Biochimica et Biophysica Acta, 944 (1988) 113–120
Elsevier

BBA 74127

Intracellular pH influences the resting membrane potential of isolated rat hepatocytes

Christine E. Bear, Joseph S. Davison and Eldon A. Shaffer

Gastrointestinal Research Group, Departments of Medicine and Medical Physiology, University of Calgary, Calgary, Alberta (Canada)

(Received 29 April 1988)

Key words: pH, intracellular; Potassium ion conductance; Quinine; Membrane potential; (Rat hepatocyte)

This study in isolated rat hepatocytes sought to determine if the changes in membrane potential associated with intracellular alkalinization or acidification could be attributed to changes in K^+ conductance. Intracellular pH_i was manipulated using the 'NH_4^+-pulse' method: inducing intracellular alkalinization with NH_4Cl (10 mM), and producing acidification by diluting the NH_4^+-loaded cells with ammonium ion-free buffer or by adding sodium proprionate. Membrane potential and resistance were measured in freshly isolated rat liver cells using intracellular microelectrodes. The results indicated that intracellular alkalinization was associated with hyperpolarization and decreased membrane resistance, whereas intracellular acidification caused depolarization with increased membrane resistance. As pH_i-mediated electrogenic responses have been related to changes in K^+ conductance in other epithelial tissues, the influence of K^+ transport inhibitors on NH_4^+-evoked electrical effects was examined. NH_4Cl-evoked membrane potential changes were inhibited by the K^+ channel blockers, quinine and barium and in potassium depolarized cells (cells bathed in a high K^+ medium where $[K^+]_{in} = [K^+]_{out} = 140$ mM). Furthermore, Rubidium-86 ($^{86}Rb^+$) efflux from preloaded hepatocytes, a measure of K^+ permeability, was enhanced following intracellular alkalinization but inhibited by intracellular acidification. Thus, these results indicate that pH_i-evoked electrogenic effects in hepatocytes are mediated through changes in K^+ conductance.

Introduction

Intracellular pH (pH_i) is involved in the control of many cellular processes including cell volume regulation in lymphocytes [1] and mitogenesis in muscle cells [2]. A role for pH_i in the regulation of hepatocellular ion transport has been proposed on the basis of microelectrode studies reported by Henderson et al. [3] in which changes in pH_i were shown to evoke changes in membrane potential. As modifications in pH_i have been shown to alter K^+ permeability in a number of cell types such as proximal tubule cells [4], gallbladder epithelial cells [5] and pancreatic B cells [6,7], we sought to determine if pH_i-mediated changes in membrane potential in hepatocytes were related to changes in K^+ conductance.

In the present investigation, intracellular pH was manipulated in isolated rat hepatocytes using the 'ammonium (NH_4^+)-pulse' method, first described by Boron [8] and the effects of changes in pH_i on membrane potential were measured using intracellular microelectrodes. The K^+ channel blockers, quinine and barium, were used in order to assess the role of K^+ conductance in pH_i-re-

Correspondence: E.A. Shaffer, Room 1751, Health Sciences Centre, The University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta, T2N 4N1 Canada.

0005-2736/88/$03.50 © 1988 Elsevier Science Publishers B.V. (Biomedical Division)

lated electrogenic responses. Furthermore, $^{86}Rb^+$ efflux from preloaded hepatocytes, an index of K^+ permeability, was measured following the production of intracellular alkalinization and acidification. The results of these studies indicate that pH_i does influence K^+ permeability in the liver cell: intracellular alkalinization enhances whereas acidification inhibits K^+ conductance.

Methods and Materials

Isolated hepatocyte suspensions were prepared from the livers of male Wistar rats (150–200 g) using the collagenase perfusion method [9]. Rats were anaesthetized by intraperitoneal injection of sodium pentobarbitone (30 mg/kg) and the liver perfused with a physiological electrolyte solution at 37 °C containing 150 units/ml collagenase (type IV). The liver was then finely minced in the same collagenase solution of 5 minutes. At the end of the digestion period, single hepatocytes or hepatocyte couplets were harvested for experimentation. Small aliquots of cells were transferred to a 3 ml chamber on the stage of a Zeiss inverted microscope and viewed at 400 × magnification. Cells, healthy in appearance without apparent blebs or vacuoles, were selected for impalement. The experiments were conducted at 37 °C. The physiological electrolyte solution contained (mM): 140 NaCl, 4.7 KCl, 1.13 $MgCl_2$, 10 glucose, 2.5 $CaCl_2$, and 10 Hepes. The pH of the solution was adjusted using 1 M NaOH to 7.2 except when the effect of different external values were assessed. Membrane potentials of isolated cells were measured using conventional microelectrodes. Microelectrodes were made by pulling borosilicate glass capillary tubes (1.2 mm o.d.; WPI, New Haven, U.S.A.) using a Haer ultrafine microelectrode puller. The microelectrodes were filled with 1 M KCl and had tip resistances of approximately 50 MΩ. The electrodes were inserted into appropriate Ag-AgCl-pellet microelectrode holders (WPI) and mounted on a Narishige hydrostatic micromanipulator. The potentials were measured using a Dagan amplifier (Model 8700) and recorded on a Gould chart recorder. A grounded, chloride-coated silver wire was used as a reference electrode. Input resistance was continuously monitored by injecting current pulses (0.2 nA) applied through the recording electrode using a Grass stimulator (Model S48). Ammonium chloride (NH_4Cl), sodium proprionate and quinine-containing solutions were prepared using the physiological electrolyte solution previously described. These solutions were administered by continuous superfusion of the cell from one of a series of line tubes permitting flow at a rate of approximately 100 μl/min. The mouth of a tube containing a particular solution was manipulated to close proximity of the impaled cell.

A fluorometric assay was used for the determination of intracellular pH (pH_i). This technique has been used in the study of pH_i in several cell types including lymphocytes and neutrophils [10,11]. Isolated hepatocytes were counted using a Coulter counter and channelyzor and resuspended in the concentration, 10^7/ml. The cells were then loaded with the probe BCECF by incubation with the acetoxymethyl ester form (3 g/ml) for 30 min at 37 °C. After two washes, 0.5 to $1.0 \cdot 10^6$ cells were used for fluorescence measurement with excitation at 485 nm and emission at 540 nm using 5 and 10 nm slits, respectively. The nigericin/potassium method of Thomas et al. [12] was used to calibrate pH_i.

To study K^+ efflux, hepatocytes were preloaded with $^{86}Rb^+$ by incubating $5 \cdot 10^6$ cells/ml in the physiological electrolyte solution which contained the isotope (15 μCi/ml of suspension) for 60 min at 37 °C. In other experiments, hepatocytes were preincubated with the radioisotope in an electrolyte solution containing NH_4Cl (10 mM). After preloading, the cells were separated from the incubation medium by centrifugation at 200 × g. The $^{86}Rb^+$ efflux study was then initiated by diluting the cells in 10 ml of isotope-free buffer, with or without NH_4Cl (10 mM). At 0, 30, 45, 60 and 75 s after efflux had been initiated, 1 ml samples were collected and immediately centrifuged at 200 × g for 60 s. The radioactivity contained in the cell supernatants was determined in a Beckman Liquid Scintillation Counter.

Materials

Collagenase (type IV) and quinine sulfate were purchased from Sigma, St. Louis, MO. Rubidium-86 was obtained from Amersham Can. Ltd., Oakville, Ontario. Nigericin was from Calbiochem-

Behring, San Diego, CA. 2,7-Biscarboxyethyl-5(6)-carboxyfluorescein (BCECF) acetoxymethyl ester was obtained from the HSC Research Development, Toronto, Canada.

Statistical methods

The results were expressed as mean ± S.E. Data were analyzed using paired or unpaired '*t*'-test when appropriate. Differences were considered significant at '*P*' values less than 0.05.

Results

The effect of extracellular pH on membrane potential

In 35 separate cell impalements the mean membrane potential and resistance measured were 25.9 ± 0.1 mV and 83.6 ± 4.5 MΩ, respectively. These values are comparable to those previously reported for isolated hepatocytes [13–15]. In all of these recordings, stable membrane potentials occurred within 30 s after impalement and were maintained for more than 4 min. As shown in Fig. 1, changes in external pH from 7.2 to 7.6 and to

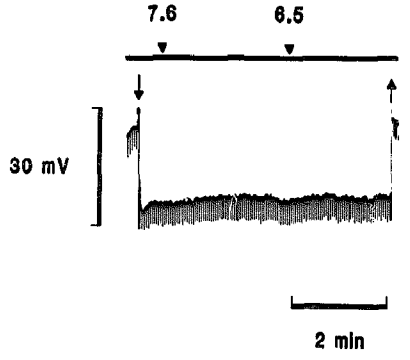

Fig. 1. Changes in external pH (pH_o) have no immediate effect on membrane potential. Potential measurements prior to and following cell impalement are shown. Hyperpolarizing current pulses (0.2 nA) applied continuously through the recording electrode assessed membrane resistance. Following cell puncture, marked by the downward arrow (↓), a sudden negative deflection in potential of 22 mV occurred, indicative of the resting membrane potential. Prior to and after impalement, the normal salt solution superfusing the cell was buffered at pH 7.2. Where the superfusion solution was changed to one buffered at pH 7.6, no change occurred. Similarly, there was no effect on membrane potential observed when the superfusion fluid was then changed to pH 6.5. The electrode was withdrawn from the cell at point marked with the upward arrow (↑).

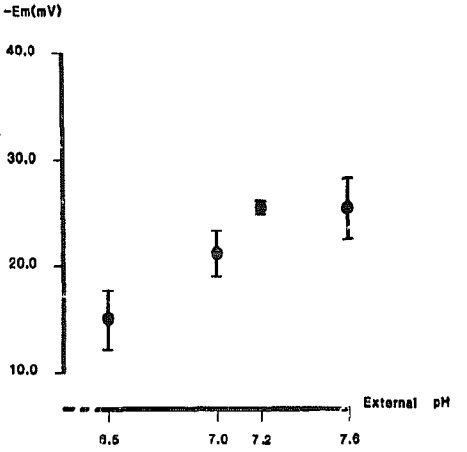

Fig. 2. Prolonged exposure to external solutions with low pH ($pH_o = 6.5$) causes reduction in membrane potential. The mean membrane potential (E_m) measured in cells has been plotted as a function of the pH of the extracellular solution. In these experiments, cells had been incubated at a particular pH; 6.5, 7.0, 7.2 and 7.6 for approx. 30 min. Five experiments were performed at pH 6.5, 8 experiments at pH 7.0, 35 and 7.2 and 22 experiments at pH = 7.6.

6.5 evoked no change in membrane potential within a two minute time period ($n = 5$). Hepatocytes were also incubated in media at pH 6.5 ($n = 5$), 7.0 ($n = 8$), 7.2 ($n = 35$) or 7.6 ($n = 22$), each for 30 min in separate experiments to determine the effect of prolonged exposure to acidic or alkaline solutions on membrane potential. Fig. 2 shows the mean membrane potential measured following exposure to media at each of the above pH values. The membrane potential measured in cells exposed to medium at pH 6.5 was significantly less ($P < 0.01$) than that measured in cells bathed in medium at pH 7.2.

The effect of intracellular pH on membrane potential

The 'NH_4Cl pulse' method, of Boron et al. [8] has been used to manipulate the intracellular pH of many cell types [16,17] and has recently been applied to the study of pH regulation in isolated rat hepatocytes [3]. Henderson [3] has shown that exposure of hepatocytes to NH_4Cl (10 mM) caused cellular alkalinization using pH-sensitive microelectrodes. Subsequent exposure of NH_4^+-loaded cells to a NH_4Cl-free solution caused intracellular

acidification as predicted by Boron [8]. We first confirmed that addition of NH_4Cl (10 mM) caused cellular alkalinization using the BCECF fluorimetric assay. The intracellular pH of hepatocytes in the control medium was 6.93 ± 0.05 ($n = 5$), a value comparable to that reported by Henderson [3] in microelectrode studies. The addition of NH_4Cl (10 mM) caused a transient increase in pH_i to 7.11 ± 0.03 ($n = 5$), a change which is statistically significant ($P < 0.05$). Furthermore, the addition of proprionate (20 mM) which is known to cause intracellular acidification in several cell types [1,16,17] evoked a decrease in pH; to 6.74 ± 0.05 ($n = 5$), ($P < 0.05$). Fig. 3 shows the effect of NH_4Cl and sodium proprionate addition on intracellular pH.

The addition of NH_4Cl caused changes in membrane potential and resistance (Fig. 4a). Exposure to NH_4Cl (10 mM) resulted in cellular hyperpolarization: 5.1 ± 1.1 mV, with a concomitant decrease in membrane resistance of 10.8 ± 4.9 MΩ in seven experiments. The changes in potential and resistance were significantly different from resting values with P values less than 0.0025 and 0.05, respectively. The subsequent superfusion of these NH_4^+-loaded cells with a NH_4Cl-free solution a maneuver which causes intracellular acidification, resulted in depolarization of 9.2 ± 1.7 mV with an increase in membrane resistance of 11.8 ± 3.8 MΩ, significantly different from values measured when the cells were exposed to the NH_4Cl-containing solution ($P < 0.0025$ and 0.05, respectively). Furthermore, the addition of proprionate

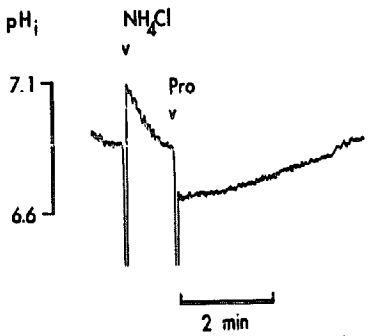

Fig. 3. Measurement of intracellular pH. The addition of NH_4Cl (10 mM) caused intracellular alkalinization while sodium proprionate (Pro) (20 mM) caused intracellular acidification.

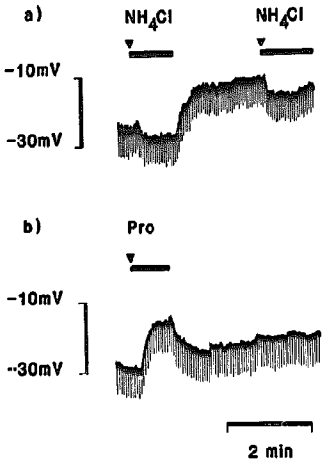

Fig. 4. (a) The 'NH_4Cl pulse' method for causing changes in pH_i evokes changes in E_m. This intracellular recording, showing a resting membrane potential of −30 mV, was obtained in a cell exposed to continuous superfusion with a normal salt solution. Changing this solution to one containing NH_4Cl (noted by solid line), a maneuver which should increase pH_i, resulted in modest hyperpolarization. Switching the superfusion solution back to the normal one without NH_4Cl (at the very end of the solid line), which should cause intracellular acidification, resulted in marked depolarization to a value less than the resting membrane potential. The difference between depolarized and resting values was significant in seven comparable experiments ($P < 0.02$). (b) Intracellular acidification caused by proprionate results in membrane depolarization. The resting membrane potential measured in this cell was −30 mV. The introduction of sodium proprionate (Pro) into the superfusion solution caused cellular depolarization, an effect which was reversed following reintroduction of the control solution.

(20 mM), which we confirmed will induce intracellular acidification, caused cellular depolarization of 20.7 ± 4.6 mV with an increase in membrane resistance, 17.2 ± 1.5 MΩ ($n = 6$) (Fig. 4b). These proprionate induced changes were significantly different from resting values ($P < 0.005$ and 0.0005, respectively). Proprionate evoked potential and resistance changes were reversed by exposure of cells to proprionate-free solutions. These results suggest that cellular alkalinization causes the increased ionic conductance leading to hyperpolarization, whereas acidification decreases ionic conductance causing depolarization.

The effect of quinine, barium and high extracellular KCl concentrations on NH_4Cl and proprionate-induced electrical effects

In order to assess the role of K^+ conductance

in pH_i-related electrical changes we studied the effect of quinine and barium, both K^+ channel blockers in many tissues [18–20], on pH_i-evoked potential changes. In these experiments, the ability of the impaled cell to respond electrically to manipulations of pH_i was first assessed using the 'NH_4Cl-pulse' method (Fig. 5a) or by superfusion of proprionate (Fig. 5b). Addition of quinine (200 μM) caused a marked depolarization of 17.4 ± 4.0 mV ($P < 0.0005$) with an increase in membrane resistance: 22.0 ± 5.4 MΩ ($n = 11$) ($P < 0.001$). These electrogenic effects indicate that that quinine causes the blockade of K^+ channels. The continued superfusion of quinine prevented further NH_4Cl and proprionate-evoked changes in potential (Figs. 5a and 5b, respectively).

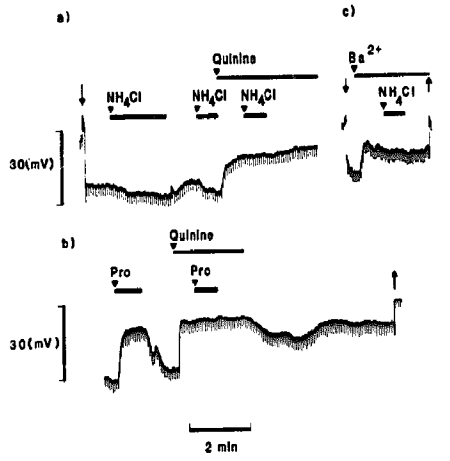

Fig. 5. (a) Quinine blocks the effect of NH_4Cl on E_m. The membrane potential measured in this particular hepatocyte is -25 mV in the presence of the continuous superfusion of normal salt solution. Superfusion with NH_4Cl caused hyperpolarization while the subsequent replacement of a NH_4Cl-free solution produced depolarization. Superfusion by quinine (200 μM) (upper solid line) caused marked depolarization. The subsequent addition of NH_4Cl failed to evoke any change in E_m. (b) Quinine blocks the effect of sodium proprionate on E_m. A membrane potential of -30 mV was measured in this cell. Exposure to proprionate (Pro) caused cellular depolarization as before. Quinine when subsequently added depolarized the cell by almost 20 mV. Proprionate when reapplied did not elicit any change in E_m during the continued superfusion of quinine. The quinine evoked depolarization could not be permanently reversed by the return to quinine-free control solution for up to 5 min. The electrode was pulled out of the cell where marked (↑). (c) Barium inhibits the effect of NH_4Cl on E_m (upper right corner). Addition of barium (Ba^{2+}) (upper solid line) caused cellular depolarization accompanied by increased membrane resistance. The continued superfusion of the Ba^{2+}-containing solution inhibited NH_4Cl electrogenic effects.

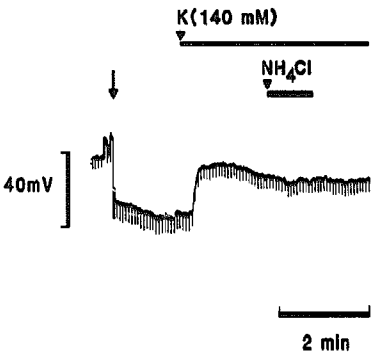

Fig. 6. High extracellular K^+ (140 mM) prevents NH_4Cl-induced changes in E_m. Cell puncture occurred where marked as before and a stable membrane potential of -30 mV was observed at 30 s. A change in the superfusion solution from that normally used, ($[K^+]$) = 3.4 mM to one with $[K^+] = 140$ mM evoked a depolarization of 25 mV. The membrane potential then stabilized at -10 mV after 1.0 min. Subsequent addition and withdrawal of NH_4Cl failed to evoke changes in membrane potential in the presence of the high $[K^+]$ solution.

As seen in Fig. 5c, the superfusion of hepatocytes with barium (Ba^{2+})-containing solution (2 mM) caused changes in membrane potential and resistance similar to those evoked by quinine. Within one minute, barium addition induced depolarization with a magnitude of 15.5 ± 2.9 mV ($P < 0.05$) and membrane resistance increased by 17.5 ± 0.9 MΩ ($P < 0.005$) ($n = 4$). As with quinine, the presence of barium inhibited NH_4Cl-evoked changes in potential and membrane resistance.

Exposure of isolated hepatocytes to an extracellular solution containing 140 mM KCl resulted in depolarization of 31.9 ± 6.5 mV ($P < 0.0005$) with a decrease in membrane resistance of 21.0 ± 4.4 MΩ ($P < 0.0025$) ($n = 7$), Fig. 6. Potassium-depolarized cells failed to respond electrically to NH_4Cl-induced pH_i changes. These experiments implicate a role for K^+ conductance in the pH_i-related electrical events.

Rubidium-86 efflux studies

In order to directly assess the effect of intracellular alkalinization on potassium permeability, we studied $^{86}Rb^+$ efflux from preloaded hepatocytes,

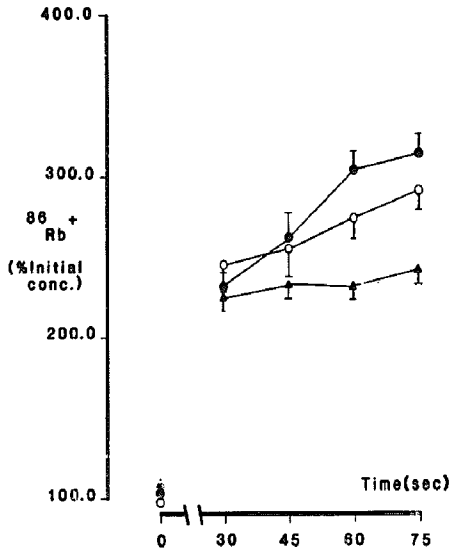

Fig. 7. Rubidium-86 ($^{86}Rb^+$) efflux. $^{86}Rb^+$ efflux from preloaded cells into isotope-free buffer was measured as the radioactivity in the cell supernatant of samples taken at 30, 45, 60 and 75 s after the stimulation of $^{86}Rb^+$ efflux. The radioactivity measured has been expressed as a percentage of the initial measurement taken immediately (0 s) after efflux initiation. $^{86}Rb^+$ efflux was determined in three separate experimental conditions: control (○), those causing cellular alkalinization (●) or intracellular acidification (▲), using five different cell preparations.

following exposure to isotope-free buffer containing NH_4Cl (10 mM). To determine the effect of cellular acidification, cells were preloaded with $^{86}Rb^+$ and exposed to NH_4Cl prior to initiation of $^{86}Rb^+$ efflux into NH_4Cl-free buffer. As a control, $^{86}Rb^+$ efflux was measured in cells that had not been exposed to NH_4Cl in either the preincubation or incubation period. All three protocols were performed simultaneously using hepatocytes from a single cell suspension. As shown in Fig. 7, the radioactivity in cell supernatants obtained at 60 and 75 s after stimulation of $^{86}Rb^+$ efflux was significantly greater in hepatocytes whose intracellular pH is alkaline compared to control cells, ($P < 0.05$). In contrast, $^{86}Rb^+$ efflux is significantly less in hepatocytes following intracellular acidification at 60 and at 75 s ($P < 0.01$). This time interval was chosen as $^{86}Rb^+$ efflux from preloaded cells has been shown to be linear for approx. 75 s (Bear, C., unpublished data). Calculation of the linear regression coefficient for each set of experimental data was performed. In control cells, the efflux curve had a linear correlation coefficient (r) of 0.86 ($P < 0.01$) with a slope of 2.19. The efflux curve for alkalinized cells had a (r) value of 0.97 ($P < 0.01$) with a slope of 2.97. In acidified cells the (r) value of the curve was 0.86 ($P < 0.01$) with the slope 1.73. The slope for $^{86}Rb^+$ efflux from the alkalinized cells was significantly lower ($P < 0.05$) whereas that from the acidified cells were greater ($P < 0.02$) than the controls. These values confirm that the efflux curves are linear. The changes in slope with changes in intracellular pH are consistent with the suggestion that cellular alkalinization stimulates K^+ conductance, or the rate of efflux, whereas acidification inhibits K^+ conductance.

Discussion

This study reveals that intracellular pH influences the membrane potential of isolated rat hepatocytes. Cellular alkalinization causes membrane hyperpolarization whereas acidification produces depolarization. Evidence is presented to suggest that pH_i exerts its effect on membrane potential by directly or indirectly modifying K^+ conductance. The membrane resistance measurements attained are consistent with the hypothesis that intracellular alkalinization enhances K^+ conductance while acidification blocks K^+ conductance. Furthermore, exposure of hepatocytes to a high extracellular concentration of KCl (140 mM), which dissipates the transmembrane K^+ gradient, inhibited pH_i-induced potential changes. Similarly, the K^+ channel blockers, quinine and barium abolished the effect of intracellular acidification on membrane potential.

Changes in the pH of the extracellular, bathing medium from 7.6 to 6.5 did not result in any immediate change in membrane potential. This observation is suggestive of a low membrane permeability to hydrogen (H^+ or OH^-). Therefore the immediate electrogenic effects induced by intracellular alkalinization or acidification do not reflect changes in extracellular pH induced experimentally. Prolonged exposure, however, had a different effect: acidic solutions (pH 6.5) caused

depolarization of hepatocytes compared to cells incubated in media at pH 7.2 or 7.6. Conceivably the cell becomes less capable of pH_i regulation with prolonged exposure to acidic media and the membrane depolarized because of eventual intracellular acidification. Ekhardt et al. [21] have reported similar results for the effect of extracellular pH on membrane potential in isolated hepatocytes.

Ammonium chloride has been used extensively in studies of intracellular pH regulation [3,8,16,17]. Exposure of cells to ammonium chloride led to intracellular alkalinization associated with cell hyperpolarization and increased membrane conductance. We have interpreted these findings as indicating that alkalinization may enhance K^+ conductance. Alternatively NH_4^+ has been found to substitute for K^+ in the Na^+/K^+-ATPase transporter so that intracellular accumulation of NH_4^+ may activate the pump transporting NH_4^+ out of the cell [16]. Such pump activation would lead to cellular hyperpolarization, as was observed in our studies but this could not account for the observed decrease in membrane conductance. Therefore, enhanced K^+ conductance rather than pump activation is the most likely explanation for the electrical effects observed with NH_4Cl addition.

Intracellular acidification was achieved through two methods: (1) NH_4^+-preloaded cells washed with the 'control', NH_4Cl-free superfusion solution and (2) cells exposed to the weak acid; proprionate. Both of these manipulations resulted in a marked depolarization with increased membrane resistance. In contrast to the effect of intracellular alkalinization, we propose that acidification inhibits K^+ conductance. Perhaps, the transport mechanism affected by changes in pH_i is sensitive to the intracellular accumulation of H^+. Binding of H^+ to a modifier site on the Na^+/H^+ antiport [22], regulates the activity of this membrane protein so it is conceivable that such a mechanism also exists for the regulation of the K^+ channel protein. Concerning the proprionate results, this weak acid has not only been shown to cause intracellular acidification but is also known to block Cl^- channels in some tissues. It is unlikely that proprionate caused the observed potential and resistance changes through an effect on Cl^- conductance as chloride channel blockade in may cell types evokes membrane hyperpolarization, not depolarization [23].

Quinine and barium abolishes pH_i-evoked changes in membrane potential, implicating a role for K^+ conductance in these events. The addition of either of these K^+ channel blockers decreases the membrane potential of impaled cells to approx. -10 mV, a value close to equilibrium potential for chloride ion. These findings indicate that most of the K^+ channels participating in the electrodiffusional control of the resting membrane potential can be inhibited by quinine (200 μM) or by barium (2 mM). Similarly, superfusion of cells with the high K^+ solution led to dissipation of the transmembrane K^+ gradient resulting in cell depolarization. No change in potential was evoked by intracellular alkalinization or acidification in K^+ depolarized cells, providing additional support for the proposed role of K^+ conductance. Furthermore, $^{86}Rb^+$ efflux from preloaded hepatocytes into isotope-free media was enhanced in cells with intracellular alkalinization but inhibited in cells with intracellular acidification. The rubidium efflux studies provide more direct evidence for involvement of pH_i in the regulation of K^+ conductance.

Recent experiments using patch-clamp electrophysiology have revealed the presence of a K^+-selective channel in the membrane of isolated rat hepatocytes which is activated by increases in ionized Ca^{2+} from 10^{-9} M to 10^{-7} M at the cytoplasmic face of the membrane [24]. Using patch-clamp techniques, Cook et al. [7] determined that Ca^{2+}-dependent K^+ channel activity in pancreatic B cells was enhanced when the pH of the solution bathing the cytoplasmic membrane surface was increased from 7.2 to 7.6 but inhibited when the pH was decreased from 7.2 to 6.5. Cook suggested that the intracellular accumulation of protons may block K^+ channel activity by competing with Ca^{2+} for regulatory sites on the channel itself. Perhaps a similar mechanism accounts for the effects of pH_i manipulation on K^+ conductance in hepatocytes in the present work.

A physiological role for pH_i-induced changes in K^+ conductance in the liver cell is unknown at present. Several hormones and neurotransmitters act to enhance K^+ conductance in hepatocytes

[25]. On the basis of the results in the present study, the responsiveness of liver cells to neurohumoral stimulation may depend in part upon the intracellular pH which can vary as a function of the cells' metabolic status.

Acknowledgement

This study was supported by the Medical Research Council of Canada (grant MT5080) and the Alberta Heritage Foundation for Medical Research.

The authors wish to acknowledge the excellent secretarial services of C. Gail Eden, and the valuable technical assistance in the preparation of isolated rat hepatocytes provided by Wanda Williams. We also appreciate the assistance of Dr. M.J. Phillips and Dr. A. Rothstein at the Hospital for Sick Children, Toronto, Canada in the performance of the intracellular pH studies.

References

1 Grinstein, S., Cohen, S. and Rothstein, A. (1984) J. Gen. Physiol. 83, 341–369.
2 Rothenburg, T., Glaser, L., Schlesinger, P. and Cassel, D. (1983) J. Biol. Chem. 258, 12644–12653.
3 Henderson, R.M., Graf, J. and Boyer, J.L. (1987) Am. J. Physiol. 252, G109–G113.
4 Wingo, C.S. (1985) Kidney Int. 27, 886–891.
5 Reuss, L. (1981) in Ion Transport by Epithelia (Schultz, S.G.), pp. 109–129, Raven Press, New York.
6 Rosario, L.M. and Rojas, E. (1986) Am. J. Physiol. 250, C90–C94.
7 Cook, D.L., Ikeuchi, M. and Fujimoto, W.Y. (1984) Nature 311, 269–271.
8 Boron, W.F. (1977) Am. J. Physiol. 233, C61–C73.
9 Berry, M.N. and Friend, D.S. (1969) J. Cell. Biol. 43, 506–520.
10 Grinstein, S. and Goetz, J.D. (1985) Biochim. Biophys. Acta 819, 267–270.
11 Grinstein, S. and Furuya, W. (1986) Am. J. Physiol. 251, C55–C65.
12 Thomas, J.A., Buchsbaum, R.N., Zimniak, A. and Racker, E. (1979) Biochemistry 18, 2210–2218.
13 Bear, C.E., Davison, J.S. and Shaffer, E.A. (1987) Biochim. Biophys. Acta 903, 388–394.
14 Graf, J., Gautam, A. and Boyer, J.L. (1984) Proc. Natl. Acad. Sci. USA 81, 105–126.
15 Graf, J., Henderson, R.M., Krumpholz, B. and Boyer, J.L. (1987) J. Membr. Biol. 95, 241–254.
16 Thomas, R.C. (1984) J. Physiol. 354, 3p–22p.
17 Vigne, P., Frelen, C. and Lazdunski, M. (1982) J. Biol. Chem. 257, 9394–9400.
18 Welsh, M.J. (1985) J. Membr. Biol. 88, 149–163.
19 Griff, E.R., Shirao, Y. and Steinberg, R.H. (1985) J. Gen. Physiol. 86, 853–876.
20 Iwatsuki, N. and Petersen, O.H. (1985) Biochim. Biophys. Acta 819, 249–257.
21 Ekhardt, V. (1984) Biochim. Biophys. Acta 775, 182–188.
22 Aronson, P.S. (1984) Soc. Gen. Physiol. Ser. 38, 49–70.
23 Bretag, A.H. (1987) Physiol. Rev. 67, 618–724.
24 Bear, C.E. and Petersen, O.H. (1987) Pflügers Arch. 410, 342–344.
25 Friedmann, N. and Park, C.R. (1968) Proc. Natl. Acad. Sci. USA 61, 504–508.