EFFECTS OF BICARBONATE REMOVAL ON THE SPONTANEOUS MECHANICAL ACTIVITY OF THE GUINEA PIG UTERUS

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Summary 1. Effects of bicarbonate removal and of decreasing external pH on the mechanical and electrical activities in isolated strips of the guinea pig uterus were investigated.

2. When bicarbonate was reduced at 37°C, a high degree of contractile activity was observed, which was associated with high frequency spike activity. However, when the same experiment was repeated at 21°C, the activity was suppressed. These sustained contractions were produced whether the HCO_3^- -free solution was acid (pH: about 6) in the presence of 3% CO_2 , or the solution was alkaline (pH: about 8) in the absence of CO_2 .

3. When the pH of the solution was decreased to 6 by 5 mM tris malate buffer, the mechanical activity was also increased in the presence of 15.5 mM HCO_3^- .

4. In low Cl^- (7 mM) Krebs solution the mechanical activity depended on the substitute for Cl^- : substitution with isethionate reduced or stopped the activity, while substitution with NO_3^- increased the activity. The increase in activity on HCO_3^- removal was less in isethionate solution and much larger in NO_3^- solution.

5. At a lower temperature (21°C), HCO_3^- removal suppressed the activity when O_2 containing 3% CO_2 was bubbled through the solution.

6. It is suggested that HCO_3^- removal and acidification increase Cl^- permeability of the membrane and that this leads to high spontaneous activity. It is also possible that modification of the external buffer system may affect the internal pH and metabolism, which in turn modifies spontaneous activity.

The smooth muscle of the guinea pig uterus has spontaneous activity, but the pattern of activity varies greatly from preparation to preparation. The ex-

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planation for this is unknown and, indeed, little information is available about factors which control spontaneous activity.

In the guinea pig taenia coli, replacement of Cl^- with a less permeant anion usually suppresses the spontaneous electrical activity, whereas replacement of Cl^- with a more permeant anion accelerates the spontaneous activity (KURIYAMA, 1963; BÜLBRING and TOMITA, 1969). This suggests a possibility that the pacemaker potential is due to movements of Cl^- ions in normal solution. It is known that foreign anions decrease the Cl^- permeability of the membrane in the frog skeletal muscle (HUTTER and PADSHA, 1959). Since a physiological Krebs solution contains between 15 and 25 mM NaHCO_3 as a buffer (BÜLBRING, 1953; GOODFORD and HERMANSEN, 1961; KREBS, 1950; KREBS and HENSELEIT, 1932), HCO_3^- ions may affect the Cl^- permeability. Therefore, in the present experiments, effects of HCO_3^- removal were studied on the isolated strip of the guinea pig uterus. It was found that a reduction of the external HCO_3^- concentration increases the spontaneous activity, as would be expected from changes in the Cl^- permeability.

METHODS

Guinea pigs weighing 250–330 g were stunned and bled. The uterus was quickly removed, and strips of uterus (20 mm long, 1–2 mm wide) were dissected. The strips were suspended in an organ bath (20 ml capacity) and connected to a strain gauge transducer to record mechanical activity isometrically.

The control Krebs solution contained NaCl 121 mM, glucose 11.5 mM, CaCl_2 2.5 mM, NaHCO_3 15.5 mM, MgCl_2 1.2 mM, and KCl 5.9 mM. A gas mixture composed of 3% CO_2 and 97% O_2 was bubbled through the solution. When NaHCO_3 was reduced, it was replaced with an equimolar concentration of NaCl. In some experiments, pure O_2 was bubbled through the solution.

Solutions flowed continuously through the organ bath at a constant speed of about 2 ml/min, and it took about 30 min for a complete change of the solution. The effects of CO_2 bubbling on the pH of the external solution were much faster than those of HCO_3^- removal because of the slow speed of changing the solution. The pH of the perfused solutions was measured by placing a pH electrode near the preparation in the organ bath. The electrical outputs of pH meter (Toa Electronics, Model HM-6A) and of the strain gauge transducer were simultaneously recorded on a potentiometric pen recorder (National Electronics Model VP-652B).

At least three preparations from different animals were used in each experiment. Occasionally a pregnant uterus was used but since the responses were not different from those obtained in uteri from non-pregnant animals, the hormonal stage of the uterus was not considered.

In some experiments, electrical recordings were made under the same experimental condition as in mechanical recordings. External electrodes (Ag-

AgCl, 100 μ in diameter) were placed across the preparation.

RESULTS

Effects of removal of HCO₃⁻

The smooth muscle of the guinea pig uterus contracts spontaneously. The pattern of the spontaneous activity was irregular and varied in different preparations. It usually appeared as a burst which occurred every few minutes. Experiments were started after equilibrating the tissue in the organ bath for at least 30 min.

Figure 1 shows an example of the mechanical response produced by removal of HCO₃⁻ from the Krebs solution. When the HCO₃⁻ concentration was reduced from 15.5 mM to 0 mM, the pH of the external solution declined from 7.5 to about 6 in the presence of 3% CO₂. The duration of a burst of spontaneous contractions was prolonged and tension was maintained at a high level during the exposure to the HCO₃⁻ deficient solution. There were many small contractions superimposed on the sustained tension. The normal pattern of activity reappeared on returning the HCO₃⁻ concentration to normal (15.5 mM), but it took about 10 min before the first burst of activity started.

Fig. 1. Effects of HCO₃⁻ removal on mechanical activity (top) and pH of the external solution (bottom) of guinea pig uterine smooth muscle. Flow of HCO₃⁻-free solution is indicated by the horizontal bar. a: a gas mixture of 3% CO₂ and 97% O₂ was bubbled through the solution. b: 3% CO₂ and 97% O₂ was changed to 100% O₂ during the flow of HCO₃⁻-free solution.

When the reduction of the HCO₃⁻ concentration was accompanied by a change in the composition of the gas from 3% CO₂ and 97% O₂ to 100% O₂, the pH of the external solution increased from 7.5 to about 8 (Fig. 1b). Although

the pH of the solution was different, the mechanical response produced by HCO_3^- reduction was essentially the same as when 3% CO_2 was used throughout the experiment. The increase in activity produced by HCO_3^- removal was stronger when the resting tension of the preparation was increased by stretching.

Small contractions during periods of high maintained tension in HCO_3^- -free solution suggest the presence of spike activity. As shown in Fig. 2, there was a good correlation between the tension development (upper trace) and the spike activity (lower trace).

Fig. 2. Effects of HCO_3^- removal on mechanical (top) and electrical (bottom) activities. Note the good correlation between tension development and spike activity in normal solution (a) and also in HCO_3^- -free solution (b, c & d).

Tris buffer experiments

The pH of the external solution was reduced to less than 6.5 by removing HCO_3^- . To examine the effect of lowering external pH, solutions containing 5 mM tris malate buffer with a given pH in place of an equivalent amount of NaCl were used. Pure O_2 was bubbled through the solution. The pH of the control solution which contained 15.5 mM HCO_3^- and 5 mM tris malate was 8.2. A slight increase in the activity was observed (Fig. 3a). This suggests that the tris buffer has some excitatory action.

When the pH was lowered to 6.0 by adjusting with tris malate buffer in the presence of normal concentration of HCO_3^- , the activity was strongly increased (Fig. 3b). Similar results were obtained in four other preparations at a pH between 6 and 6.5. When HCO_3^- was removed, the sustained activity was always produced regardless of the external pH.

Effects of HCO_3^- removal in Cl^- -deficient solution

It is possible that removal of HCO_3^- increases the Cl^- permeability of the membrane, and that the mechanical response produced by reducing the HCO_3^-

Fig. 3. Effects of pH adjustment by tris malate (5 mM) buffer in the presence of 15.5 mM HCO₃⁻. 100% O₂ was bubbled throughout. a: pH was kept constant at 8.2. b: pH was changed from 8.2 to 5.9. Horizontal bars indicate flow of the solution containing 5 mM tris malate with a given pH (8.2 in (a) and 5.9 in (b)).

Fig. 4. Effects of HCO₃⁻ removal in Cl⁻-deficient isethionate solution (7 mM Cl⁻). a: mechanical activity in normal solution. b: after 30 min in isethionate solution, and when HCO₃⁻ was removed from the solution (horizontal bar). The pH of the isethionate solution was 7.8.

concentration is somehow mediated through the change in the Cl^- permeability. Lowering the pH of the external solution may also affect the Cl^- permeability, since it has been reported that pH influences the Cl^- permeability of the membrane in frog skeletal muscle (HUTTER and WARNER, 1967), barnacle muscle (HAGIWARA *et al.*, 1968) and cardiac muscle (CARMELIET and BOSTEELS, 1969).

When NaCl and KCl in Krebs solution were replaced with an equimolar concentration of isethionate salts, leaving 7 mM Cl^- as CaCl_2 and MgCl_2 , the activity was suppressed after a period of about 20 min during which irregular bursts of high activity appeared. Activity was again produced by removing HCO_3^- , but the tension was not maintained as in the normal solution containing Cl^- (Fig. 4). In three other preparations similar results were obtained. In two preparations, some spontaneous activity remained in the Cl^- -deficient solution, but its frequency was much slower than in normal Krebs solution.

In contrast to isethionate, substitution of Cl^- with NO_3^- increased the spontaneous activity. Removal of HCO_3^- from the Cl^- -deficient NO_3^- solution further increased the activity so that a strong sustained contraction was produced (Fig. 5).

Fig. 5. Effects of HCO_3^- removal in Cl^- -deficient NO_3^- solution (7 mM Cl^-). a: Mechanical activity in normal solution. b: After 30 min in NO_3^- solution, HCO_3^- was removed (horizontal bar).

Effects of temperature

It may be that removal of HCO_3^- reduces the intracellular pH and that the spontaneous activity is produced as a result of a change in cell metabolism following the pH change. If this is true, a large effect of temperature would be expected.

Figure 6 shows the effect of a removal of HCO₃⁻ at two different temperatures. At 37°C (a), a sustained increase in tension was produced by removing HCO₃⁻. This response was essentially the same as that shown in Fig. 1a. However, when the same experiment was repeated at 21°C (b), the removal of HCO₃⁻ suppressed the activity. The activity quickly reappeared on admission of 15.5 mM HCO₃⁻. Thus, lowering the temperature reversed the effect of HCO₃⁻-free solution.

Fig. 6. Effects of HCO₃⁻ removal at 37°C (a) and 21°C (b). After recording the response (a) at 37°C the organ bath was cooled down to 21°C and equilibrated at this temperature for 30 min before taking record (b).

The activity was increased when the pH was lowered to 6–6.5 by tris malate buffer (Fig. 3b and Fig. 7a). In this experiment, the initially applied solution contained 15.5 mM HCO₃⁻ and was bubbled with 100% O₂. The excitatory effect of the tris malate buffer was not essentially modified by lowering the temperature from 37°C to 21°C (Fig. 7b). Thus, at 37°C the effect of HCO₃⁻ removal was the same as that of acidification with tris malate buffer, while at 21°C their effects were different.

The absence of suppression by acidification with tris buffer at the lower temperature could be related either to the presence of HCO₃⁻ or to the absence of CO₂. In order to discriminate between these possibilities, the following experiments were carried out.

The pH of the solution was about 8.2 since pure oxygen was used instead of 3% CO₂ and 97% O₂. At 37°C removal of HCO₃⁻ increased the activity, although the pH did not fall much below 8.0. This is in accord with the result shown in Fig. 1b. Suppression of activity by removal of HCO₃⁻ was very weak or

Fig. 7. Effects of acidification (pH: 6.7) at 37°C (a) and 21°C (b). pH was adjusted with 5 mM tris malate buffer (horizontal bar). 100% O₂ was bubbled throughout.

absent at 21°C, and a transient suppression appeared on readmission of 15.5 mM HCO₃⁻ (Fig. 8b). In the HCO₃⁻-free solution, the activity was inhibited by bubbling 3% CO₂ at 21°C (Fig. 8c), as expected from the result shown in Fig. 6b, and an increase in activity quickly appeared when CO₂ supply was stopped.

DISCUSSION

Removal of HCO₃⁻ produces sustained increase in tension due to a high spontaneous spike activity in the smooth muscle of the guinea pig uterus. Acidification of the external solution is not an essential factor for this response, since the sustained tension is also produced by HCO₃⁻ removal at pH 8.0 when 3% CO₂ is omitted (Fig. 1b and Fig. 8a). Acidification may be an additional factor increasing the activity. This is suggested by the sustained high mechanical activity observed in the presence of 15.5 mM HCO₃⁻ when the pH is adjusted to 6.0 by tris malate buffer.

High spontaneous activity produced by HCO₃⁻ removal or by acidification may be a result of an increase in Cl⁻ permeability. Substitution of Cl⁻ with a large anion, isethionate, reduces or stops the spontaneous activity. On the other hand, substitution of Cl⁻ with NO₃⁻, which is probably more permeant, increases the activity. In the smooth muscle of the guinea pig taenia coli, the high permeability of NO₃⁻ compared with Cl⁻ has been demonstrated by measurements of volume changes produced by osmotic pressure (BRADING and TOMITA, 1968). Thus, it seems that the anion permeability controls, in some way, the generation of spontaneous activity. For example, an increase in anion permeabi-

Fig. 8. Effects of HCO₃⁻ removal in the absence of CO₂ at 37°C (a) and 21°C (b), and effects of 3% CO₂ in the absence of HCO₃⁻ at 21°C (c).

lity may contribute to the depolarization of the pacemaker potential, as proposed for cardiac muscle (HUTTER and NOBLE, 1961).

It has been suggested that foreign anions suppress the Cl⁻ permeability in the frog striated muscle, probably due to some interaction with the anion channels (HUTTER and PADSHA, 1959). It is possible that HCO₃⁻ acts in this way also in smooth muscle and that removal of HCO₃⁻ increases the Cl⁻ permeability.

In the frog skeletal muscle the Cl⁻ permeability decreases with decreasing the external pH (HUTTER and WARNER, 1967). In contrast, the Cl⁻ permeability increases with decreasing the external pH in crustacean muscle fibers (DE MELO and HUTTER, 1966; HAGIWARA *et al.*, 1968; REUBEN *et al.*, 1962). In the guinea pig taenia coli, a recovery of tissue weight in hypertonic solution made by adding KCl or KNO₃ is accelerated by acidification (M. Ohba and T. Tomita, personal communication), suggesting an increase in anion permeability with decreasing pH, as in the crustacean muscle. This property may also be applicable to the uterine smooth muscle. Then, it is reasonable to assume that an increase in the Cl⁻ permeability is at least partly responsible for the high activity in the HCO₃⁻-

free and acid solutions.

The excitatory effect of HCO_3^- removal is reversed to an inhibitory effect when the temperature is lowered to 21°C . The presence of CO_2 seems necessary for this suppression. However, it is difficult to elucidate the mechanism of the HCO_3^- effect on spontaneous activity at different temperatures. It is possible that intracellular Cl^- concentration is reduced at low temperature shifting the equilibrium potential for Cl^- to a more negative level than the resting potential, and that the contribution of Cl^- conductance increase on HCO_3^- removal to the membrane potential is reversed. It is also possible that changes in intracellular pH may affect the cellular metabolism which regulates in a complicated way the spontaneous activity. For example, it is reported that in the frog skeletal muscle, glucose-6-phosphate increases, and creatinephosphate and the efflux of lactate decrease on reducing the external HCO_3^- concentration (MAINWOOD *et al.*, 1972). These biochemical changes may also occur in the uterine smooth muscle and may affect the membrane properties.

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