Modulation of K^+ conductance by intracellular pH in pancreatic β-cells

Luis M. Rosario and Eduardo Rojas

Laboratory of Cell Biology and Genetics, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Building 4, Room 312, Bethesda, MD 20205, USA

Received 17 March 1986

Measurements of the effects of NH_3/NH_4^+ on glucose-induced electrical activity in β-cells from microdissected mouse islets of Langerhans and on intracellular pH in single collagenase-isolated islets pre-loaded with a fluorescent pH probe were performed and are reported here. Application of NH_3/NH_4^+ (15 mM) in the presence of glucose (11 mM) promptly hyperpolarized the β-cell membrane, reduced input resistance by 60% and blocked electrical activity. These changes were paralleled by an increase in islet fluorescence indicative of a cytosolic pH increase. Removal of NH_4Cl initially stumulated electical activity, which returned to resting level with a time constant of 51 s. Concomitant with the removal of NH_4Cl there was a drop in pH_i followed by a slow return to resting level with a time constant of 83 s. The results suggest that the $[Ca^{2+}]$-dependent K^+ channel in the β-cell membrane is activated by a rise in cytosolic pH.

Sulfofluorescein diacetate intracellular pH K^+ conductance (Pancreatic β-cell)

1. INTRODUCTION

It is becoming increasingly clear that at least two types of K^+ channels are present in the pancreatic β-cell membrane. One type (about 250 pS single channel conductance) is activated by an increase in $[Ca^{2+}]_i$, voltage-sensitive [1,2] and inhibited by lowering pH on the cytoplasmic side of the plasma membrane [1]. The other type (about 50 pS) is blocked by an increase in $[ATP]_i$, voltage-, pH_i- and $[Ca^{2+}]_i$-insensitive [3], and blocked by glucose metabolism [4,5]. The relative contributions of these two types of K^+ channels to the resting membrane potential are still unclear [1–5].

The slow oscillations in potential and the burst pattern [6] of glucose-evoked electrical activity in pancreatic β-cells have been modelled [7] assuming a feedback regulatory cycle involving $[Ca^{2+}]_i$ and activation/deactivation of the $[Ca^{2+}]$-dependent K^+ channel. The excellent agreement between the theoretical prediction and the experimental results [8] strongly supports the view that the $[Ca^{2+}]$-dependent K^+ channel plays a key role in the regulation of glucose-induced electrical activity in mouse pancreatic β-cells.

Earlier studies of the effects of weak acids and bases on glucose-induced electrical activity as well as of anions thought to be involved in cytosolic pH (pH_i) regulation suggested that pH_i might modulate K^+ conductance [9,10]. This idea has gained further support from the observation that the $[Ca^{2+}]$-dependent K^+ channel is blocked by lowering the pH on the cytoplasmic side of excised patches of β-cell membrane from neonatal rat islets [1]. We report here the results of experiments in which pH_i was perturbed by application of the permeant base NH_3 and its weak conjugate acid NH_4^+. Changes in pH_i in islet cells were monitored by measuring pH-related fluorescence of sulfofluorescein. Parallel measurements of membrane potential and β-cell input resistance were used to evaluate the effects of NH_3/NH_4^+ on K^+ conductance. Taken together, the data suggest that under physiological conditions the $[Ca^{2+}]$-dependent K^+ channel can be activated by cytosolic alkalinization.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

2. MATERIALS AND METHODS

The modified Krebs solution used had the following composition (mM): 120 NaCl, 25 $NaHCO_3$, 5 KCl, 2.6 $CaCl_2$, 1.1 $MgCl_2$. The solution was pre-warmed in a water bath and continuously equilibrated with CO_2/O_2 (5%:95%) to give a pH of 7.4 at 37°C.

The membrane potential was recorded using high-resistance microelectrodes as described [11].

The fluorescence of islets preloaded with 5(6)-sulfofluorescein diacetate (SFA_2) was measured using a fluorescence microscope (Leitz Diavert) provided with a xenon light source (75 W) and an epifluorescence attachment. Islets were isolated using a standard collagenase technique [12]. After 60 min incubation in modified Krebs medium containing 22 mM glucose, the islets were loaded with SFA_2 (100–200 μM) during a further 60 min incubation in Krebs medium containing 16.7 mM glucose. A few islets were then transferred to a small perfusion chamber provided with a thin glass bottom and a single islet was selected for fluorescence recording. The volume of the chamber was 110 μl and that of the chamber used in the electrophysiological experiments was 70 μl. The transit time for the solution to flow from the stopcock to the chamber was less than 2 s in both cases. A glass micropipette was used to hold the islet in place within the light path. The pipette was filled with Krebs solution and could be positioned using a micromanipulator. The following optical filters were used (Leitz): BP 530-560 (excitation), LP 580 (suppression) and RKP 580 (beam-splitting mirror). To minimize photobleaching, 1 s pulses of the exciting light were used. An electronically controlled shutter provided the desired pulse sequence. The photomultiplifer output was fed into a 4-channel analogue tape recorder (Racal Thermoionic) and a fast chart recorder (Gould 2400S). The excitation spectra of the pH_i probe in vitro were recorded using a Spex spectrofluorometer (Fluorolog 2) provided with a 560 nm cut-off long-pass filter in the emission path to reduce scattered light and equipped with a DM1B processor unit.

SFA_2 was obtained from Molecular Probes (Junction City, USA). The hydrolysis of SFA_2 was carried out at room temperature in water containing 0.1 M NaOH. NH_4Cl and sodium succinate were added as small aliquots of concentrated stock solutions (pH 7.4) and had no measurable effects on the pH of the Krebs solution. All other chemicals were from Sigma (St. Louis, MO).

3. RESULTS

Fig.1A shows the pH dependency of the excitation spectrum of the end-product of in vitro hydrolysis of SFA_2 (SF). At the concentration used to obtain these spectra several pH-sensitive bands with one isosbestic point at 454 nm can be resolved. The most pH-sensitive spectral region

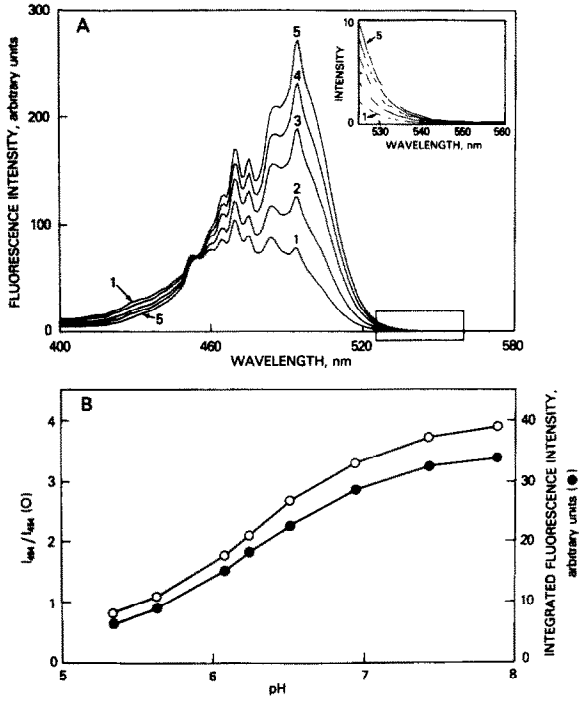

Fig.1. pH dependency of 5(6)-sulfofluorescein fluorescence. (A) The excitation spectra of the end-product of in vitro hydrolysis of 5(6)-sulfofluorescein diacetate (2 μM) were measured in a lightly buffered solution containing (mM): 145 NaCl, 5 KCl and 1.5 Hepes. The emission wavelength was set at 590 nm. The pH of the solution was varied by adding 1 M 2-(*N*-morpholino)ethanesulfonic acid (Mes), pH 2.0. From 1 to 5, the pH of the solution was 5.63, 6.07, 6.53, 6.96 and 7.88, respectively. Inset: tail of the excitation spectra (see box) in the range used for the single islet fluorescence measurements on expanded *X*-*Y* axis. (B) (○) Ratio between the fluorescence intensity at 494 nm (I_{494}) and that at the isosbestic point (I_{454}) as a function of pH. (●) Integrated light intensity in the range 525–560 nm as a function of pH (see inset in A).

occurred above 480 nm with an excitation maximum near 494 nm. The fluorescence ratio I_{494}/I_{454} is shown to increase with pH in a sigmoidal-like fashion (fig.1B, empty circles). The integrated intensity (area underneath the curves) in the range covered by the excitation filter used for the single islet fluorescence measurements, i.e. 525–560 nm (see inset in fig.1A), displayed a similar pH dependency (fig.1B, filled circles). At a lower SF concentration (0.2 μM) the resolution from the excitation spectra was partially lost below 480 nm, but either the fluorescence ratio I_{494}/I_{454} or the integrated fluorescence intensity in the range 525–560 nm bore an identical relationship with pH (not shown).

To minimize photobleaching, which is probably the cause of the slow decline in fluorescence observed in the experiments with islets of Langerhans, SF was excited in a low absorption region (see fig.1A). The effects of NH_4Cl (15 mM) on the SF fluorescence recorded from single SFA_2-loaded islets, in the presence of 11 mM glucose, are shown in fig.2. Points represent 1 s readings of the fluorescence. After 23 s exposure to NH_4Cl there is a substantial increase in SF fluorescence. The maximum increase in fluorescence averaged 5.4%. The rate of decay of SF fluorescence in the presence of NH_4Cl was slightly higher than that measured in its absence. Removal of NH_4Cl elicited a rapid drop in SF fluorescence (average value 15.0%), which reached a minimum in less than 60 s. After this minimum the SF fluorescence returned slowly to the baseline (straight line). Semi-logarithmic plots of the difference between SF fluorescence values after removal of NH_4Cl minus basal values (not shown) gave reasonably straight lines with time constants in the range 53–140 s (mean 83 s).

The effects of NH_4Cl (15 mM) on glucose-induced electrical activity are illustrated in fig.3. The initial part of the record shows the oscillatory burst pattern of electrical activity [11] consisting of a silent phase at about −45 mV and an active phase at about −27 mV. Addition of NH_4Cl induced a hyperpolarization of the membrane to a level 12 mV more negative than the silent-phase potential (~−57 mV), and the electrical activity ceased. The initial increase in resting potential (−57 mV at 50 s) was followed by a slow decrease in the membrane potential reaching ~−45 mV by the end of exposure to NH_4Cl. Removal of NH_4Cl

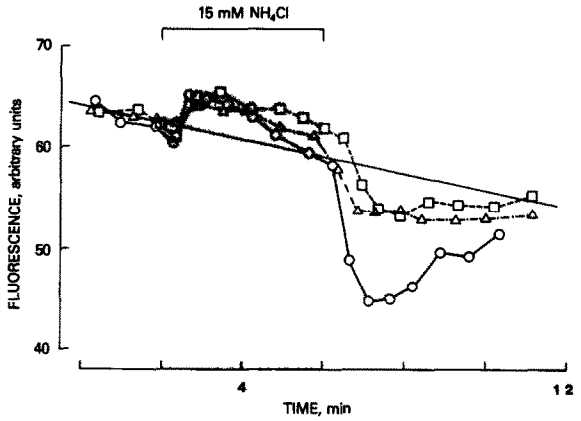

Fig.2. Effects of NH_4Cl on the fluorescence recorded from single islets loaded with 5(6)-sulfofluorescein diacetate. Each symbol represents one experiment on a single islet pre-loaded with 5(6)-sulfofluorescein diacetate (see section 2). The medium contained 11 mM glucose in all cases. The temperature in the chamber was 37°C. NH_4Cl (15 mM) was applied as indicated by the shadowed area. The following factors were used to normalize the fluorescence data: (○) 1.00, (□, △) 0.80. The straight line was fitted by a least-squares fitting procedure to the data points taken before NH_4Cl addition.

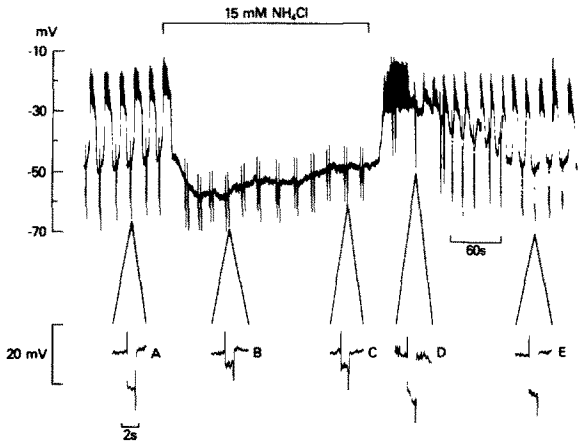

Fig.3. Effects of NH_4Cl on the input resistance to the β-cell membrane. Islet exposed to NH_4Cl as indicated. Shown beneath are the membrane potential responses to the current pulses (0.1 nA) on an expanded time base (× 30). The upward and downward deflections seen at the beginning and end of each current pulse, respectively, represent capacitive transients. Concentration of glucose in the solution was 11 mM.

was followed by membrane depolarization accompanied by a long-lasting burst of electrical activity. The average duration of this burst, measured from the onset of the depolarization to the time of appearance of the second burst, was 47.7 ± 23.4 s (± SD, $n = 20$). In this experiment, recovery of the control burst pattern was achieved 3 min after removal of NH_4Cl. The effects of NH_4Cl on input resistance were evaluated from the size of the membrane responses to hyperpolarizing pulses of current (0.1 nA). Prior to NH_4Cl exposure the size of the voltage deflections (see expanded record A in fig.3) averaged 11.2 ± 0.5 mV, corresponding to an average input resistance of 111.7 ± 5.2 MΩ ($n = 6$). The size of the voltage deflections (see record B) in the presence of NH_4Cl after 45 and 97 s NH_4Cl exposure was 3.8 ± 1.3 mV ($n = 7$) and the input resistance 38.0 ± 13.0 MΩ, a 66% decrease ($p < 0.001$). The input resistance calculated for the last 6 current pulses was 40.8 ± 6.6 MΩ, not significantly different from the input resistance calculated for the initial period of NH_4Cl exposure ($p > 0.5$; cf records B,C). Upon return to NH_4Cl-free solution, the input resistance increased rapidly (see record D) towards an average value of 162.0 ± 18.2 MΩ ($n = 5$) 55–110 s after NH_4Cl withdrawal, a value 45% higher than the initial control value ($p < 0.001$). Thereafter, input resistance decreased with time (see record E) towards an average value of 120.0 ± 4.1 MΩ for the last 4 current pulses shown in fig.3. Input resistance in the same β-cell decreased from 108.8 ± 8.8 MΩ ($n = 8$), measured in presence of glucose, to 82.1 ± 6.4 MΩ ($n = 7$) measured in its absence, a 24.5% change ($p < 0.001$).

The fraction of time in the active phase (Δt_a) can be compared with the changes in single islet fluorescence in fig.4. Each column represents the average value of 10 values of Δt_a from different islets. It may be seen that electrical activity ceased in less than 30 s following NH_4Cl addition to the medium. In some experiments, electrical activity actually resumed before NH_4Cl had been withdrawn from the medium. As shown in fig.3, electrical activity was strongly stimulated by NH_4Cl removal, Δt_a reaching a maximum in less than 60 s. Δt_a then decayed to near-basal levels, a relaxation process which could be described by a single exponential (time constant 51 s). A clear undershoot in Δt_a is also apparent in fig.4, and the

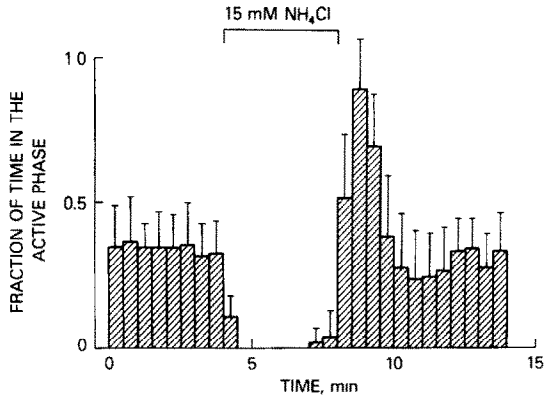

Fig.4. Effects of NH_4Cl on the fraction of time in the active phase. Each column represents the average of 10 experiments calculated for a 30 s sampling period from membrane potential records similar to that in fig.3. Vertical bars represent the SD ($n = 10$). Concentration of glucose in the solution was 11 mM.

resting level (~0.35) was reached 5–6 min after removal of NH_4Cl.

Since it is known that the mitochondrial enzyme glutamate dehydrogenase (GDH) is allosterically inhibited by the end-products of the deamination of glutamate, namely ammonia [13,14], NH_4Cl may interfere with β-cell mitochondrial function. Islets were pre-incubated with succinate, a substrate of the tricarboxylic acid cycle. This substrate enters the tricarboxylic acid cycle two steps after α-ketoglutarate, which is partially supplied by oxidative deamination of glutamate in a reaction catalyzed by GDH. Exposure to sodium succinate (20 mM) for 10 min resulted in overall stimulation of electrical activity. Note in fig.5 that the silent-phase potential is less negative in the presence of sodium succinate than in its absence (beginning of middle record). Exposure to NH_4Cl in the presence of sodium succinate induced hyperpolarization of the membrane (fig.5, middle). A second control application of NH_4Cl, 11 min after removal of sodium succinate from the medium (fig.5, lower) gave a membrane potential response similar to that recorded at the beginning of the experiment. A higher concentration of sodium succinate (40 mM) also failed to impair the hyperpolarizing effect of NH_4Cl (not shown).

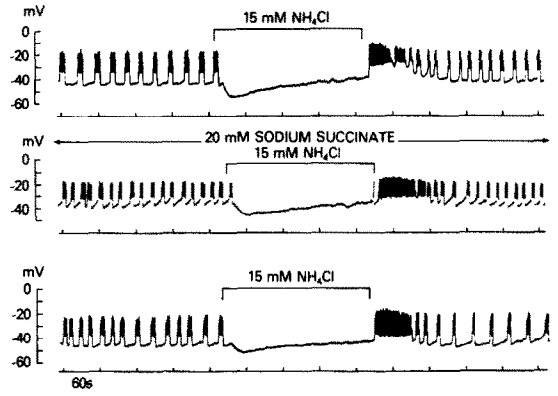

Fig.5. Effects of NH_4Cl on glucose-induced electrical activity in the presence of exogenous succinate. Same cell throughout in 11 mM glucose. The islet was exposed to NH_4Cl (15 mM) as indicated. 5 min of the record were omitted between the upper and middle parts. Sodium succinate (20 mM) was added 10 min before the second exposure to NH_4Cl (middle). Sodium succinate was removed 11 min before the third exposure to NH_4Cl (lower). The time calibration is denoted by the segments on the horizontal axis (60 s).

4. DISCUSSION

The highly fluorescent polyanion resulting from intracellular hydrolysis of the pH probe 5(6)-carboxyfluorescein diacetate (CF) has been reported to remain confined mostly to the cytosol [15,16]. Although the possible compartmentalization of the end-product of intracellular hydrolysis of its analogue SFA_2 (SF), a pH_i probe used here for the first time, has not been examined in any detail, the lower pK_a of sulfonic acid compared to carboxylic acid makes it even less likely that SF will be incorporated into a cytoplasmic alkaline compartment (e.g. mitochondria). In a solution of NH_4Cl at pH 7.4 about 0.5% is in the form of the weak base NH_3. Since NH_3 is permeant across the plasma membrane, NH_3 is protonized giving rise to alkalinization of the cytosol. However, NH_4^+ also crosses the plasmalemma, presumably by active transport [17] and/or by permeation of K^+ channels [18,19]. This additional influx results in a further build up of $[NH_4^+]$ in the cells. For this reason prolonged exposure to NH_4Cl will eventually cause a drop in pH_i [20]. Removal of NH_4Cl from the medium leads to a fall in pH_i below the resting levels. This technique has often been used to load cells with protons (see [20]). Therefore, the multiphasic perturbation of cell pH brought about by addition as well as removal of NH_4Cl is likely to represent pH changes occurring in the cytosol of the islet cells. As the proportion of β-cells in normal mouse islets is close to 80% (see [21]), the fluorescence measurements carried out in this work represent the pH_i changes of the insulin-secreting cells.

The results showed that NH_4Cl hyperpolarized the β-cell membrane and suppressed glucose-induced electrical activity while the β-cell cytosol remained alkaline. Conversely, the transient cytosolic acidification observed after NH_4Cl removal was accompanied by a transient depolarization and stimulation of electrical activity. Moreover, the time course of the pH_i changes was similar to that of the membrane potential changes. In this respect, it is interesting to compare the time courses of the pH_i and Δt_a following an acute acid load (NH_4Cl removal). The apparent time constant for the former process (83 s) was slightly higher than the time constant for the change in Δt_a (51 s). It is conceivable that the stimulated Ca^{2+} influx due to spike activity brought about by removal of NH_4Cl might increase $[Ca^{2+}]_i$. This elevated $[Ca^{2+}]_i$, in turn, would activate $[Ca^{2+}]$-dependent K^+ conductance known to exist in pancreatic β-cells [1,2]. Activation of K^+ conductance would induce a transition of the β-cell membrane towards more negative potentials and would cause the end of the burst. This tentative explanation may also account for the undershoot of Δt_a that preceded the recovery of electrical activity towards baseline (see fig.4) – a phenomenon that resembles the reduction of glucose-induced electrical activity induced by short exposures to a high-K^+ solution [22].

In the presence of glucose, application of NH_4Cl hyperpolarized the β-cell membrane. This hyperpolarization can be impaired [23] by quinine [24] and glibenclamide [25]. Although the specificity of these drugs is not known, it is likely that the NH_4Cl-induced hyperpolarization reported here results from the action, either direct or indirect, of pH_i on K^+ permeability. This conclusion is reinforced by the observation that cytosolic alkalinization was paralleled by a significant decrease in the input resistance to the β-cell membrane. However, cell-to-cell electrical coupling has been

demonstrated in mouse islets of Langerhans [26] and junctional conductance has been shown to be modulated by pH_i in other tissues [27]. Thus, the possibility that other factors, apart from K^+ permeability changes, may also play a role in the NH_4Cl-induced decrease of the input resistance cannot be ruled out.

As to the possible role of the $[Ca^{2+}]$-dependent K^+ channel, the range of the NH_4Cl-induced pH_i changes has to be considered. Taking 7.30 as an upper limit for pH_i in the presence of 11 mM glucose [28–30] and assuming that the pH_i increase induced by NH_4Cl amounts to 0.19 units [31] and, furthermore, that the pH dependency of SF fluorescence in vitro is similar to that measured in the islet cells as reported for CF in other cell types [15], the results suggest that the maximum pH_i reached during the exposure to NH_4Cl was 7.49 and that the minimum pH_i reached after NH_4Cl removal was about 6.9. Hence, NH_4Cl would perturb the β-cell pH_i in the range 6.9–7.5, a range where pH affects the activity of the $[Ca^{2+}]$-dependent K^+ channel [1], but not that of the ATP-blockable K^+ channel [3]. Thus, the results suggest that cytosolic alkalinization activates the $[Ca^{2+}]$-dependent K^+ channel operating under physiological conditions. The pH sensitivity of the $[Ca^{2+}]$-dependent K^+ channel is not unique to the pancreatic β-cell. Indeed, the red cell $[Ca^{2+}]$-dependent K^+ channel as well as other K^+ channels in different cell types have also been reported to exhibit pH dependency [32,33]. The idea that the $[Ca^{2+}]$-dependent K^+ channel is pH-sensitive is reinforced by the observation that, in the presence of 11 mM glucose, the ATP-blockable K^+ channel is already blocked [34].

It is possible that ammonia interferes with the tricarboxylic acid mitochondrial cycle. This action may result in a reduction of cytosolic ATP levels and hence in the activation of the ATP-blockable K^+ channel. Although glycolysis represents the major glucose-metabolism pathway in islet cells, the oxidation rate of both exogenous and endogenous nutrients and the related ATP generation seem to match the rate of ATP utilization (see [35]), suggesting that phosphorylative oxidation may be the major metabolic pathway for overall ATP generation. Although little is known in islet cells on the properties of the GDH catalyzed oxidative deamination of glutamate to α-ketoglutarate, this reaction could be regarded as the best a priori candidate to account for the possible inhibitory effects of ammonia on the tricarboxylic acid cycle, which would lead to inhibition of the transfer of reducing equivalents to the respiratory chain and, ultimately, to a drop in cytosolic ATP. However, our control experiments with succinate, a mitochondrial substrate that permeates the β-cell plasma membrane [36], strongly suggest that this is not the case. The exposure to succinate could support 3 out of 4 tricarboxylic acid cycle reactions involved in the generation of reducing equivalents. The results showed that pre-incubation of the islets with sodium succinate failed to impair the hyperpolarizing effect of NH_4Cl.

In conclusion, we propose that cytosolic pH modulates the β-cell $[Ca^{2+}]$-dependent K^+ channel. The consequences of this finding for the physiological responses during glucose-induced electrical activity still remain to be assessed.

ACKNOWLEDGEMENTS

The authors thank Drs I. Atwater, I. Cabanchick and H. Pollard for suggestions and discussion. Thanks are also due to A. Markowitz (Biomedical Engineering Instrumentation Branch, NIH) for electronic design and to Dr E. Forsberg for revision of the manuscript.

REFERENCES

[1] Cook, D.L., Ikeuchi, M. and Fujimoto, W.Y. (1984) Nature 311, 269–271.
[2] Findlay, I., Dunne, M.J. and Petersen, O.H. (1985) J. Membrane Biol. 83, 169–175.
[3] Cook, D.L. and Hales, N. (1984) Nature 311, 271–273.
[4] Ashcroft, F.M., Harrison, D.E. and Ashcroft, S.J.H. (1984) Nature 312, 446–448.
[5] Rorsman, P. and Trube, G. (1985) Pflügers Arch. 405, 305–309.
[6] Atwater, I., Dawson, C.M., Scott, A., Eddlestone, G. and Rojas, E. (1980) Horm. Metab. Res. (suppl.) 10, 100–107.
[7] Chay, T.R. and Keizer, J. (1983) Biophys. J. 42, 181–190.
[8] Perez-Armendariz, E., Atwater, I. and Rojas, E. (1985) Biophys. J. 48, 741–749.
[9] Tarvin, J.T., Sachs, G. and Pace, C.S. (1981) Am. J. Physiol. 241, C264–C268.

[10] Eddlestone, G.T. and Beigelman, P.M. (1983) Am. J. Physiol. 244, C188–C197.
[11] Atwater, I., Ribalet, B. and Rojas, E. (1978) J. Physiol. 278, 117–139.
[12] Lacy, P.E. and Kostianovsky, M. (1967) Diabetes 16, 35–39.
[13] Brown, A., Colen, A.H. and Fisher, H.F. (1979) Biochemistry 18, 5925–5928.
[14] LaNoue, K.F., Schoolwerth, A.C. and Peage, A.J. (1983) J. Biol. Chem. 258, 1726–1734.
[15] Thomas, J.A., Buchsbaum, R.N., Zimniak, A. and Racker, E. (1979) Biochemistry 18, 2210–2216.
[16] Grinstein, S. and Furuya, W. (1984) Biochim. Biophys. Acta 803, 221–228.
[17] Aikin, C.C. and Thomas, R.C. (1977) J. Physiol. 267, 791–810.
[18] Edwards, C. (1982) Neuroscience 7, 1335–1366.
[19] Rosario, L.M. and Rojas, E. (1986) Am. J. Physiol. 250, C90–C94.
[20] Boron, W.F. (1983) J. Membrane Biol. 72, 1–16.
[21] Hedeskov, C.J. (1980) Physiol. Rev. 60, 442–509.
[22] Dawson, C.M., Atwater, I. and Rojas, E. (1984) Q. J. Exp. Physiol. 69, 819–830.
[23] Rosario, L.M., Atwater, I. and Rojas, E. (1986) Biophys. J. 49, 162a.
[24] Atwater, I., Dawson, C.M., Ribalet, B. and Rojas, E. (1979) J. Physiol. 288, 575–588.
[25] Ferrer, R., Atwater, I., Omer, E.M., Gonçalves, A.A., Croghan, P.C. and Rojas, E. (1984) Q. J. Exp. Physiol. 69, 831–839.
[26] Eddlestone, G.T., Gonçalves, A., Bangham, J.A. and Rojas, E. (1984) J. Membrane Biol. 77, 1–14.
[27] Spray, D.C. and Bennett, M.V.L. (1985) Annu. Rev. Physiol. 47, 281–303.
[28] Lindstrom, P. and Sehlin, J. (1984) Biochem. J. 218, 887–892.
[29] Deleers, M., Lebrun, P. and Malaisse, W.J. (1983) FEBS Lett. 154, 97–100.
[30] Hellman, B., Sehlin, J. and Täljedal, I.-B. (1972) Endocrinology 90, 335–337.
[31] Reber, W.R. and Weingart, R. (1982) J. Physiol. 328, 87–104.
[32] Stampe, P. and Vestergaard-Bogind, B. (1985) Biochim. Biophys. Acta 815, 313–321.
[33] Moody, W. jr (1984) Annu. Rev. Neurosci. 7, 257–278.
[34] Ashcroft, F.M., Harrison, D.E. and Ashcroft, S.J.H. (1986) in: Biophysics of the Pancreatic B-cell (Rojas, E. and Atwater, I. eds) Plenum, New York, in press.
[35] Sener, A. and Malaisse, W.J. (1984) Experientia 40, 1026–1035.
[36] Hellman, B., Sehlin, J. and Täljedal, I.-B. (1971) Biochem. J. 123, 513–521.