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VARIATIONS IN ATP-SENSITIVE K^+ CHANNEL ACTIVITY PROVIDE EVIDENCE FOR INHERENT METABOLIC OSCILLATIONS IN PANCREATIC β-CELLS

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The cell-attached configuration of the patch clamp technique was used for studying slow variations in the activity of the ATP-sensitive K^+ channels in pancreatic β-cells isolated from mouse and man. In 0 or 3 mM glucose, the fraction of time the channels were open exhibited oscillations with frequencies in the 0.25-0.40 /min range. This phenomenon is a strong argument for inherent fluctuations in the ATP production of the β-cells. Variations in metabolism may thus be a major determinant for the characteristic large amplitude oscillations of cytoplasmic Ca^{2+} with equivalent frequency.

Glucose-induced insulin release is mediated by a rise of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) following increased entry of the ion into the pancreatic β-cells (1). The glucose-stimulated β-cell exhibits characteristic slow large amplitude oscillations of $[Ca^{2+}]_i$ (2,3), which are synchronized with pulsatile release of insulin (4). Since pulsatile secretion is believed to be significant for counteracting diabetes (5), it is fundamental to explore the mechanism underlying the generation of the $[Ca^{2+}]_i$ oscillations.

It has previously been shown that individual β-cells exposed to glucose exhibit slow bursts of action potentials with similar frequency as the large amplitude oscillations in $[Ca^{2+}]_i$ (6,7). Although these observations indicate that periodic depolarization underlie the oscillations in $[Ca^{2+}]_i$, it is not evident how the rhythmicity is generated. Some observations support the idea that rise in $[Ca^{2+}]_i$ above a a critical level initiates hyperpolarization with feed-back inhibition of further Ca^{2+} entry (3,8). However, since glucose-induced depolarization is mediated by closure of the ATP-sensitive K^+ channels (K^+_{ATP}) (9,10), it is also possible that the cycles of depolarization are determined by oscillations in β-cell

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metabolism. The latter alternative has now been investigated by recording the activity of the K^+_{ATP} channels under conditions eliminating feed-back effects of high $[Ca^{2+}]_i$. We report that the activity of the K^+_{ATP} channels in pancreatic β-cells from mice and man is subject to cyclic variations with frequencies equivalent to the large amplitude oscillations of $[Ca^{2+}]_i$. Since these observations were made at lower concentrations of glucose than required for the generation of action potentials, it is evident that a rhythmic behavior is an intrinsic property also of the non-stimulated β-cell.

MATERIALS AND METHODS

Islets of Langerhans were isolated by collagenase digestion from four adult non-inbred *ob/ob*-mice (11) and two human cadaveric organ donors. Single cells were prepared according to previously described protocols (2,12) and suspended in RPMI 1640 medium supplemented with 10 % fetal calf serum, 100 I.U. penicillin, 100 μg/ml streptomycin and 10 μg/ml gentamicin. The cells were allowed to attach to circular 25 mm cover slips during culture for 1-3 days at 37° C in an atmosphere of 5 % CO_2 in humidified air. The cover slips were rinsed and used as the bottoms of an open chamber designed for microscopic work (13).

Single channel K^+ currents were recorded at 37° C from individual β-cells using the cell-attached configuration of the patch clamp technique (14) with an EPC-9 amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany). The temperature was controlled by thermostatically heating the objective of the microscope, the perifusion inlet and the cell chamber. It was checked that the temperature variations ($\pm$ 0.5° C) did not coincide with the observed periodicity of the channel activity. The cells were bathed in a solution containing (mM): NaCl 138, KCl 5.6, $MgCl_2$ 1.2, $CaCl_2$ 2.6, and HEPES 10 titrated to pH 7.4 with NaOH. For the pipette medium all Na^+ of the above solution was replaced with equimolar K^+.

The currents were digitized (VR-10B digital data recorder; Instrutech Corp., Great Neck, NY) and stored on video tape for later use. Analyzing the data, records were digitized at 5 kHz and filtered at 2 kHz using an Axolab interface and pCLAMP 5.5 software (Axon Instruments Inc., Foster City, CA). Histograms of all data points during consecutive periods of 20 s were formed and fitted to Gaussian distributions (Fig.1). Data points representing the mean amplitudes of K^+_{ATP} channels ($\pm$ 2 SD) were selected and used to calculate the fraction of time the channels were in the open state. Channel activity was expressed as the percentage open time during each 20 s period.

Reagents of analytical grade and deionized water were used. Collagenase and bovine serum albumin were obtained from Boehringer Mannheim GmbH, Mannheim, Germany. Fetal calf serum was provided by Gibco Ltd, Paisley, UK.

RESULTS

Recordings at 37° C of the activity of the K^+_{ATP} channels in mouse β-cells revealed periodic variations of the time in the open state (Fig. 2). The frequencies noted in the absence of glucose for β-cells from two mice were 0.27 and 0.29 /min, and at 3 mM glucose the frequencies were 0.32 and 0.40 /min in cells from another two animals. Analyses of

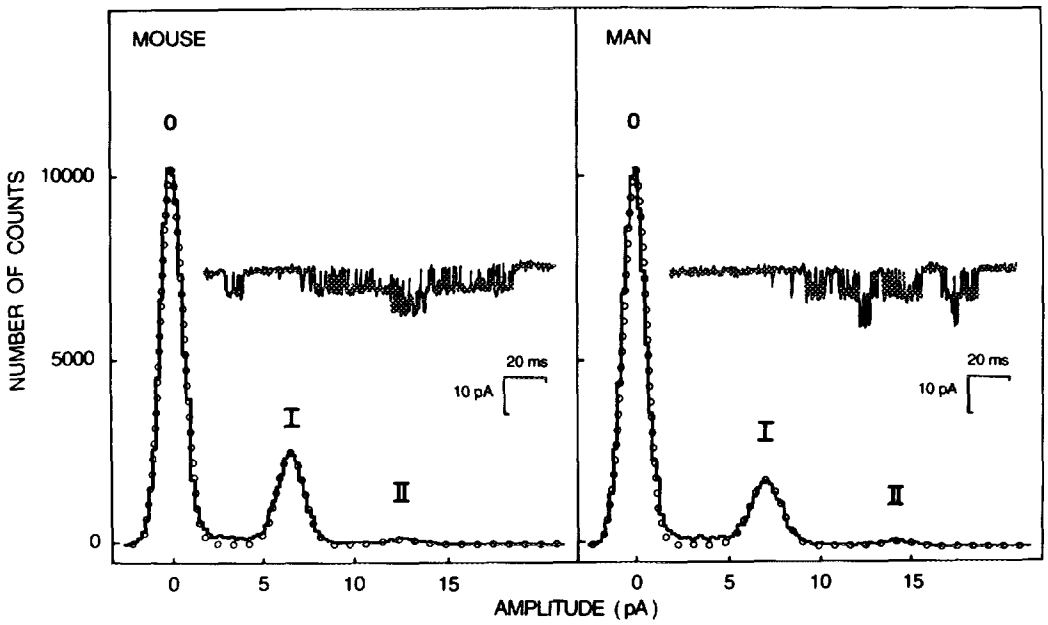

Fig. 1. Histograms of current levels encountered when studying K^+_{ATP} channels (inset) at 37 °C in a mouse (left) and a human (right) β-cell using the cell-attached configuration of the patch clamp technique. The histograms are fitted with Gaussian functions (open symbols) and above each peak the number of K^+_{ATP} channels simultaneously open are indicated. Data points representing the mean amplitudes ± 2 SD were taken for further analyses.

human β-cells from two organ donors revealed a similar pattern of oscillatory activity (Fig 3). In the latter case the frequencies observed in 3 mM glucose were 0.23 and 0.25 /min.

DISCUSSION

The glucose-induced large amplitude oscillations in $[Ca^{2+}]_i$ of individual β-cells coincide with rhythmic depolarization and entry of Ca^{2+} through voltage-dependent channels (3,6,7,15). Since the glucose-induced depolarization is mediated by closure of the K^+_{ATP} channels (9,10), it has been suggested that fluctuations in metabolism determine the oscillations in $[Ca^{2+}]_i$ (16). Evidence favoring oscillatory metabolism have been obtained using perifused batches of pancreatic islets. It was found that oxygen consumption (16) and lactate production (17) oscillate with frequencies similar to the pulsatile release of insulin. However, it is difficult to judge the significance of these observations, since they represent the concerted action of many pancreatic islets without functional coupling. Oscillations in metabolism should in principle be possible to demonstrate also in individual β-cells by recording changes in the fluorescence of reduced pyridine nucleotides. Using this approach

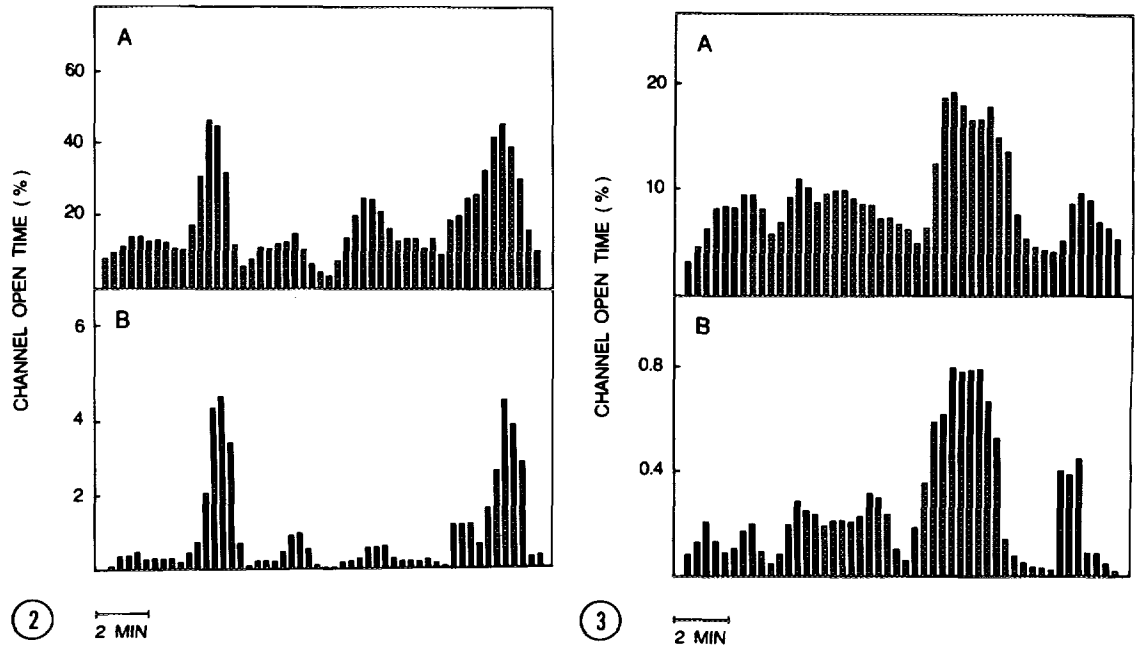

Fig.2. Periodic fluctuations in the K^+_{ATP} channel activity of a mouse β-cell in the absence of glucose. The upper panel shows the percentage open time recorded during 20 s intervals. In the lower panel data for two simultaneous channel openings are presented. Noise is reduced by plotting three-point moving averages.

Fig.3. Periodic fluctuations in the K^+_{ATP} channel activity of a human β-cell in 3 mM glucose. The upper panel shows the percentage open time recorded during 20 s intervals. In the lower panel data for two simultaneous channel openings are presented. Noise is reduced by plotting three-point moving averages.

the β-cells only occasionally exhibited some oscillations (18). The difficulties to demonstrate sustained rhythmicity in the redox state may have technical reasons. Previous studies have revealed that intense UV-light induces photodamage of the β-cells manifested as a loss of their $[Ca^{2+}]_i$ oscillations (19).

Although it has been found that the metabolic events precede the initial rise in $[Ca^{2+}]_i$ in response to glucose (18), $[Ca^{2+}]_i$ oscillations are not necessarily driven by an oscillatory metabolism. Alterations of $[Ca^{2+}]_i$ in β-cells are rapidly reflected in the mitochondrial free Ca^{2+} concentration (20), which determines the activity of various dehydrogenases (21). Accordingly, periodic variations in the metabolism may result from stimulated entry of Ca^{2+} rather than causing the $[Ca^{2+}]_i$ oscillations. In the present study the problems associated with feed-back effects of Ca^{2+} were avoided by studying the K^+_{ATP} channel activity under conditions when the voltage-dependent Ca^{2+} channels are closed.

With the demonstration of cyclic variations in the K^+_{ATP} current at a concentration of glucose lower than required for promoting entry of Ca^{2+} it seems likely that the $[Ca^{2+}]_i$ oscillations have a metabolic origin. The observation of oscillations even in a glucose-free medium cannot be taken as an argument against cyclic variations in the energy metabolism. As indicated from measurements of the oxygen consumption (22) and heat production (23) in isolated islets, the pancreatic β-cells modify their metabolism when deprived of glucose to utilize endogenous substrates as fuels. Since glycolysis is a prototype of an oscillatory metabolic pathway (24), it is noteworthy that the β-cells effectively degrade glycogen when exposed to a glucose-free medium (25). The observed oscillations in the open state of the K^+_{ATP} channels had similar frequencies as noted for the cyclic changes in $[Ca^{2+}]_i$ in individual β-cells from mice (3) and man (12). This similarity and the fact that the frequency of the $[Ca^{2+}]_i$ oscillations was unaffected by the glucose concentration (3) indicate that the β-cell rhythm is independent of the time-average metabolic rate.

It was evident from the discovery of glucose-induced $[Ca^{2+}]_i$ oscillations that a rhythmic behavior is an intrinsic property of the β-cells and not the result of neural pacemaker activity (2). Studying the open state of K^+_{ATP} channels it has now been shown that the β-cells oscillate even when they are not electrically active. Our observations have implications beyond the understanding of the mechanisms behind the $[Ca^{2+}]_i$ cycles initiating pulsatile release of insulin. In addition to affecting the K^+ permeability regulating entry of Ca^{2+}, fluctuations of membrane-associated ATP may periodically activate ion pumping by the Na^+/K^+ and Ca^{2+} ATPases.

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