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A possible role of the ATP-sensitive potassium ion channel in determining the duration of spike-bursts in mouse pancreatic β-cells

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Abstract

The pancreatic β-cell displays an electrical activity consisting of spike bursts and silent phases at glucose concentrations of about 10 mM. The mechanism of initial depolarization induced by glucose is well defined. However, the mechanism inducing the silent phase has not been fully elucidated. In the present study, the possibility of involvement of ATP-sensitive K^+ channels in repolarization was examined using the patch-clamp technique in the cell-attached recording configuration. Ouabain (0.1 mM), an inhibitor of Na^+/K^+-ATPase, caused a complete suppression of ATP-sensitive K^+ channel activity followed by typical biphasic current deflections, which were due to action potentials. The channel activity was also inhibited by removal of K^+ from a perifusion solution. Furthermore, the activity of ATP-sensitive K^+ channels was markedly inhibited either by replacement of external NaCl with LiCl or by addition of amiloride (0.2 mM), a blocker of Na^+/H^+ antiport. Addition of L-type Ca^{2+} channel blockers such as Nifedipine or Mn^{2+} induced the complete suppression of K^+ channel activity. These findings strongly suggest that a fall in ATP consumption results in sustained depolarization, and that the repolarizations interposed between spike-bursts under normal ionic conditions are due to the periodical fall of ATP concentration brought about by periodical acceleration of ATP consumption at Na^+/K^+-pumps. It is concluded that the elevation of intracellular Na^+ concentration as a consequence of accelerated Na^+/Ca^{2+}-countertransport during the period of spike-burst enhances ATP consumption, leading to a fall in ATP concentration which is responsible for termination of spike-burst and initiation of repolarization.

Keywords: ATP; Beta cell; Electrical activity; Potassium channel; (Mouse pancreas)

1. Introduction

The main function of the pancreatic β cell has been known to be the provision of insulin output in response to a rise in blood glucose concentration. Studies using microelectrodes have shown that when stimulated with intermediate-high glucose concentrations (7–17 mM) the β-cell displays a gradual depolarization followed by a spike train and subsequent periodic electrical activity [1–4]. An important characteristic of this electrical activity is its rhythmicity. It consists of regular slow waves of the membrane potential superimposed with bursts of Ca^{2+} spikes (spike-burst phase) [4,5]. It has been reported that periodical spike-bursts seem to underlie both the cyclic oscillation of intercellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and the insulin release in an isolated whole islet of Langerhans [6–8]. Elevation of external glucose concentration prolongs the duration of the spike-burst phase, and shortens the silent phase interposed between spike-bursts, whereas the frequency of single spikes within the spike-burst periods does not depend on the external glucose concentration [9]. Therefore, $[Ca^{2+}]_i$ is intrinsically associated with the duration of spike-bursts.

It is known that when pancreatic β-cells are exposed to glucose in high concentrations the K^+ permeability of the plasma membrane markedly decreases [10,11]. This decrease in K^+ permeability is due to a closure of ATP-sensitive K^+ channels [12,13]. The ATP-sensitive K^+ channel will be referred to as the K(ATP) channel in this article. It is currently believed that an elevation of external glucose concentration causes a rise in ATP/ADP ratio in the cytoplasm which is responsible for the suppression of K(ATP) channel activity [14,15]. Although it is generally accepted that the depolarization from resting potential to the threshold potential is due to the closure of K(ATP)

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channels, the mechanism determining the duration of the spike-burst phase has not been sufficiently studied.

Pharmacological evidence [16,17] seems to rule out participation of the Ca^{2+}-activated K^+ channel (maxi K(Ca) channel) in the termination of spike-burst. The regulation of glucose-induced bursting has also been attributed to the activity changes of ionic channels other than the K(ATP) channel [9] and of distinct maxi K(Ca) channel, or interpreted by $[Ca^{2+}]_i$-dependent slow processes of inactivation of Ca^{2+} currents [18]. Recently we have reported that a decreased Na^+ influx might cause a slowing of ATP-consumption at the site of Na^+/K^+-pumps [19]. This idea is supported by the previous findings that lowering external Na^+ concentration [20,21], amiloride, an inhibitor of Na^+/H^+-antiport [22,23], or ouabain, an inhibitor of Na^+/K^+-ATPase [2], has been reported to induce a persistent depolarization superposed with continuous spike activity. Thus, we have speculated that under physiological conditions a rise in $[Ca^{2+}]_i$ during the spike-burst causes an increase in Na^+ influx through the Na^+/Ca^{2+}-counter transporters [24,25], resulting in a rise in intracellular Na^+ concentration ($[Na^+]_i$) and an acceleration of ATP-consumption at Na^+/K^+-pumps which produces a fall in intracellular ATP level responsible for the interruption of the spike-burst.

The experiments reported in this article were performed to elucidate more systematically whether a change in intracellular ATP-consumption exerts a modulatory influence on the cyclic occurrence of spike-burst. We recorded K(ATP) channel currents using a cell-attached patch configuration to trace variation in the intracellular ATP level.

2. Materials and methods

The experiments were performed using either islets or single β-cells of male ICR mice (6–10 weeks) killed by cervical dislocation.

2.1. Preparation of single cells

Pancreatic tissue was minced with scissors and digested by collagenase (2 mg/ml) for 30 min at 37°C. Islets were picked up with a small pipette. The method used to isolate pancreatic islet cells has been described elsewhere [26]. In brief, after exposure to a Ca^{2+}- and Mg^{2+}-deprived medium, the islets were disrupted using dispase (Godo Shusei, Tokyo). After isolation, the cells were seeded onto poly-D-lysine-coated coverslips and cultured for 2–6 days in RPMI 1640 medium containing 11.1 mM glucose supplemented with 10% fetal calf serum (Flow Lab, USA), 100 IU/ml penicillin and 100 μg/ml streptomycin. The cells immunohistochemically positive to anti-insulin antiserum were large in size, and contained many granules, whereas the cells immunohistochemically positive to anti-glucagon antiserum were small in size. On the basis of these observations, it was not difficult to identify β-cells under ordinary illumination.

2.2. Patch-clamp recording

A standard cell-attached patch configuration technique [27] was used to record the K(ATP) channel currents. The patch pipettes were pulled from borocillicate glass capillaries and then heat-polished just before use. They had resistances of 6–10 MΩ when filled with 140 mM-K^+ solution having the following composition (in mM): 140 KCl, 2 $CaCl_2$, 2 $MgCl_2$ and 10 Hepes (pH 7.2 adjusted with KOH). The bath contained a standard Krebs-Ringer-Bicarbonate (KRB) solution consisting of (mM) 110 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.1 $MgCl_2$, 25 $NaHCO_3$ and 5 Hepes. The bath solution was gassed continuously with 95% O_2 + 5% CO_2 (pH 7.4). Larger single islet cells (with diameter > 10 μm) were selected because smaller cells were potentially considered to be non-β cells on the basis of immunohistochemical observations. Measurement of channel currents was performed at room temperature (about 25°C). In the cell-attached mode, those cells that responded to glucose of 11.1 mM with a suppression of K^+ channel currents and the development of biphasic current deflections due to action potentials [12,13] were judged to be β-cells. Single channel currents did not show time-dependent decay for about 30 min in the bath solution. Currents were recorded through a patch clamp amplifier (CEZ-2200 Nihon Kohden, Tokyo) and were stored on a video tape after being digitized by a PCM device (SONY, PCM-801 modified) for later analysis. For drawing current records, analog-output voltage was re-digitized using a 14-bit ADC (TEAC, Tokyo) and plotted on an XY-plotter (Roland DXY-980A, Hamamatsu). The resting potential of β-cells is close to −70 mV in standard external solution [12] so that the potential across the patch membrane is thus $-(70 + V_p)$ mV. The pipette potential was zero in all experiments. Inward currents across the membrane are displayed conventionally as downward deflections in all current traces.

2.3. Membrane potential recording

A small piece of pancreatic tissue containing two or three islets was mounted on a small ball in a chamber. The islets were perifused with KRB solution gassed with 95% O_2 + 5% CO_2. The membrane potential of β-cells was recorded using a microelectrode (300–400 MΩ). The electrode was connected to a high input impedance bridge-amplifier (MEZ-8301 Nihon Kohden, Tokyo) via Ag/AgCl electrode. The analog-output was stored in a videotape after being digitized by a PCM device. For plotting records, analog-output voltage of the PCM device was re-digitized using a 14-bit ADC (Canopus, Kobe) controlled by a personal computer (NEC PC-98, Tokyo) and plotted on an XY-plotter. The temperature of the

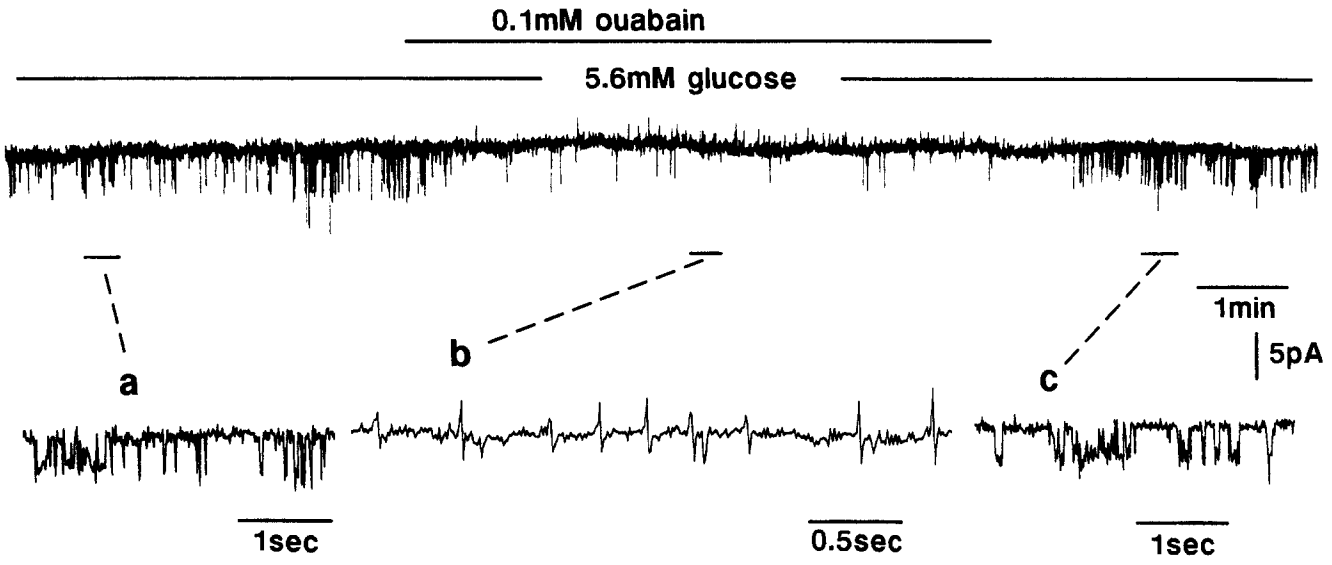

Fig. 1. Effect of ouabain on the activity of K(ATP) channels. Both glucose and ouabain were added during the period indicated by horizontal bars. Pipette potential was 0 mV. Lower traces show details of electrical activity in a 60-fold (a and c of lower traces) and 120-fold (b of lower traces) expanded time scale. Addition of ouabain caused abolishment of K(ATP) channel current, and induced biphasic deflections of the baseline current level due to the action potentials of β-cells (lower b).

solution was controlled at 36°C by a thermomodule. The pancreatic β-cells in the islet were identified by showing the typical electrical activity that they display in the presence of 11.1 mM glucose.

2.4. Chemicals

Ouabain, amiloride and nifedipine were purchased from Sigma (St. Louis, USA). Collagenase was obtained from Wako Pure Chemicals Ltd (Osaka, Japan). Other chemicals were purchased from Nacalai Tesque (Kyoto, Japan).

3. Results

3.1. The effect of Na^+/K^+-pump inhibition on K(ATP) channel activity

Fig. 1 shows K(ATP) channel currents recorded in the cell-attached configuration. In the presence of 5.6 mM glucose, we observed openings of a K^+ channel which had a unit amplitude of 4.3 pA. The single channel conductance was calculated to be 61 pS, assuming the resting potential to be −70 mV and intracellular potassium con-

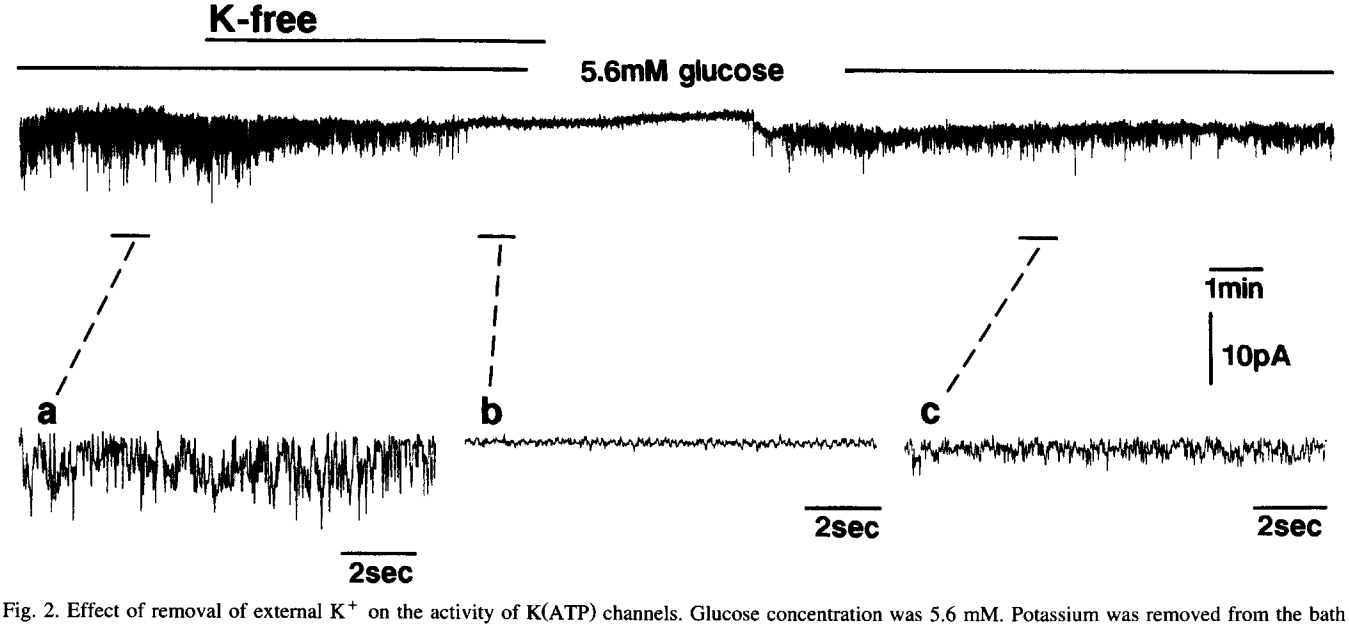

Fig. 2. Effect of removal of external K^+ on the activity of K(ATP) channels. Glucose concentration was 5.6 mM. Potassium was removed from the bath solution during the period indicated by a horizontal bar. Details of electrical activity are shown in a 45-fold expanded time scale (lower; a, b and c).

centration to be 140 mM. This value is in agreement with that of the K(ATP) channel previously reported in pancreatic β-cells [12,13]. At present, as there is no method to directly trace a change in intracellular ATP concentration, the activity of the K(ATP) channel was adopted as an indicator of intracellular ATP-level.

K(ATP) channel activity was gradually inhibited after exposure to ouabain (Na^+/K^+-pump inhibitor) of 0.1 mM, resulting in almost complete closure of the K(ATP) channel. We found biphasic current defections from the baseline during the periods of ouabain application. The biphasic deflections are considered to be due to action potentials elicited on depolarization [12,13]. After washing out ouabain, the channels recovered their activity ($n = 5$, different cells). This finding agrees well with the results of microelectrode studies that ouabain (0.1 mM) has evoked depolarization and almost completely abolished the silent phase [2,28]. Thus, it may be said that a decrease in ATP-consumption as a result of an inhibition of Na^+/K^+-pumps by ouabain leads to an elevation of intracellular ATP concentration. To further examine the effect of inhibition of Na^+/K^+-pumps on K(ATP) channel activity, potassium was removed from a perifusion solution. As shown in Fig. 2, K(ATP) channel activity was gradually suppressed after exposure to K^+-free solution, and finally disappeared ($n = 4$, different cells).

Intracellular ATP concentration depends on both the consumption and production rates of ATP. This means the effect of ouabain or K^+-removing on membrane potential might be modified by changing the production of ATP. To verify this view, the K^+-free experiments were repeated at various concentrations of glucose using a microelectrode. The upper trace in Fig. 3 shows an experiment conducted at 11.1 mM glucose. Removing external K^+ almost completely abolished the silent phase. However, at 8.4 or 5.6 mM glucose (Fig. 3, middle and lower traces), external K^+

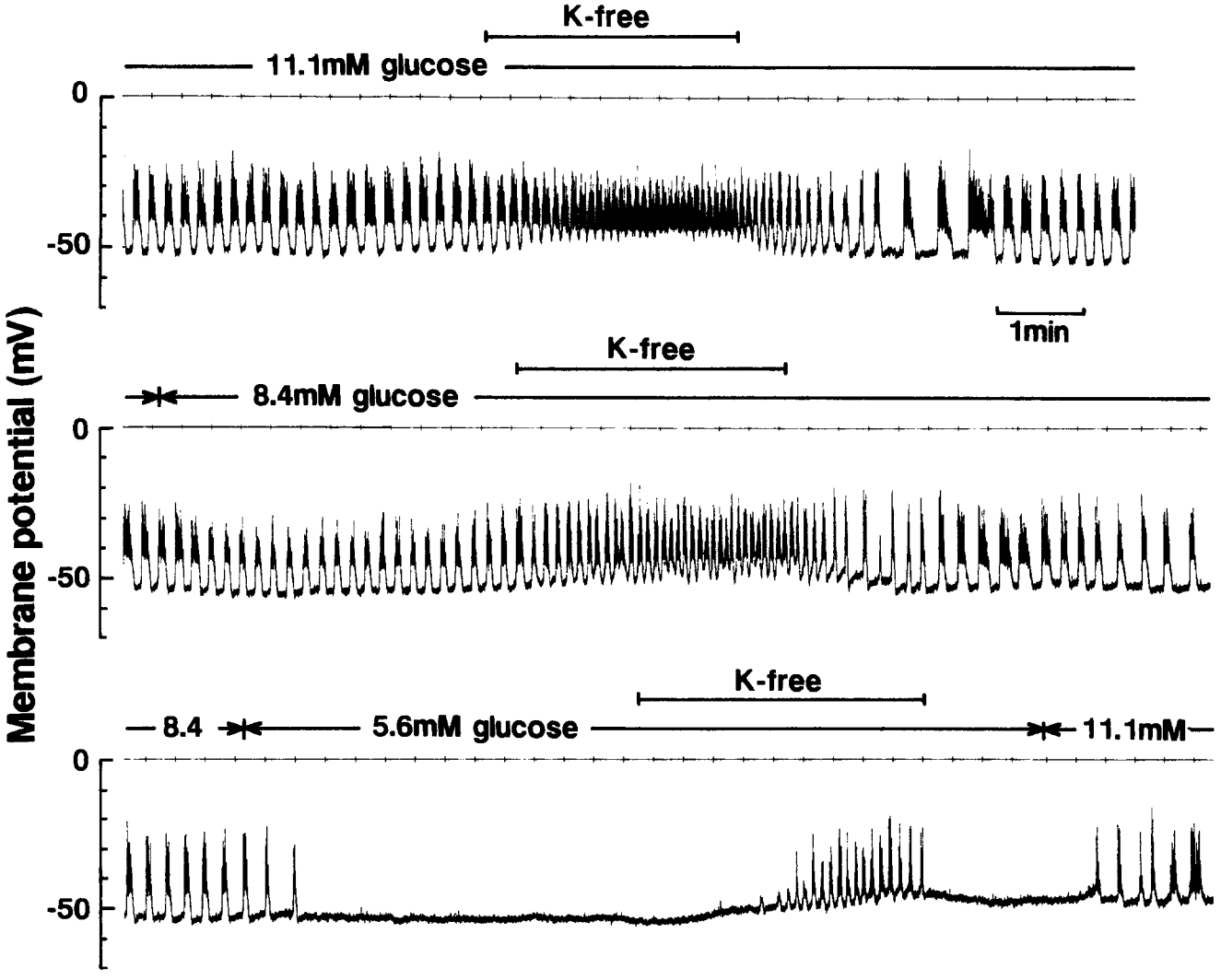

Fig. 3. Changes in the pattern of electrical activity induced by a removal external K^+ at different glucose concentrations. Glucose concentration was varied from 11.1 mM to 8.4 mM, 5.6 mM and then to 11.1 mM as denoted on horizontal bars. Potassium was removed from the bath solution during the period indicated by a bar.

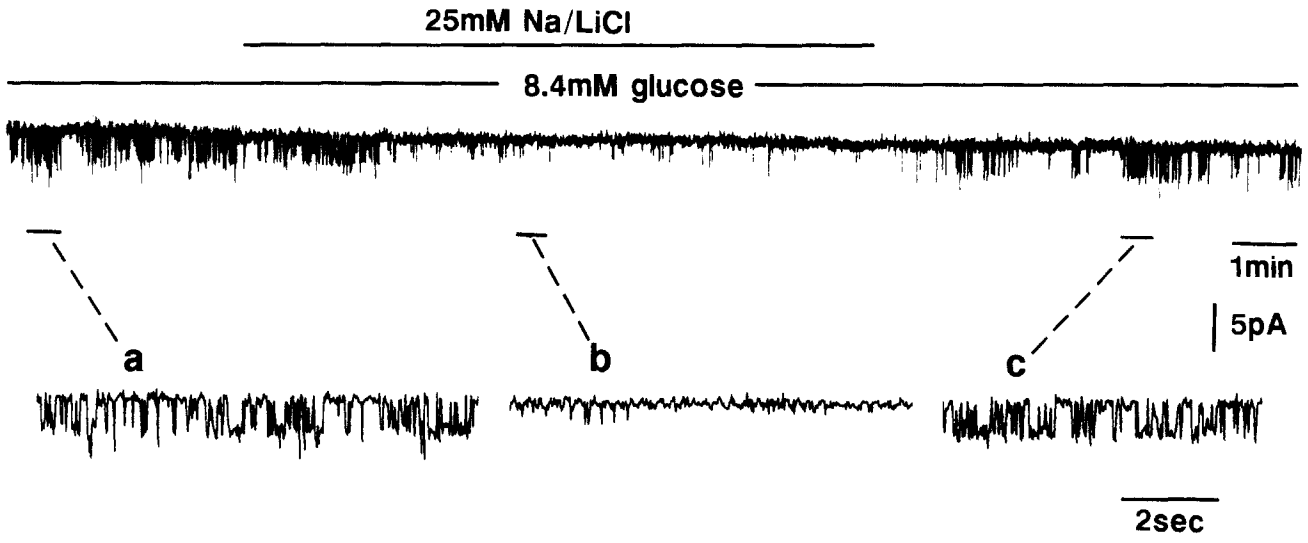

Fig. 4. Effect of lowering external Na^+ concentration from 135 mM to 25 mM on the activity of K(ATP) channels. Glucose concentration was 8.4 mM. Na^+ was lowered from 135 mM to 25 mM during the period indicated by a bar. Low Na^+ solution was prepared by replacing 110 mM of NaCl with the same moles of LiCl. Details are shown in a 45-fold expanded time scale (lower; a, b and c).

removing was less effective ($n = 4$, different islet). This finding strongly suggests that the change in electrical activity induced by removal of external K^+ depends on the balance between production and consumption of ATP.

3.2. The effect of low Na^+ on the activity of the K(ATP) channel

It has been reported that Na^+/Ca^{2+}-counter transport plays a role in extruding Ca^{2+} from cytosol to the extracellular side of the pancreatic β-cell [25]. Under conditions where Na^+/Ca^{2+}-exchange is taking place, an increase in $[Ca^{2+}]_i$ resulting from continuous generation of Ca^{2+} spikes is expected to induce an elevation of $[Na^+]_i$. If a fall in ATP level resulting from an elevation of $[Na^+]_i$ is responsible for initiating an observed repolarization, the omission of extracellular Na^+ may prevent an initiation of repolarization. As shown in Fig. 4, the activity of K(ATP) channels was markedly suppressed when lowering external Na^+ concentration from 135 mM to 25 mM by replacing NaCl with LiCl ($n = 4$, different cells). This finding accords with our very recent report that lowering external Na^+ concentration has caused gradual depolarization and continuous spike activity recorded using the microelectrode method [19].

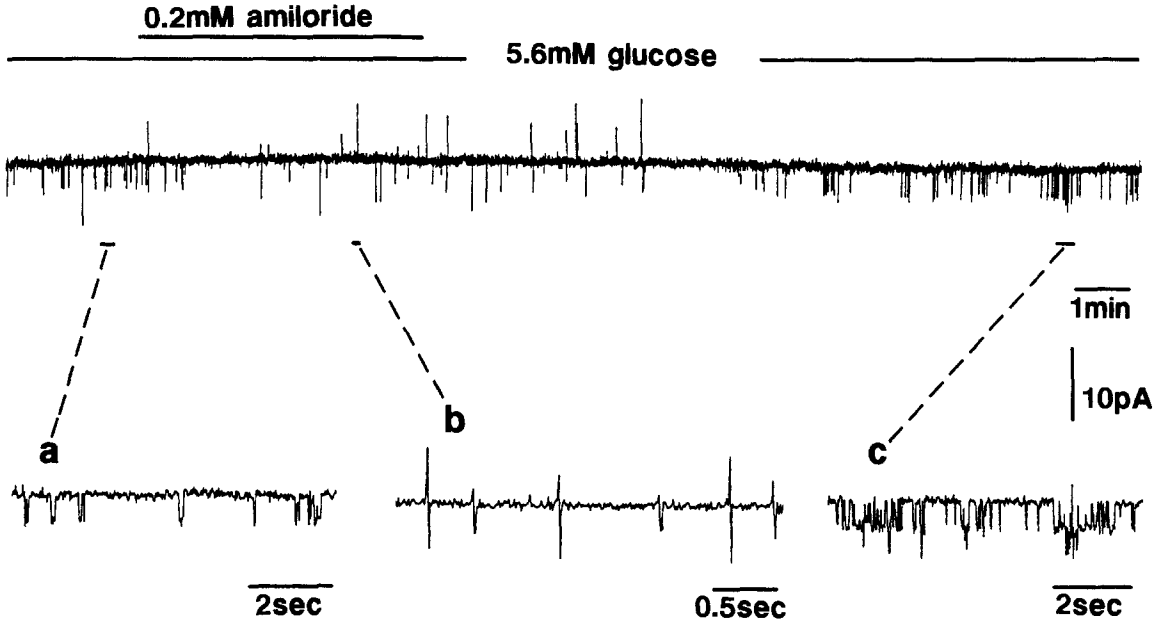

Fig. 5. Effect of amiloride on the activity of K(ATP) channels. Glucose concentration was 5.6 mM. Amiloride was applied during the period indicated by a horizontal bar. Details are shown in 45-fold (a, c) and 180-fold (b) expanded time scales.

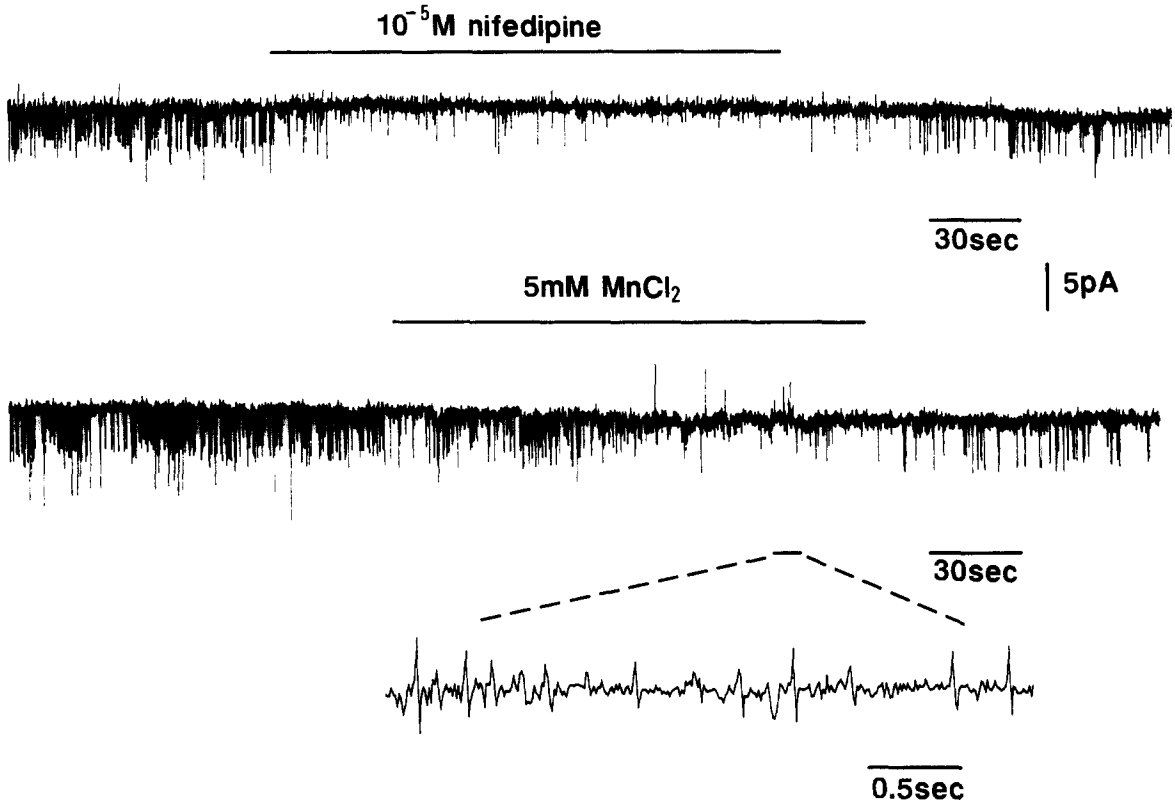

Fig. 6. Effects of nifedipine and $MnCl_2$ on the activity of K(ATP) channels. The perifusion solution contains 8.4 mM glucose. Nifedipine and $MnCl_2$ were added during the period indicated by horizontal bars. Details of electrical activity induced by $MnCl_2$ are shown in a 60-fold expanded time scale (Lower trace).

Amiloride, a specific blocker of transmembrane sodium proton exchange, has also been reported to induce a persistent depolarization with continuous spike activity [22,23]. Thus, we consider that this phenomenon also is due to decreased ATP consumption by Na^+/K^+-pumps as a result of a decrease in $[Na^+]_i$. To further confirm this idea we examined the effect of amiloride on the activity of the K(ATP) channel. Addition of 0.2 mM amiloride brought about a complete inhibition of K(ATP) channel activity, following which, biphasic current deflections due to action potentials were elicited (Fig. 5). After switching back to the control perifusion solution, the channel activity was completely restored ($n = 4$, different cells).

3.3. The effect of Ca^{2+} channel blocker on the activity of the K(ATP) channel

The results presented above show that all the procedures which would induce a decrease in Na^+ influx cause a suppression of K(ATP) channel activity. Lowering $[Ca^{2+}]_i$ by Ca^{2+} channel blocker might also result in a decrease in Na^+ influx through Na^+/Ca^{2+}-counter transporters, leading to a fall in $[Na^+]_i$. As shown in Fig. 6, addition of Nifedipine (10 μM), a blocker of L-type Ca^{2+} channel [29], brought about a complete suppression of K(ATP) channel activity ($n = 4$, different cells).

The result in Fig. 6 also indicates that applying 5 mM Mn^{2+}, a competitive Ca^{2+} channel blocker [30], caused a complete inhibition of K(ATP) channel activity accompanied by typical biphasic current deflections (Fig. 6, Lower trace) which are due to action potentials as mentioned above. After the removal of the Ca^{2+} channel blocker, the K^+ channel activity was partially reversed ($n = 4$, different cells). Thus, the results presented above strongly suggest that a decrease in ATP-consumption causes an elevation in intracellular ATP concentration.

4. Discussion

Pancreatic β-cells exhibit periodical electric activity consisting of a spike-burst phase and a silent phase in response to external glucose of intermediate-high concentrations [1–4]. Following the elevation of glucose concentration, the duration of the spike-burst becomes longer; and conversely, the duration of the silent phase becomes shorter. It has been shown that the length of spike-burst is closely associated with the amount of insulin release [31]. However, the mechanism underlying the periodicity of the spike-burst still remains to be clarified. Rosario and his collaborators have mentioned that channel activity underlying spike-burst is sensitive to Ca^{2+} influx [18]. Other

studies have indicated that the degree of Ca^{2+} sequestration in the endoplasmic reticulum is related to repolarization [32]. Present observations do not support the idea that elevated $[Ca^{2+}]_i$ is directly involved in the initiation of the repolarization which terminates the spike-burst. Recently, we have suggested that decreased Na^+ influx produced a continuous spike activity as a result of decreased ATP-consumption at the Na^+/K^+-pump sites [19].

It is well known that the Na^+/K^+-pump is blocked by both ouabain and a potassium-free mediums [33]. Ouabain induces a depolarization in pancreatic β-cell. The depolarization induced by ouabain has been interpreted to be due to a suppression of electrogenic Na^+/K^+-pump [34]. In our cell-attached patch experiments, the K(ATP) channel is clearly demonstrated to be completely suppressed under conditions where the activity of Na^+/K^+-pumps is suppressed (Figs. 1 and 2). Similar observation on the K(ATP) channel activity in the presence of ouabain was made by Grappengiesser et al. [35], although they had no attempt to analyze the relationship between the ouabain-induced depolarization and ATP-consumption rate. The ouabain-induced suppression of K(ATP) channel activity suggests that decreasing the ATP-consumption rate at Na^+/K^+-pump sites is enough to induce an elevation of intracellular ATP concentration which leads to a closure of K(ATP)channels. Furthermore, the fact that removal of external K^+ is found to be more pronounced in higher glucose concentrations strengthens the view that the balance between production and consumption of ATP might be important in regulating intracellular ATP level.

The rate of ATP-consumption by the Na^+/K^+-pump is dependent on intracellular Na^+ concentration [36]. This is the first report of demonstrating that both lowering external Na^+ concentration and removing Ca^{2+} cause suppression of K(ATP) channel activity in β-cells. We have also found that the intracellular ATP concentration of isolated islets is significantly increased after 20 min-incubation in a low Na KRB solution or in a Ca^{2+}-free KRB solution [37]. Measurement of $[Na^+]_i$ using a sodium sensitive fluorescent probe has indicated that acetylcholine which facilitates spike generation causes an elevation both in intracellular Na^+ and Ca^{2+} concentrations [38]. Although acetylcholine receptor-mediatedly causes increases in both Na^+ and Ca^{2+} influxes leading to a depolarization, some part of increased Na^+ influx may be mediated by an accelerated Na^+/Ca^{2+}-counter transport as a result of an elevation of intracellular Ca^{2+} concentration. In connection with this report, lowering external Na^+ concentration which may suppress the Na^+/Ca^{2+}-counter transport has caused a marked increase in $[Ca^{2+}]_i$ [19]. These findings fully support the view that the Na^+/Ca^{2+}-counter transporter plays an important role in maintaining $[Ca^{2+}]_i$ at a low level, and that an elevation in intracellular Na^+ concentration mediated by accelerated Na^+/Ca^{2+}-counter transport during the period of spike-bursts stimulates ATP consumption at Na^+/K^+-pump sites. Grapengiesser et al. [35] reported that inhibition of the Na^+/K^+-pump by ouabain or removal of K^+ transformed the Ca^{2+}-oscillation into a sustained increase of $[Ca^{2+}]_i$, the level reached exceeded that obtained in response to a rise of glucose alone, while at low concentrations ouabain reduced the frequency of the glucose-induced oscillations with an increase in the amplitude an half-widths of the Ca^{2+} peaks. On the basis of observations presented in this study, the increase of intracellular Ca^{2+} concentration is accounted for by the continuous Ca^{2+}-spike generation superposed on a sustained depolarization brought about from an elevation of the ATP/ADP ration which is the result of a fall in the ATP consumption rate at Na^+/K^+-pump sites. Thus, it may be said that a depolarization induced by ouabain is not a result of a suppression of electrogenic Na^+/K^+-pump but is due to a closure of K(ATP) channels caused by a rise in ATP/ADP ratio as a result of a fall in ATP consumption.

TEA is known to develop an elongated plateau phase of action potential [39]. This phenomenon is accounted for by a blockade of voltage-dependent K^+ channels, which results in an increase in the amplitude of spikes. In a KRB solution containing TEA, the inter-spike potential is close to the resting potential attained at low glucose concentrations [40], suggesting low intracellular ATP concentration during this period. It is thought that during the elongated plateau phase just after the crest of the spike, a small but continuous Ca^{2+} influx causes a gradual rise in $[Ca^{2+}]_i$, leading to a rise in $[Na^+]_i$ through Na^+/Ca^{2+} exchange. The gradual rise in $[Na^+]_i$ will cause a fall in the ATP/ADP ratio which is responsible for the repolarization. Since in the plateau phase many voltage-dependent Ca^{2+} channels are considered to be in an inactivated state, a small increase in K^+ conductance may be enough to cause the repolarization. Probably, the single channel conductance of K(ATP) channels will increase during the process of repolarization, contributing to an acceleration in repolarization. The large repolarization during the inter-spike periods makes it possible for Ca^{2+} channels to recover so rapidly from an inactivated state that a large action potential can be elicited by gradual depolarization after a relatively short interval. When TEA concentration is low, blocking of voltage-dependent K^+ channels is incomplete. The incompleteness makes the induced phenomenon complex [41]. Tolbutamide enhances the sensitivity to ATP of K(ATP) channels [42]. The finding that this drug induces continuous spike activity [41,43] is also consistent with our view.

It has once been proposed that H^+ yielded in the oxidative breakdown of glucose is responsible for the depolarization induced by external glucose [44]. However, another group has reported that no substantial change in intracellular pH is caused by elevating external glucose concentration [45]. We have recently reported that both manipulations of replacing external Na^+ with either Li^+ or $Tris^+$ cause a persistent depolarization with continuous

spike activity, whereas these manipulations have distinct effects on pH_i [19]. Patch clamp experiments in isolated patch membranes have revealed that intracellular pH has little effect on K(ATP) channel activity until it is reduced below 6.5 [46]. In our experiments, however, the lowest pH attained by replacing NaCl with LiCl has been 6.8 [19]. These facts indicate that changes in electrical activity induced by low Na^+ are not due to the change in intracellular pH but rather due to a fall in $[Na^+]_i$ as a result of a decrease in Na^+ influx.

In conclusion, the present study demonstrates that maintenance of higher ATP concentration induced by low Na^+ influx is enough to close K(ATP) channels. Thus, under normal ionic circumstances a possible mechanism is suggested as follows: an increased influx of Ca^{2+} during the spike-burst period causes a gradual elevation of $[Ca^{2+}]_i$ which results in a rise in $[Na^+]_i$ through Na^+/Ca^{2+}-counter transport; the rise in $[Na^+]_i$ accelerates the consumption of ATP at Na^+/K^+-pumps; the fall in ATP concentration results in opening of K(ATP) channels which terminates spike-generation and initiates a silent phase; during the silent phase the ATP concentration gradually increases because no Ca^{2+}-spike is elicited; this increased ATP concentration causes a depolarization and then spike-burst is evoked again. This cycle may be the mechanism underlying the periodic spike-burst activity.

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