is used (Heaton & Wray, 1991). Cyanide inhibited the development of both spontaneous and high-K^+ evoked contractions (cf. Wray *et al.* 1992). Also in agreement with previous work, the effects of cyanide on force were more pronounced in the non-pregnant compared to pregnant uterus (Wray *et al.* 1992).

Cyanide produced a large increase in the K^+ efflux from the tissue. The results obtained with Ca^{2+}-free and high-K^+ solution confirmed that this was a direct effect of cyanide on the efflux, and not secondary to any changes in membrane excitability following alteration of the contractile response. The increase in K^+ efflux with cyanide was not maintained. Since the cyanide-induced increase of efflux was also transient in high-K^+ and Ca^{2+}-free solutions, the transient nature of the response is unlikely to be a consequence of the decrease of electrical activity.

As discussed already, the uterus possesses more than one K^+ channel. Glibenclamide-sensitive K^+_{ATP} channels have been identified in other smooth muscles (Standen *et al.* 1989). Recently Piper *et al.* (1990) showed that glibenclamide could antagonize the effects of cromakalim and other K^+ channel openers *in vitro* and *in vivo* in the myometrium. In our experiments glibenclamide produced a significant reduction in the myometrial K^+ efflux in cyanide. This suggested that part of the increased efflux is via K^+_{ATP} channels. It should, however, be noted that, at least in neuroblastoma cells, glibenclamide may not be selective for the K^+_{ATP} channel and can inhibit voltage-gated K^+ channels (Reeve, Vaughan & Peers, 1992). Although uterine [ATP] in cyanide falls to around 1 mM (Wray, 1990), this is still well above

their dissociation constant (K_D) for [ATP] (Noma, 1983). However, it is possible that only a small fraction of the total K^+_{ATP} channels need to be activated to produce electrophysiological changes in cells (Cook, Satin, Ashford & Hales, 1988; Nichols, Ripol & Lederer, 1991). In addition the increased free [ADP] which occurs during hypoxia, may shift the sensitivity of K^+_{ATP} channels to [ATP] (Weiss, Venkatesh & Lamp, 1992). Finally Davies, Standen & Stanfield (1992) have shown that intracellular acidification reduces the effectiveness of ATP in blocking these K^+ channels in skeletal muscle. As pH_i as well as [ATP] falls in hypoxia it may be that this, along with increased [ADP], causes the opening of these channels in the uterus. As will be discussed later, intracellular acidification *per se* did not elevate K^+ efflux.

Glibenclamide appears to have little if any effect on the spontaneous contractions. This is similar to results obtained in guinea-pig bladder (Fujii, Foster, Brading & Parekh, 1990). Glibenclamide (20 μM) reduced K^+ efflux in cyanide by around 50%, and higher doses were without further effect. This decrease is similar to that reported in cardiac hypoxia where glibenclamide reduced K^+ efflux by 10–50% (Venkatesh, Lamp & Weiss, 1991; Weiss *et al.* 1992). It has been suggested by these authors that elevated free ADP may limit the efficacy of glibenclamide. It is also possible that there is another route by which the remainder of the K^+ leaves. No evidence could be found in our studies for a role of Ca^{2+}-activated K^+ channels (K_{Ca}) contributing to the K^+ efflux in cyanide, since neither Ca^{2+}-free solution nor Ca^{2+}-free solution with glibenclamide produced any additional effect on K^+ efflux from the uterus. However, the direct effect of blockers of K^+_{Ca} channels was not examined. There have been no measurements of $[Ca^{2+}]_i$ during hypoxia in the uterus and so it is not known whether it is elevated, as has been reported for other tissues under these conditions (Cobbold & Bourne, 1984). Thus the pathways carrying the remaining K^+ efflux are currently unclear, and further work is needed to uncover them. One possibility is that it may be linked to anion transport. In studies on cardiac muscle it has been suggested that K^+ efflux in hypoxia may be coupled to lactate efflux, but this remains controversial (Kleber, 1984; Gasser & Vaughan-Jones, 1990).

Does increased K^+ efflux in hypoxia contribute to the depression of force?

A large increase in K^+ efflux occurs during cyanide application to the uterus – does it have any functional significance? The K^+ efflux will tend to cause a hyperpolarization of the smooth muscle membrane, which will make it harder to excite. Spontaneous uterine contractions depend upon pacemaker depolarization. The ionic mechanisms underlying pacemaker activity have not been fully characterized, but probably involve decreased K^+ permeability and increased Na^+ or Ca^{2+} permeability (Parkington & Coleman, 1990). By increasing K^+ permeability, hypoxia would tend to reduce the frequency of contractions or abolish them, as is observed. The effects on contraction of a decrease in membrane excitability were tested directly by depolarizing the myometrial membrane. When spontaneous contractions had been abolished by cyanide, a depolarizing solution (60 mM K^+) was able to elicit contraction, in the continued presence of cyanide (Fig. 1). This result suggests that although cyanide may well have effects on other stages in contraction, the decrease in membrane excitability, resulting from the increased K^+ efflux, may lead to a depression of force, before other mechanisms act. However, in the presence

of cyanide, the high-K^+ response is not maintained. This may be because the energy cost of the contracture decreases [ATP] and pH_i and increases inorganic phosphate. In support of this, even in the absence of cyanide, elevating external K^+ decreases [ATP] (Wray, 1990) and a larger K^+-induced fall of [ATP] is to be expected in the presence of cyanide. Thus ultimately, neither spontaneous nor high-K^+-elicited contractions can be maintained in cyanide. It would appear that cyanide exerts its effects at several places in the contraction process but that reduction in pacemaker activity may be the initial cause of the fall in contraction frequency. It is worth noting that the abolition of contraction due to the increased K^+ permeability will protect the uterus by limiting the metabolic demand and thence changes of metabolite concentrations. Glibenclamide reduced the K^+ efflux by around 50%, but there was no functional improvement in cyanide (Fig. 4), i.e. blocking K^+_{ATP} channels is not sufficient to ameliorate the depression of force in hypoxia. This suggests that even modest increases in K^+ permeability may depress spontaneous uterine contractions.

The increase in K^+ efflux was similar in pregnant and non-pregnant uterine preparations. Thus although gestational conditions may alter K^+ channel conductances in the uterus (Toro *et al.* 1990), such changes were not influencing the response to cyanide. The greater depression of contraction by cyanide in non-pregnant compared to pregnant uteri must reside in some other effect of cyanide; for example, the greater fall in [ATP] and [PCr] (Wray, 1990).

Effects of pH_i on K^+ efflux

As Fig. 5 shows, alkalinization increases the frequency of uterine contractions. However, the increased frequency is not due to changes in K^+ efflux, because efflux was unaltered by alkalinization, after secondary changes due to increased contractile activity had been removed. Thus some other ionic mechanism susceptible to pH must be affected. It is possible that, as in other tissues, alkalinization increases the calcium current (Mironov & Lux, 1991). It was also found that intracellular acidification produced by butyric acid had no effect on K^+ efflux from the myometrium. The transient and small increase in efflux seen in some preparations with rebound acidification (produced by withdrawal of base), may indicate that large decreases in pH_i have a slight effect on the efflux, since this acidification is greater than that occurring with addition of butyrate (60 mM) (M. J. Taggart & S. Wray, preliminary observations). It is unlikely that the acidification which occurs in hypoxia will contribute to the increased K^+ efflux. As mentioned above, the acidification in hypoxia may indirectly contribute to K^+ efflux by reducing the activation of K^+_{ATP} channels, although there is no evidence for this mechanism in the uterus so far.

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