Volume 332, number 1,2, 9–13 FEBS 13054 October 1993

Differential modulation of pancreatic β-cell bursting by intracellular pH in the presence and absence of a K-ATP channel blocker

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Received 11 August 1993

The study of the influence of intracellular pH (pH_i) changes on the mechanism underlying pancreatic β-cell bursting has been hampered by concomitant effects on the activity of background ATP-dependent K^+ (K-ATP) channels. β-cells were made to burst in the absence of active K-ATP channels by raising external Ca^{2+} in the presence of 11 mM glucose and tolbutamide. An alkalinizing pH_i shift (exposure to 20 mM NH_4Cl) increased the burst active phase duration. Conversely, an acidifying shift (NH_4Cl withdrawal) suppressed the electrical activity. This is the mirror image of the effects recorded in the absence of tolbutamide. Glibenclamide and quinine suppressed the alkalinization-evoked hyperpolarization. This study emphasizes the differential sensitivity of different β-cell ion channels to pH_i and the prevalent role of K-ATP channels as electrical transducers of cytoplasmic pH changes under regular physiological conditions.

Pancreatic β-cell; Islet of Langerhans; Intracellular pH; Bursting electrical activity; ATP-dependent K^+ channel; Extracellular Ca^{2+}

1. INTRODUCTION

The main function of the pancreatic β-cell is to provide an effective insulin output in response to a rise in blood glucose concentration. When stimulated with intermediate-high glucose concentrations (7–17 mM) the β-cell displays a bursting pattern of electrical activity consisting of alternating depolarized (active) and hyperpolarized (silent) phases [1]. This bursting pattern appears to underlie both the oscillations of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and the high frequency oscillatory insulin output that have been measured from single whole islets of Langerhans [2–4].

Glucose metabolism is known to close K-ATP channels, thereby depolarizing the cells and activating the voltage-dependent Ca^{2+} and K^+ channels underlying the electrical activity [5]. We have previously provided evidence against a fundamental role for the K-ATP channels in the generation of the burst [6,7]. However, knowledge of the modulation of the mechanism underlying the burst by some drugs and intracellular factors implicated in the physiological response to glucose has been hampered by the concomitant effect of these agents on the activity of background K-ATP channels. For example, the latter channels are exquisitely sensitive to intracellular pH (pH_i) changes around physiological levels [8]. Clearly, this may prevent the pH_i sensitivity of the bursting mechanism to be determined using intracellular recording techniques.

In the present study, we have used the bursting pattern evoked by high Ca^{2+} in the presence of glucose and tolbutamide [6,7] as an experimental model to assess the sensitivity of the bursting mechanism to pH_i during blockade of K-ATP channels.

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Abbreviations: pH_i, intracellular pH; K-ATP channels, ATP-dependent K^+ channels; K-Ca channels, Ca^{2+}-activated K^+ channels; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; BCECF, 2′,7′-bis-carboxyethyl-5(6)-carboxyfluorescein; BCECF/AM, acetoxymethyl ester of BCECF.

2. MATERIALS AND METHODS

The membrane potential was recorded from microdissected mouse islets of Langerhans using a high-impedance amplifier essentially as previously recorded [7]. Briefly, microdissected islets were pinned to the plastic bottom of a fast perifusion chamber through which modified Krebs' solution flowed at a rate of ca. 2 ml/min at 37°C. The solution had the following composition (mM): 125 NaCl, 5 KCl, 25 $NaHCO_3$, 2.56 $CaCl_2$, 1.1 $MgCl_2$ and 11 glucose. The solution was constantly gased with 95% O_2/5% CO_2 for a final pH of 7.4. Other electrophysiological methods as in [7].

Normal albino mice (Charles Rivers breeding) were used in most experiments. In some experiments (e.g. Fig. 4), homozygous ob/ob obese brown mice [9] originated from the Institute of Animal Genetics at the University of Edinburgh (UK) were used.

The pH_i was recorded from single collagenase-isolated mouse islets of Langerhans using the pH_i indicator BCECF [10] and a dual excitation epifluorescence system [7] supplied by PTI (Princeton, New Jersey, USA). Briefly, the islets were incubated with 2 μM BCECF/AM for 30 min at 37°C, after which they were transferred to a fast perifu-

sion chamber placed on the stage of an inverted epifluorescence microscope. The islets were excited at 440 and 500 nm via two monochromators. The fluorescence was detected by a photomultiplier after passing through a band-pass interference filter centered at 535 nm. The data were automatically corrected for background fluorescence and acquired at 5 Hz by a computer. The fluorescence ratio F_{500}/F_{440} was converted into pH_i values using an in vitro calibration procedure (fluorescence recorded from droplets of BCECF-containing solutions at known pHs). This effectively corrected for the BCECF photobleaching revealed by the individual F_{440} and F_{500} traces (Fig. 1A). Other microfluorescence methods as in [7].

3. RESULTS

We have assessed the perturbing effect of ammonium on pH_i using the fluorescent pH probe BCECF. In these experiments, the pH_i was recorded from single BCECF-loaded mouse islets of Langerhans using a ratiometric fluorescence microscopy technique. Fig. 1A depicts the effect of 20 mM NH_4Cl on the BCECF fluorescence recorded at 440 (F_{440}) and 500 nm (F_{500}). Exposure to NH_4Cl increased F_{500} but did not affect F_{440}, an effect consistent with the known spectral sensitivity of BCECF to pH [11]. The effect of NH_4Cl on the calibrated fluorescence ratio F_{500}/F_{440} is depicted in Fig. 1B. NH_4Cl exposure caused a pronounced cytoplasmic alkalinization, which was followed by a slow pH_i decrease throughout the pulse. Ammonium removal evoked a pronounced cytoplasmic acidification, followed by a slow pH_i recovery towards basal levels. While the alkalinizing shift originates from the intracellular protonation of freely diffusible ammonia molecules, the acidification response is caused by the sudden displacement of the NH_3/NH_4^+ equilibrium towards NH_3 following NH_4Cl withdrawal [12].

The experiments depicted in Fig. 2 have been designed to assess the role of the K-ATP channel in the NH_4Cl-evoked hyperpolarization [13] recorded in 11 mM glucose. Addition of 15 mM NH_4Cl to the perifusion medium hyperpolarized the β-cell membrane and suppressed bursting electrical activity (Fig. 2A, upper panel). NH_4Cl removal triggered the immediate reappearance of the electrical activity, with the first burst having a distinctly higher duration compared to control bursts. Both 4 μM glibenclamide (a specific K-ATP channel blocker [14]) and 100 μM quinine (a non-specific K-ATP channel blocker [15]) induced continuous electrical activity firing from a level close to the burst active phase. Importantly, both agents suppressed the NH_4Cl-evoked hyperpolarization (Fig. 2A, middle

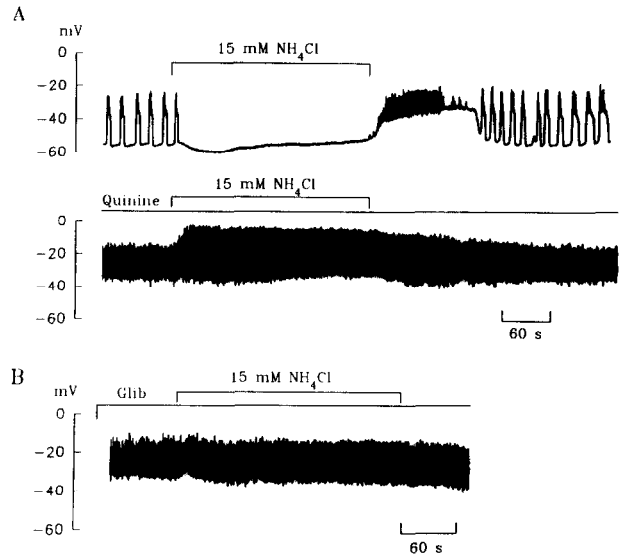

Fig. 2. Alkalinization-evoked hyperpolarization is suppressed by K-ATP channel blockers. Microdissected islets were exposed to NH_4Cl in the absence (A, upper panel) and in the presence of quinine (A, lower panel) or glibenclamide (B). Panels A and B depict two different islets. Glucose concentration was 11 mM throughout. The effects depicted are representative of 4–6 identical experiments on different islets.

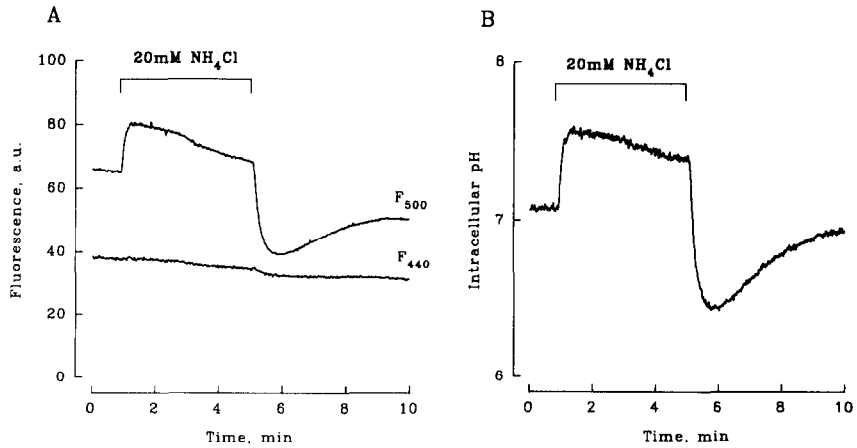

Fig. 1. Ammonium-evoked alkalinization and acidification in single islets of Langerhans. A single BCECF-loaded islet was exposed to NH_4Cl as indicated. The pH_i was recorded using the fluorescent indicator BCECF. Glucose concentration was 11 mM throughout. (A) Individual fluorescence traces recorded at 440 nm (F_{440}) and 500 nm (F_{500}) excitation. Baseline drift is due to BCECF photobleaching. (B) Fluorescence ratio F_{500}/F_{440} calibrated in pH units.

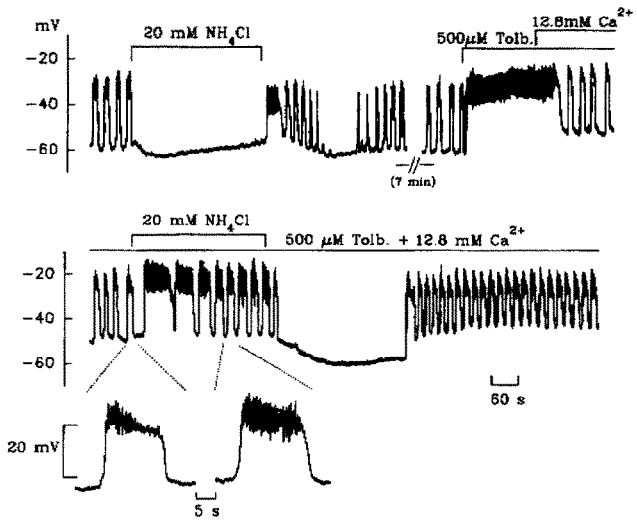

Fig. 3. Differential effects of NH_4Cl on bursting electrical activity in the presence and absence of tolbutamide. The islet was exposed to NH_4Cl in the presence of 11 mM glucose and 2.56 mM Ca^{2+} (upper panel). Exposure to tolbutamide evoked a continuous electrical activity pattern, which changed into a bursting pattern following a rise in external Ca^{2+} concentration to 12.8 mM. The islet was then exposed to NH_4Cl in the presence of tolbutamide and 12.8 mM Ca^{2+} (lower panel). Individual bursts recorded before and during the latter NH_4Cl exposure are also shown on an expanded time basis. The effects depicted are representative of 4 identical experiments on different islets.

panel and Fig. 2B). It is also apparent in Fig. 2A that exposure to NH_4Cl caused a pronounced increase in spike amplitude in quinine-treated islets. Interestingly, this enhancement of spike amplitude was not immediately apparent in glibenclamide-treated islets.

We have carried out experiments designed to assess the effect of NH_4Cl on bursting electrical activity evoked by high Ca^{2+} in the presence of the K-ATP channel blocker tolbutamide. Addition of 500 μM tolbutamide to the perifusion medium abolished the silent phases of the bursts and evoked continuous electrical activity firing at the level of the active phase (Fig. 3, upper panel). Raising external Ca^{2+} concentration ($[Ca^{2+}]_o$) to 12.8 mM in the presence of the sulphonylurea restored the ability of the cell to display bursting electrical activity, as previously described [6]. Surprisingly, challenging the cell with NH_4Cl in the presence of tolbutamide and 12.8 mM Ca^{2+} evoked a pronounced increase in burst duration, an effect that tended to dissipate throughout the pulse but remained pronounced towards the end of the 4 min exposure period (Fig. 3, lower panel). NH_4Cl removal generated a pronounced membrane hyperpolarization, which was accompanied by suppression of bursting electrical activity. Regular bursting electrical activity resumed ca. 5 min after NH_4Cl withdrawal. The effects of NH_4Cl recorded in the presence of tolbutamide and high Ca^{2+} are the mirror image of the effects recorded in control conditions (i.e. 11 mM glucose and 2.56 mM Ca^{2+}), as assessed by inspection of the records obtained from the same cell (Fig. 3). It should be noted that, following NH_4Cl removal in the experiment depicted in the upper panel of Fig. 3 there was a slight hyperpolarization of the burst silent phase, during which the electrical activity was transiently suppressed. This trough was, however, not evident in all the experiments performed (see Fig. 2 and [13]).

We have also examined the effect of NH_4Cl on the electrical activity of β-cells from homozygous obese (ob/ob) mice of the UK strain [9,16]. These cells appear to have an altered K^+ conductance, as suggested by their poor sensitivity to sulphonylureas and quinine and by their reduced sensitivity to glucose [9,16]. Interestingly, the prevalent effect of NH_4Cl in these cells was qualitatively similar to the effect described above for normal cells in the presence of tolbutamide and high Ca^{2+}. Indeed, challenging the ob/ob β-cells with NH_4Cl in the presence of 11 mM glucose evoked a pattern of continuous electrical activity (Fig. 4). Furthermore, resembling the experiment depicted in the lower panel of Fig. 3 NH_4Cl withdrawal was followed by a period with no electrical activity.

4. DISCUSSION

We have shown that intracellular alkalinization increases the time spent in the active phase of the bursts evoked by high Ca^{2+} in the presence of the K-ATP channel blocker tolbutamide. Conversely, intracellular acidification hyperpolarizes the β-cell membrane and suppresses the electrical activity. This stands in sharp contrast to the effect of alkalinizing and acidifying pH_i

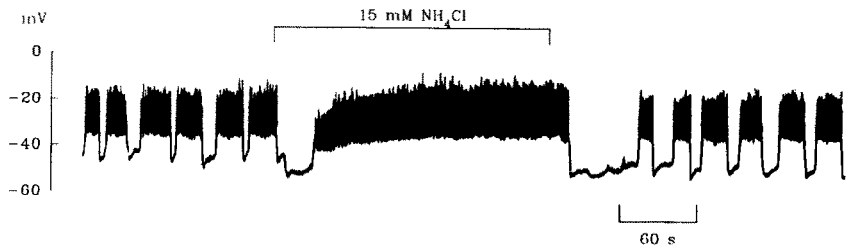

Fig. 4. Stimulatory effect of NH_4Cl on bursting electrical activity recorded from an ob/ob mouse islet (UK strain). NH_4Cl was applied as indicated. Glucose concentration was 11 mM. The effects depicted are representative of 3 identical experiments on different islets.

shifts on the bursting electrical activity recorded under control conditions (i.e. 2.56 mM Ca^{2+} and 11 mM glucose), thus emphasizing the differential sensitivity of different β-cell ion channels to pH_i.

The K-ATP channel present in the pancreatic β-cell has a sharp pH sensitivity in the physiological range, with a pH rise increasing the open probability of the channel [8]. We have shown that two different K-ATP channel blockers (i.e. tolbutamide and quinine) impair the hyperpolarizing effect of NH_4Cl exposure. Thus, the K-ATP channel appears to have a prevalent role as an electrical transducer of intracellular pH shifts. Glucose is known to increase the pH_i in isolated β-cells [17], an effect that we have recently confirmed using BCECF-loaded whole islets (Santos, Salgado and Rosario, manuscript in preparation). Thus, the K-ATP channel may play a role in the physiological regulation of electrical activity by metabolism-supported pH_i changes.

Interestingly, the response of ob/ob mouse islets (UK strain) to NH_4Cl resembles qualitatively that of normal mouse islets treated with tolbutamide and high Ca^{2+}. These ob/ob β-cells[1] are poorly sensitive to sulphonylurea drugs and quinine and have a decreased sensitivity to glucose when compared with normal β-cells [9,16]. Furthermore, resting membrane potential of ob/ob β-cells at low glucose concentrations is clearly less negative than normal cells [9], indicating an increased resting K^+ conductance in the latter cells. These observations may be accounted for by a decreased density, or even total absence of sulphonylurea-sensitive K-ATP channels in ob/ob β-cells. Under these conditions, the effect of NH_4Cl-evoked alkalinization on the channels underlying the burst may become preponderant, with a corresponding net depolarizing action on the β-cell membrane.

The molecular and ionic mechanisms underlying the bursts of electrical activity remain essentially unknown. K-ATP channel blockers of the sulphonylurea family change the regular bursting pattern into a continuous firing pattern, an effect probably accounted for by the suppression of residual K-ATP channel activity observed at intermediate-high glucose concentrations [21]. We have previously shown that several drugs and/or conditions known to enhance Ca^{2+} influx across the plasma membrane can restore the ability of sulphonylurea-treated cells to display bursting electrical activity [6,7]. This indicates that the channel underlying the burst is highly sensitive to $[Ca^{2+}]_i$ changes supported by Ca^{2+} influx. Moreover, we found that the bursts evoked by high Ca^{2+} in the presence of tolbutamide are not impaired by charybdotoxin and quinine [7]. Thus, the repolarization that terminates the burst is highly unlikely to be provided either by activation of charybdotoxin- and tetraethylammonium-sensitive large-conductance K-Ca channels or by the activation of K-ATP channels. There have been suggestions that the pacemaker current underlying the burst may be carried by a K-Ca channel distinct from the charybdotoxin-sensitive channel [21] and by the voltage-sensitive Ca^{2+} channel [22,23], but the possibility that distinct Ca^{2+} channels or non-selective cation channels might be involved cannot be ruled out.

We have seen that the bursting mechanism is intrinsically sensitive to intracellular pH, with an alkalinizing shift promoting a net depolarization to the burst active phase. Thus, the future study of the pH sensitivity profile of channels putatively implicated in the burst may be instrumental for the assignment of specific roles for these channels in the bursting mechanism.

Acknowledgements: The authors thank Prof. A.P. Carvalho for continued support. The help of Miss Amélia Silva in the collagenase-isolation of the islets is gratefully acknowledged. This work was partially financed by grants from JNICT and Calouste Gulbenkian Foundation (Portugal).

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[1]It should be noted that β-cells from other strains of ob/ob mice may behave differently with respect to K^+ channel blockers and NH_4Cl. For example, the C57BL/6J-ob strain from Jackson Laboratories (USA) has seemingly normal electrical responses to glucose, quinine and NH_4Cl (Rosario, unpublished observations). Furthermore, the latter cells appear to have a normal set of K^+ channels [18]. Since the expression of the ob gene depends on the background genome carrying the gene [19,20], any functional interstrain differences may relate primarily to the particular genetic backgrounds of the ob/ob mice investigated.

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