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The role of plasma membrane K^+ and Ca^{2+} permeabilities for glucose induction of slow Ca^{2+} oscillations in pancreatic β-cells

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Abstract

In individual pancreatic β-cells the rise of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$), induced by 11 mM glucose, is manifested either as oscillations (0.2–0.5 min^{-1}) or as a sustained elevation. The significance of the plasma membrane permeability of Ca^{2+} and K^+ for the establishment of these slow oscillations was investigated by dual wavelength microfluorometric measurements of $[Ca^{2+}]_i$ in individual *ob/ob* mouse β-cells loaded with fura-2. Increasing the extracellular Ca^{2+} to 10 mM or the addition of Ca^{2+} channel agonist BAY K 8644 (1 μM) or K^+ channel blocker tetraethylammonium$^+$ (TEA; 10–20 mM) caused steeper rises and higher peaks of the glucose-induced oscillations. However, when extracellular Ca^{2+} was lowered to 0.5 mM the oscillations were transformed into a sustained suprabasal level. When the β-cells exhibited glucose-stimulated sustained elevation of $[Ca^{2+}]_i$ in the presence of a physiological Ca^{2+} concentration (1.3 mM), it was possible to induce slow oscillations by promoting the entry of Ca^{2+} either by raising the extracellular Ca^{2+} concentration to 10 mM or adding TEA or BAY K 8644. The results indicate that glucose-induced slow oscillations of $[Ca^{2+}]_i$ depend on the closure of ATP-regulated K^+ channels and require that the rate of Ca^{2+} influx exceeds a critical level. Apart from an inherent periodicity in ATP production it is proposed that Ca^{2+}-induced ATP consumption in the submembrane space contributes to the cyclic changes of the membrane potential determining periodic entry of Ca^{2+}.

Keywords: Membrane permeability; Potassium ion; Calcium ion; Glucose-induced oscillation; Plasma membrane; (Pancreatic β-cell)

1. Introduction

The action of glucose as a physiological stimulus of insulin release is mediated by an increase of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) in pancreatic β-cells. At a threshold concentration (7–20 mM) characteristic for the individual β-cell, glucose induces oscillations of $[Ca^{2+}]_i$ with a frequency of 0.2–0.5 min^{-1} [1,2]. With an additional increase of glucose the oscillations are essentially unaltered or transformed into a sustained elevation of $[Ca^{2+}]_i$. Glucose can also induce fast oscillations (4–25 min^{-1}) superimposed on the slow ones or on the elevated steady-state level of $[Ca^{2+}]_i$. The two types of $[Ca^{2+}]_i$ oscillations are seen both in individual β-cells [1,3–6] and intact islets [7–12].

The ability to respond to glucose with oscillations is critically dependent on the condition of the β-cell. Actually, disappearance of $[Ca^{2+}]_i$ oscillations is a sensitive indicator of β-cell damage [13,14]. In generating pulsatile secretory activity it is likely that the slow $[Ca^{2+}]_i$ pulses produce the fluctuations of insulin known to occur in circulating blood [12,15,16]. It has been reported that the regular oscillations of plasma insulin disappear during the development of both type 1 [17] and type 2 diabetes [18].

While representing a fundamental aspect of glucose-stimulated insulin release it is important to analyze the mechanisms behind the oscillatory $[Ca^{2+}]_i$ activity in the β-cell. In the present study attention has been given to how the plasma membrane permeabilities of K^+ and Ca^{2+} affect the glucose-induced slow oscillations. It will be shown that such oscillations depend on the closure of ATP-regulated K^+ channels and appear when the rate of Ca^{2+} influx is sufficient to result in a temporary imbalance between the addition and removal of Ca^{2+} from the cytoplasm.

2. Materials and methods

2.1. Materials

Reagents of analytical grade and deionized water were used. Fura-2 and its acetoxymethyl ester were supplied by

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Molecular Probes, Eugene, OR, USA. Collagenase and Hepes were obtained from Boehringer Mannheim, Germany. Tetraethylammonium chloride (TEA), quinine and bovine serum albumin (fraction V) were supplied by Sigma, St. Louis, MO, USA. (±) BAY K 8644 was provided by Calbiochem, San Diego, CA, USA. Tolbutamide was kindly donated by Hoechst AG, Frankfurt/Main, Germany.

2.2. Preparation of β-cells

Islets of Langerhans were isolated by collagenase digestion of pancreas from 10-month-old *ob/ob* mice taken from a non-inbred colony [19]. Previous studies have indicated that these islets contain more than 90% β-cells [19], which respond normally to glucose and other stimulators of insulin release [20]. Single cells were prepared by shaking in a Ca^{2+}-deficient medium [21] and suspended in RPMI 1640 medium containing 10% fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin and 30 μg/ml gentamicin. The cells were allowed to attach to circular cover glass and were incubated at 37°C in an atmosphere of 5% CO_2 in humidified air for 24–48 h. The selection of β-cells for analysis was based on their large size and low nuclear/cytoplasmic volume ratio compared with the islet cells secreting glucagon and somatostatin [22].

2.3. Measurements of cytoplasmic Ca^{2+}

Subsequent experimental handling was performed with a medium containing in mM: NaCl 125, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$, 1.3, Hepes 25, adjusted to pH 7.40 with NaOH and supplemented with 0.5 mg/ml bovine serum albumin. Measurements of $[Ca^{2+}]_i$ in single β-cells were done by dual wavelength microfluorometry adhering to a previously described protocol [14]. The cells were loaded with fura-2 for 30–40 min by incubation with 0.5 μM of its acetoxymethyl ester. The loaded cells were studied in a perfusion chamber placed on the stage of an inverted microscope within a climate box maintained at 37°C. The indicator was excited at 340 and 380 nm and the fluorescence emitted at 510 nm was measured with a photomultiplier. $[Ca^{2+}]_i$ was calculated from the 340/380 nm fluorescence excitation ratio as previously described [1,23].

2.4. Statistical analysis

Results are expressed as means ± S.E. Statistical comparisons were made using Student's *t*-test for paired data.

3. Results

Under basal conditions (3 mM glucose, 1.3 mM Ca^{2+}) cytoplasmic Ca^{2+} remained stable in the range of 60–90 nM. Representative responses of individual β-cells to

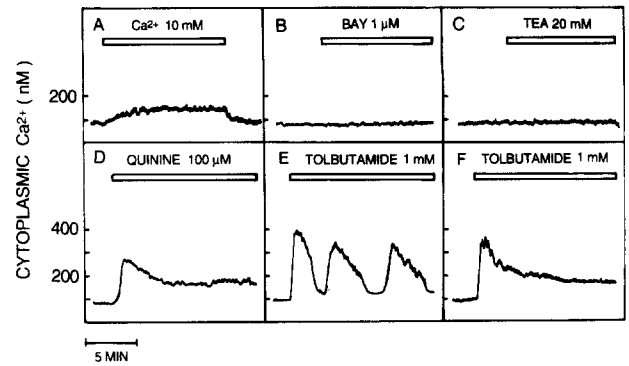

Fig. 1. Effects on $[Ca^{2+}]_i$ obtained in the presence of 3 mM glucose after raising extracellular Ca^{2+} or adding BAY K 8644, TEA, quinine and tolbutamide. The responses are representative for (A) 7 of 7 cells, (B) 5 of 7 cells, (C) 6 of 6 cells (D) 18 of 18 cells, (E) 4 of 17 cells and (F) 13 of 17 cells.

agents with different potencies to promote the entry of Ca^{2+} are demonstrated in Fig. 1. After raising external Ca^{2+} to 10 mM there was an increase of $[Ca^{2+}]_i$ by 55 ± 6 nM ($n = 7$). Addition of L-type Ca^{2+} channel agonist BAY K 8644 did not affect $[Ca^{2+}]_i$ except for an occasional sustained elevation of 10–20 nM. While testing K^+-permeability blockers there was no effect with TEA but a substantial increase with tolbutamide and quinine. Whereas some β-cells responded to tolbutamide with slow oscillations, the remaining cells (80%) reacted with a sustained elevation of $[Ca^{2+}]_i$.

Slow oscillations of $[Ca^{2+}]_i$ appeared in 80% of the β-cells responding to 11 mM glucose. The oscillations originated from the basal level, reached peak values of 200–500 nM and had frequencies of 0.2–0.5 min^{-1}. The characteristics of the oscillations in the individual β-cell

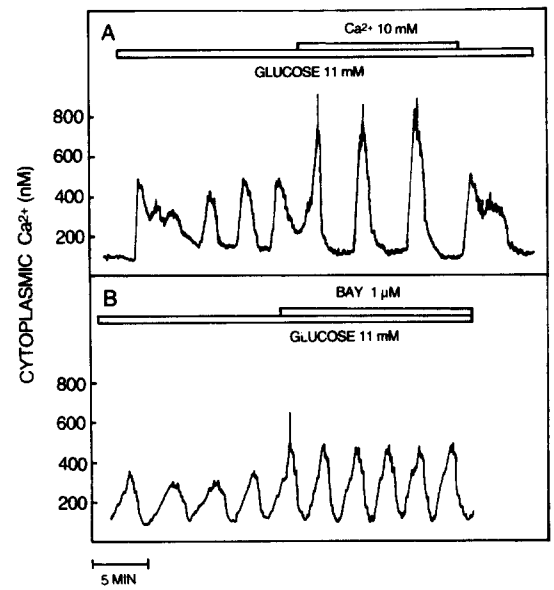

Fig. 2. Effects on glucose-induced oscillations of $[Ca^{2+}]_i$ after raising extracellular Ca^{2+} or adding BAY K 8644. The responses are representative for (A) 6 of 6 cells and (B) 14 of 15 cells.

Table 1
Effects on glucose-induced oscillations of $[Ca^{2+}]_i$ after raising extracellular Ca^{2+} or adding BAY K 8644 or TEA

		Ca^{2+}	BAY	TEA
Amplitude (nM)	Before	300 ± 40	270 ± 20	250 ± 20
	After	580 ± 90	420 ± 40	380 ± 20
	Effect	+280 ± 60 **	+150 ± 30 **	+130 ± 10 ***
Frequency (min^{-1})	Before	0.31 ± 0.01	0.29 ± 0.03	0.32 ± 0.02
	After	0.26 ± 0.02	0.36 ± 0.03	0.51 ± 0.06
	Effect	−0.05 ± 0.02 *	+0.07 ± 0.01 ***	+0.19 ± 0.05 **
Slope at half-rise (nM s^{-1})	Before	7.6 ± 1.2	7.5 ± 1.3	6.4 ± 0.6
	After	40.0 ± 9.0	19.3 ± 3.7	24.6 ± 3.5
	Effect	+32.4 ± 8.9 *	+11.8 ± 3.4 **	+18.2 ± 2.6 ***
Slope at half-decline (nM s^{-1})	Before	−6.0 ± 0.7	−7.2 ± 1.3	−8.9 ± 1.0
	After	−11.6 ± 3.1	−13.3 ± 1.5	−15.0 ± 2.5
	Effect	−5.6 ± 3.0	−6.1 ± 1.9 **	−6.1 ± 2.4 *

Oscillations of $[Ca^{2+}]_i$ were induced in individual pancreatic β-cells by 11 mM glucose before raising extracellular Ca^{2+} from 1.3 to 10 mM (6 cells) or adding 1 μM BAY K 8644 (14 cells) or 10–20 mM TEA (20 cells). The amplitudes (height above basal $[Ca^{2+}]_i$ at 3 mM glucose), frequencies and slopes are given as mean values ± S.E.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

remained essentially unaltered during more than 60 min. When stimulating the influx of Ca^{2+} either by raising the extracellular concentration to 10 mM or adding BAY K 8644 the peaks of the glucose-induced oscillations became higher (Fig. 2; Table 1). Whereas the frequency increased in the presence of BAY K 8644, the opposite was seen after raising extracellular Ca^{2+} (Fig. 2; Table 1). K^+ channel blockers modified the oscillations in different ways (Fig. 3). The presence of 10–20 mM TEA resulted in an increase of both the amplitude and the frequency (Table 1), but quinine transformed the $[Ca^{2+}]_i$ oscillations into a sustained elevation with superimposed transients. Two types of responses to tolbutamide were observed. In 7 out of 15 cells there was transformation of the glucose-induced

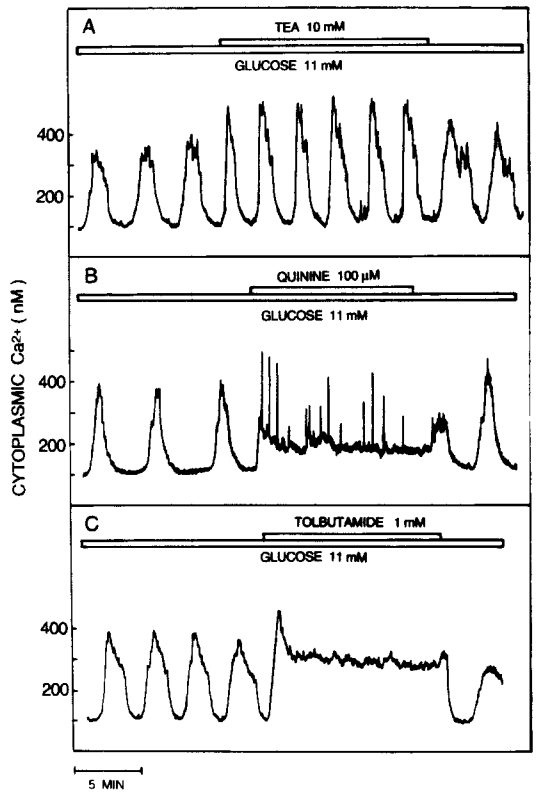

Fig. 3. Effects on glucose-induced oscillations of $[Ca^{2+}]_i$ after addition of TEA, quinine and tolbutamide. The responses are representative for (A) 20 of 20 cells, (B) 16 of 16 cells and (C) 7 of 15 cells.

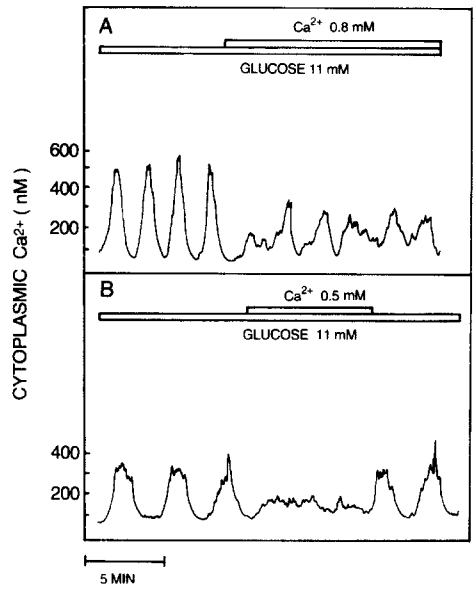

Fig. 4. Effects on glucose-induced oscillations of $[Ca^{2+}]_i$ after lowering extracellular Ca^{2+} from 1.3 to 0.5–0.8 mM. The cells reacted either with (A) decrease of the amplitudes of the oscillations or with (B) transformation of the oscillations into sustained elevation of $[Ca^{2+}]_i$ above the basal level. The latter reaction was seen in 9 of 17 cells at 0.8 mM Ca^{2+} and in 11 of 12 cells at 0.5 mM Ca^{2+}.

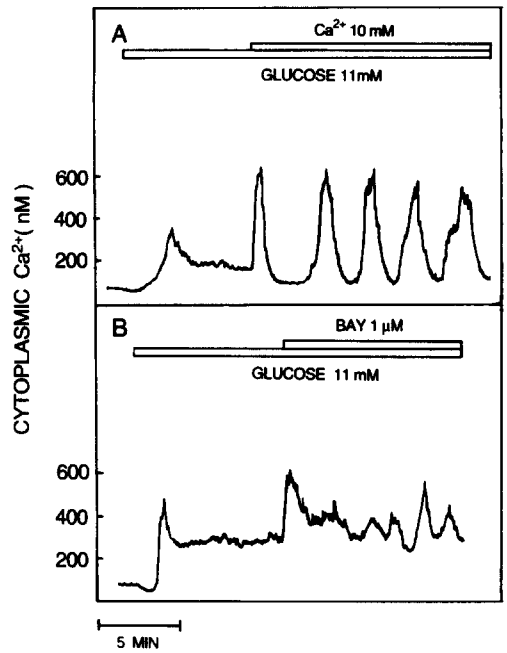

Fig. 5. Effects on glucose-induced sustained elevation of $[Ca^{2+}]_i$ after raising extracellular Ca^{2+} or adding BAY K 8644. The responses are representative for (A) 8 of 8 cells and (B) 6 of 6 cells.

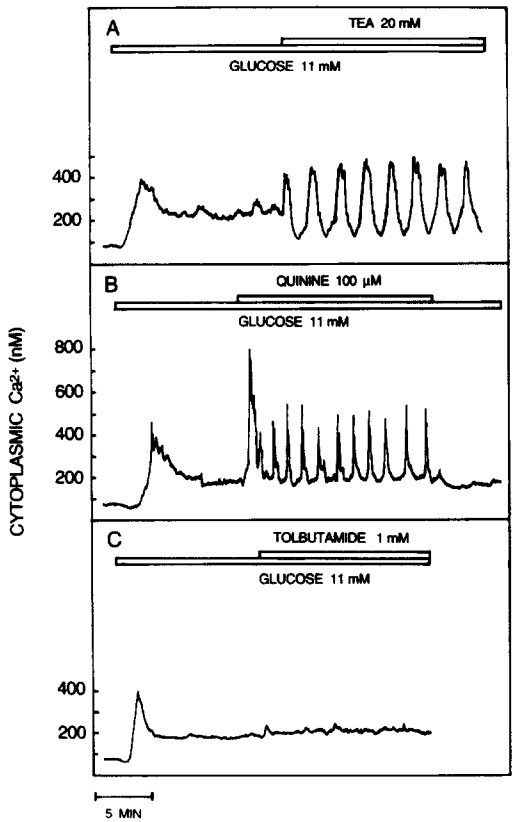

Fig. 6. Effects on glucose-induced sustained elevation of $[Ca^{2+}]_i$ after addition of TEA, quinine and tolbutamide. The responses are representative for (A) 14 of 15 cells, (B) 11 of 11 cells and (C) 7 of 7 cells.

$[Ca^{2+}]_i$ oscillations into a sustained elevation and in the remaining cells the oscillations were essentially unchanged. Stimulating Ca^{2+} entry by raising the extracellular concentration or adding BAY K 8644 or TEA increased the steepness of the rising phase more than that of the descending phase (Table 1). It was also tested how the suppression of Ca^{2+} influx affected the glucose-induced $[Ca^{2+}]_i$ oscillations. Lowering the extracellular Ca^{2+} concentration to 0.8 or 0.5 mM resulted either in decreased amplitudes of the oscillations or in their transformation into a sustained elevation slightly above the basal value (Fig. 4).

A minority of the β-cells (11%) responded to 11 mM glucose with a sustained elevation of $[Ca^{2+}]_i$ instead of oscillations. It was tested whether such a sustained elevation could be transformed into slow oscillations by promoting the entry of Ca^{2+}. This was indeed the case. Whereas raising external Ca^{2+} to 10 mM induced oscillations from the baseline, oscillations obtained by an addition of 1 μM BAY K 8644 occurred from an elevated level (Fig. 5). Different types of reactions were noted when K^+ channel blockers were added. Whereas TEA induced characteristic slow oscillations from the basal level (Fig. 6A), quinine triggered pronounced transients superimposed on the sustained elevation (Fig. 6B). It was not possible to affect glucose-induced sustained elevation of $[Ca^{2+}]_i$ by the addition of tolbutamide (Fig. 6C).

4. Discussion

It is well established that periodic entry of Ca^{2+} via voltage-dependent channels underlies the glucose-induced slow oscillations of $[Ca^{2+}]_i$ in individual β-cells [2]. After the observations that glucose induces periodic variations in oxygen consumption [8] and lactate production [24] in batches of islets as well as cyclic changes of reduced pyridine nucleotides in individual β-cells [25], it has been suggested that the $[Ca^{2+}]_i$ oscillations are determined by variations in the metabolism. Considering the fundamental role of ATP-sensitive K^+ channels (K_{ATP}) for the membrane potential, cyclic variations in the activity of these channels may account for a rhythmic depolarization resulting in periodic entry of Ca^{2+}. Actually, the recent demonstration of an inherent periodic activity of the K_{ATP} channels supports the idea that periodic fluctuations of the metabolism is a primary event and does not result from Ca^{2+} influx [26]. In the present study the mechanism for glucose generation of slow oscillations of $[Ca^{2+}]_i$ was approached by modifying the rate of Ca^{2+} entry either directly or by blocking the K^+ permeability.

In accordance with previous observations [2,27] it was found that closure of K_{ATP} channels by elevation of the glucose concentration or the addition of tolbutamide often results in slow oscillations of $[Ca^{2+}]_i$ in isolated β-cells. The addition of L-leucine, which generates ATP through

metabolism, has also been found to induce such oscillations [28]. However, depolarization by other means, e.g. raising the K^+ concentration or adding the positively charged amino acid arginine, is known to result in a sustained elevation of $[Ca^{2+}]_i$ without oscillations [2,28]. Since quinine is a potent blocker of K_{ATP} channels [29], it may appear surprising that this inhibitor did not induce oscillations but a sustained elevation of $[Ca^{2+}]_i$ in the presence of 3 mM glucose. This may be due to the action of quinine in blocking additional types of K^+ channels [30] and the fact that the inhibition is less reversible than that obtained with glucose or tolbutamide [29]. The observation that a high concentration of TEA was unable to raise $[Ca^{2+}]_i$ in the presence of 3 mM glucose is consistent with its relatively weak blocking of the K_{ATP} channels [29].

A prerequisite for glucose-induction of slow $[Ca^{2+}]_i$ oscillations was that the extracellular Ca^{2+} concentration exceeded 0.5–0.8 mM. At this threshold there was usually a suprabasal elevation, implying that the stimulated entry of Ca^{2+} is precisely balanced by removal of the ion from the cytoplasm. In support for the idea that a certain rate of Ca^{2+} entry is required to upset such a balance, the glucose-induced sustained elevation of $[Ca^{2+}]_i$ obtained at 1.3 mM Ca^{2+} was transformed into oscillations when promoting the entry of Ca^{2+} with high extracellular Ca^{2+}, BAY K 8644 or TEA. Whereas BAY K 8644 enhances the entry of Ca^{2+} by prolonging the open state of the voltage-dependent Ca^{2+} channels [31], TEA counteracts the termination of the action potentials by blocking Ca^{2+}-activated and delayed rectifying K^+ channels [29,32,33]. It is of interest that a threshold concentration of extracellular Ca^{2+} is required not only for inducing slow $[Ca^{2+}]_i$ oscillations in the isolated β-cell but also for observing bursting electrical activity in β-cells situated in intact islets [9]. Moreover, it has been reported that stimulation of the entry of Ca^{2+} by raising the extracellular Ca^{2+} concentration or adding BAY K 8644 or TEA results in transformation of continuous electrical activity observed in the presence of tolbutamide into a phasic pattern [34]. The influence of the Ca^{2+} influx rate on the glucose-induced slow oscillations of $[Ca^{2+}]_i$ was also apparent from the preferential acceleration of the rising phases of the oscillations when Ca^{2+} entry was stimulated by high external Ca^{2+}, addition of BAY K 8644 or TEA. It is notable that a rise of the extracellular Ca^{2+} concentration had opposite actions to TEA or BAY K 8644 in reducing the frequency of the $[Ca^{2+}]_i$ oscillations. A prolongation of the intervals between the oscillatory $[Ca^{2+}]_i$ peaks after raising extracellular Ca^{2+} has also been demonstrated in β-cells from intact islets [9,35]. Such an effect may arise from Ca^{2+} binding to the cell surface, resulting in a stabilization of the closed state of the Ca^{2+} channels [36].

The present study illustrates the critical role of Ca^{2+} entry for the glucose generation of the slow oscillations of $[Ca^{2+}]_i$. It can be anticipated that the rise of $[Ca^{2+}]_i$ influences both the formation and breakdown of ATP. An increase of Ca^{2+} concentration in the β-cell cytoplasm is rapidly reflected in the mitochondrial matrix [37], with resulting stimulation of NADH-producing dehydrogenases [38,39]. However, it still remains to be shown whether oscillations of the mitochondrial ATP production can be amplified by the $[Ca^{2+}]_i$ oscillations [40–42]. In the submembrane space the increase of $[Ca^{2+}]_i$ results in an enhanced ATP hydrolysis related to the outward transport of Ca^{2+} by the plasma membrane Ca^{2+}-ATPase and maintenance of the Na^+ gradient used for the Na^+/Ca^{2+} exchange. Accordingly, it can be expected that Ca^{2+} has a negative feedback effect on its own entry mediated by the activity of the K_{ATP} channels. In support for the idea that the activity of ion transporting pumps affects the ATP concentration in the submembrane space of the β-cells, it has been found that inhibition of the Na/K-ATPase results in a closure of the K_{ATP} channel [43].

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