

A new class of calcium channels activated by glucose in human pancreatic β -cells

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Single calcium-channel currents were recorded from membrane patches of cultured β -cells dissociated from human islets of Langerhans. In the absence of exogenous glucose, low frequency spontaneous calcium-channel openings of small amplitude (-0.34 ± 0.02 pA at 0 mV pipet potential) were observed in all membrane patches examined (25 mM Ca^{2+} in the patch pipet). The frequency of channel openings was rather insensitive to the membrane potential across the patch (range from ca 0 to 60 mV pipet potential; chord conductance 4.9 ± 0.2 pS). Addition of glucose induced a dose-dependent increase in the frequency of openings of the Ca^{2+} -channel (from now on referred to as the Ca_G -channel). A few minutes after the addition of glucose (≥ 11 mM), bursts of action potentials were often observed which were elicited only if Ca^{2+} was present in the solution bathing the β -cells. Application of glucose in the presence of mannoheptulose (11 mM), a blocker of the hexokinase controlling the first stage of glycolysis, had no effect and the activity of the Ca_G -channel remained at its resting level. The readily permeant mitochondrial substrate 2-ketoisocaproate (KIC, 10 mM) was as effective as glucose in eliciting action potentials from cells forming part of cell aggregates. The activity of the Ca_G -channel was significantly increased by KIC (11 mM). Although spike and Ca^{2+} -channel activity were markedly stimulated by glucose or KIC in all cells examined, regular bursts of action potentials were seen only if the patch was formed on β -cells which were part of a cell aggregate. Mannoheptulose (11 mM) prevented the activation of the Ca_G -channel by glucose (11 mM) but not by KIC (11 mM). Once activated, the Ca_G -channel remained active even after excision of the patch. We propose that the physiological control of this Ca^{2+} -channel is mediated by one or more products of glucose metabolism.

Pancreatic β -cell; Ca^{2+} -glucose channel; Glucose sensing; Ca^{2+} -channel

1. INTRODUCTION

Glucose-induced insulin secretion from mouse islets of Langerhans is closely correlated with the ability of the β -cell to generate regular bursts of Ca^{2+} action potentials [1]. In the absence of glucose, the β -cell membrane potential remains constant at about -70 mV. Addition of glucose (< 7 mM) evokes a dose-dependent depolarization of the membrane to about -55 mV. Higher concentrations of glucose induce fluctuations of the membrane potential between two levels, one at about -55 mV and the other at about -40 mV during which Ca^{2+} action potentials occur. Upon removal of glucose the electrical activity ceases, only to return when glucose is reapplied. The link between the secretory and electrical responses appears to be the modulation by glucose metabolism of the β -cell membrane potential which in turn is controlled by at least two K^{+} -channels. The activity of one of these K^{+} -channels, which is voltage-insensitive and blocked by glucose ($< \text{ca } 7$ mM in cell-attached patches), ATP and sulfonylureas (in inside-out patches) [2–5] has been implicated in the control of the membrane potential

from -70 to -55 mV. The properties of another K^{+} -channel, which is activated by membrane depolarization and $[\text{Ca}^{2+}]_i$ [6], have been used to model the burst pattern of electrical activity induced by higher concentrations of glucose. Several studies have shown the inhibitory effects of glucose on the activity of these two K^{+} -channels [2–6] and have emphasized their possible role in glucose sensing.

In contrast with the already important volume of work on K^{+} -channels, very little is known about the effects of glucose on the activity of various types of Ca^{2+} -channels present in the β -cell membrane [7–9]. Evidence in support of the idea that Ca^{2+} -channels may be involved in glucose sensing was provided by the observation of two distinct modalities of glucose-evoked Ca^{2+} entry in rat pancreatic islet cells [10]. Our idea that glucose might modulate Ca^{2+} entry by direct control of voltage-insensitive Ca^{2+} -channels originated in this classical study. We show here that at least one such Ca^{2+} -channel exists in the human pancreatic β -cell. Several distinctive properties, including a weak voltage sensitivity of the channel gating and resistance to blockade by exposure of the inner aspect of the membrane of the patch to a dihydropyridine (nifedipine) or high $[\text{Ca}^{2+}]_i$, distinguish this Ca^{2+} -channel from other types of voltage-gated Ca^{2+} -channels already described [7–9].

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2. MATERIALS AND METHODS

Human pancreatic β -cells for culture were prepared from collagenase-isolated islets from 4 different non-diabetic donors, 3 adults and 1 infant. Cell cultures were maintained for 2–10 days in a medium supplemented with glucose (5.6 mM). After a few days, cells were firmly attached to the bottom of the tissue culture dishes and were ready for patch clamp experiments. For the experiment, islet cells in the tissue culture dish were kept in a modified Krebs medium (in mM: 135 NaCl, 5 KCl, 2.5 CaCl_2 , 1.1 MgCl_2 , 10 Na-Hepes, 2.5 NaHCO_3 , pH 7.4 at 22°C).

Single Ca^{2+} -channel *inward* currents were recorded under voltage-clamp conditions described elsewhere [6]. Single Ca^{2+} -channel currents (cell-attached configuration) were recorded using a high Ca^{2+} solution in the patch clamp pipet (in mM: 140 tetramethylammonium chloride (TMACl), 25 CaCl_2 , 10 Na-Hepes, 10–20 tetraethylammonium (TEA) chloride, pH 7.4). The tip of the patch pipets was fire-polished and coated almost to the tip with Sylgard. These pipets had an open tip resistance in the range of 3–5 M Ω and when cell-attached patches were formed, the resistance rose to 10–20 G Ω .

3. RESULTS

3.1. Glucose activates a small conductance Ca^{2+} -channel

In the absence of exogenous glucose, records from cell-attached patches on single cells or on cells forming part of clumps of cells, always ($n = 49$ cells) showed infrequent Ca^{2+} -channel events (average amplitude of -0.32 ± 0.04 pA at a pipet potential of 0 mV). Ap-

plication of glucose (≥ 11 mM) almost always (13 out of 14 cells) induced a significant increase in the frequency of Ca^{2+} -channel openings. In addition, glucose-evoked electrical activity in all the cells examined ($n = 58$ cells from 4 different donors) and, in nearly all the cells forming part of small clusters (5–20 cells in the cluster), the electrical activity consisted of bursts of action potentials. Once activated, the Ca_G -channel remained active on excised patches.

Fig.1 illustrates the time course of the activation of the Ca_G -channel and electrical activity by glucose (pipet potential set at 20 mV throughout). The control record in fig.1A, made 20 s prior to the application of glucose, shows 3 single channel events (brief downward deflections) representing the flow of positive charge (presumably Ca^{2+}) across the patch from the solution in the pipet into the cell.

Record B in fig.1 was made 40 s after the addition of glucose to the dish (11 mM). It can be seen that glucose-activated channel events appeared as brief bursts separated by longer periods in the non-conducting state (time elapsed between segments B–E varied from 20 to 80 s to include portions in which the membrane patch remained silent). Bursts of channel openings occurred in synchrony with the action potentials (fig.1D,E). While electrical activity ceased ~ 15 s

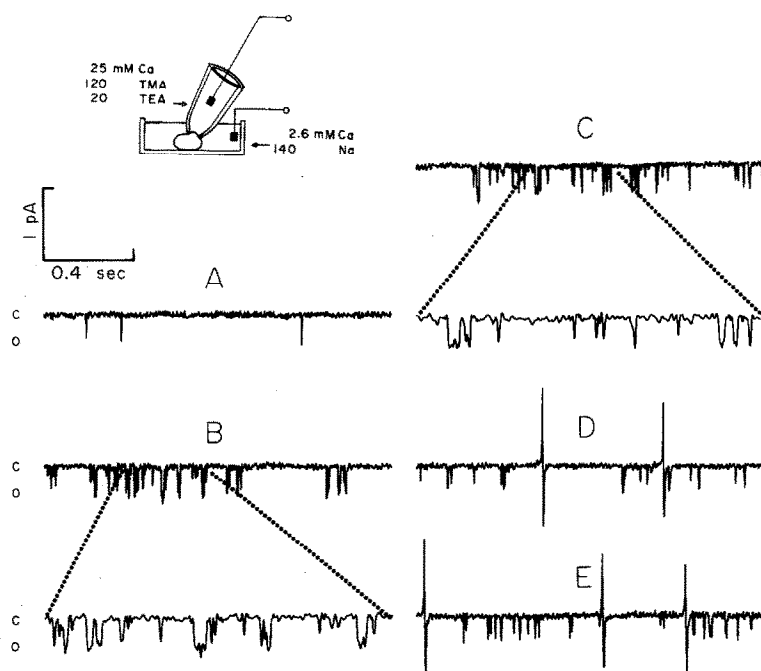


Fig.1. Calcium-channel and electrical activity induced by glucose in human pancreatic β -cells. Segments of a continuous record of the currents across a cell-attached membrane patch from a β -cell forming part of a small cluster. Potential in the solution inside the pipet was 20 mV with respect to the solution bathing the cells. Time elapsed between the segments shown varied from record to record and included long lasting (2–20 s) silent periods. Solution in the dish was Krebs [11] at 20°C. Solution in the pipet contained 25 mM Ca^{2+} [11]. Record A was made 20 s after the application of glucose (11 mM). Records B–E were made in the presence of glucose 40, 60, 80 and 240 s after record A was made. Time scale for segments shown under B and C was expanded 4-fold. Ca^{2+} -channel openings are shown as downward deflexions. D exhibits two records of action potentials occurring in the cells of the cluster. Notice that in record E the amplitude of the capacity currents representing the action potentials is not constant, suggesting that the action potentials were generated in different cells in the cluster. Pipet potential was 20 mV throughout. Records were filtered (8-pole Bessel, Frequency Devices, model 902-LPF, Haverhill, MA) using a cutoff frequency of 500 Hz.

after the addition of a Ca^{2+} chelator (3 mM NaEGTA), channel activity remained unaffected (not shown).

Glucose causes a dose-dependent blockade of K^+ -channels in pancreatic β -cells [2–6]. The immediate consequence of this effect is the depolarization of the β -cell membrane. Since the potential across the patch follows the changes in potential of the membrane outside the patch, glucose-induced stimulation of the Ca_G -channel could be the result of this membrane depolarization. However, similar stimulation of the Ca_G -channel by glucose was recorded from cell-attached membrane patches in which we hyperpolarized the patch by making the potential in the pipet solution 20 mV positive with respect to the potential outside the β -cells (fig.1).

Single Ca_G -channel conductance, obtained from the current–voltage relationship (fig.2) was 4.9 ± 0.1 pS in the presence of exogenous glucose. Although mean values of the single channel current in the absence of glucose ($\circ$) were smaller than in its presence ($\bullet$), the differences were not significant.

3.2. Glucose metabolism is required for the activation of the Ca_G -channel

Addition of glucose in the presence of mannoheptulose (11 mM) failed to activate the Ca_G -channel and electrical activity. In 5 out of 6 β -cells examined using this protocol, Ca_G -channel activity remained at its non-stimulated resting level (data not shown). In one cell,

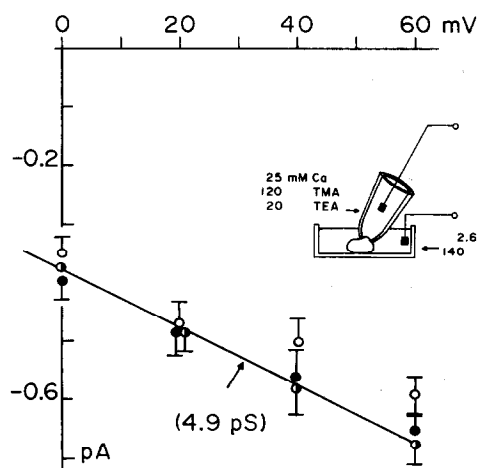


Fig.2. Current–voltage relationships and single-channel conductance for calcium-channels activated by either glucose or KIC. Single Ca^{2+} -channel currents were measured from records digitized using a digital storage oscilloscope (Nicolet Instrument, Madison, WI). They were filtered at 500 Hz and a signal-to-noise ratio of 3 was considered as acceptable to include the event in the statistics. The abscissa represents the potential in the solution in the pipet with respect to that of the solution in the bath. Extrapolated straight line cut the abscissa at -74 mV. Taking the β -cell resting potential as -60 mV and $[\text{K}^+]_i$ as 120 mM, we estimate a permeability ratio $P_{\text{Ca}}/P_{\text{K}}$ of ~ 6 (see section 4). Room temperature throughout was $\sim 20^\circ\text{C}$. Symbols represent: ($\circ$ – $\circ$) no exogenous glucose added; ($\bullet$ – $\bullet$) 11 mM glucose; ($\bullet$ – $\bullet$) 11 mM KIC.

mannoheptulose per se drastically reduced the basal activity of the Ca^{2+} -channels. Reversing this sequence, i.e. application of glucose (11 mM) followed by mannoheptulose (11 mM), caused cessation of the electrical activity in all the cells examined ($n = 3$), it did not affect the activity of the Ca_G -channel. In another cell, mannoheptulose (11 mM) caused an increase in duration of the silent periods between bursts of channel activity and in one cell a complete blockade of the electrical activity evoked by glucose. As illustrated in fig.3, mannoheptulose (11 mM) caused a moderate reduction in the frequency of Ca_G -channel openings and a complete blockade of the electrical activity evoked by glucose. Segments of the continuous record labelled A–D show the activity induced by glucose (11 mM) and segments E–J show the time course of the effect of mannoheptulose (11 mM). The mean amplitude of the single channel currents at 20 mV pipet potential in the presence of glucose was -0.45 ± 0.03 pA. The corresponding value for record J in the presence of mannoheptulose was -0.49 ± 0.05 pA also at 20 mV pipet potential.

3.3. Aerobic metabolism of glucose is necessary for the activation of the Ca_G -channel

The results presented so far suggest that glucose metabolism is required for the initial activation of the Ca_G -channels. To determine if the anaerobic metabolism of glucose was indispensable, we tested the effects of 2-ketoisocaproate, KIC, a readily permeant mitochondrial substrate which, therefore, bypasses glycolysis (fig.4). In the presence of mannoheptulose (11 mM), KIC was as effective as glucose (when tested in the absence of mannoheptulose) to activate the Ca_G -channel (records very similar to those shown in fig.1). KIC (11 mM) in the presence of glucose plus mannoheptulose (11 mM each) not only activated the Ca_G -channel but also reactivated electrical activity.

Since mannoheptulose (11 mM) impaired the activation of the Ca_G -channel by glucose (11 mM) and, as the mitochondrial substrate KIC activates the Ca_G -channel even in the presence of the inhibitor of the hexokinase, glucose action may be mediated by one or more metabolic products of mitochondrial metabolism. The conductance of the Ca^{2+} -channel activated by KIC is 4.8 ± 0.1 pS (fig.2; $\bullet$) and this conductance was not affected by glucose. Because the conductance of the channel is similar whether activated by glucose or KIC, we propose that there is only one type of Ca^{2+} -channel which might be activated by one or several products of mitochondrial function.

3.4. The Ca_G -channel is cation selective

To verify that the channel activated by glucose was indeed a cation-selective channel, we used Cl^- -free solutions either in the dish or in the pipet. The size of the single channel events in cell-attached patches (pipet

solution: 50 mM CaHepes, pH 7.4; $[Ca^{2+}] = 27$ mM measured with a Ca^{2+} electrode) was -0.55 ± 0.06 pA (20 mV pipet potential). This value is slightly greater than the control value obtained with Cl^- in the pipet (fig.2), although the difference is not significant.

After activation of the Ca_G , caused by either glucose or KIC, we observed that channel activity continued over long periods of time (~ 30 min) in inside-out, excised patches exposed to 2.6 mM Ca^{2+} . The channel activity remained upon subsequent exposure of the inside-out patch to a Ca^{2+} - and Cl^- -free Cs^+ solution (mM: 100 CsPipes, CsEGTA at pH 6.8). At a pipet potential of 60 mV single channel currents were in the inward direction with a mean value of -0.38 ± 0.1 pA. This result, together with the observation of channel activity in the complete absence of Cl^- in the dish (see above), demonstrates that the channel is cation selective.

To further characterize the Ca_G -channel, we used excised inside-out patches, exposed to different Ca^{2+} -free solutions and controlled the potential across the patch at -60 mV in an effort to mimic the cell-attached configuration. In the presence of high $[K^+]$ medium (in mM: 75 KCl, 75 Kglutamate, 5 Mg ATP, 5 NaPipes at

pH 7.0, 0.1 NaEGTA) single channel current was -0.35 ± 0.06 pA ($n = 2$ cells) at a pipet potential of 60 mV. With Cs^+ in place of K^+ , the mean value of the single channel current rose to -0.50 ± 0.08 at the same membrane potential.

To further distinguish the Ca_G -channel from other voltage-dependent Ca^{2+} -channels known to be membrane resident, we tested permeation by Ba^{2+} and Cs^+ . The experimental protocol was similar to that illustrated in fig.1, but with either Ba^{2+} (in mM: 50 $BaCl_2$, 10 CsHepes) or Cs^+ (in mM: 100 CsCl, 10 CsHepes) in place of Ca^{2+} in the pipet. In both instances we observed a basal channel activity which was profoundly increased by the addition of glucose (11 mM). Furthermore, electrical activity appeared in the records 30–45 s after the addition of glucose ($n = 3$ cells). Mean single channel current was -0.7 ± 0.1 pA with Ba^{2+} and -0.9 ± 0.07 pA with Cs^+ in the pipet. The size of the single channel current was not affected by glucose (11 mM).

We then studied the selectivity of the Ca^{2+} -channel to different divalent cations in the presence of high $[Ca^{2+}]$ in the pipet. The reversal potential V_{rev} for the Ca_G^{2+} -channel current on cell-attached patches ($n = 3$)

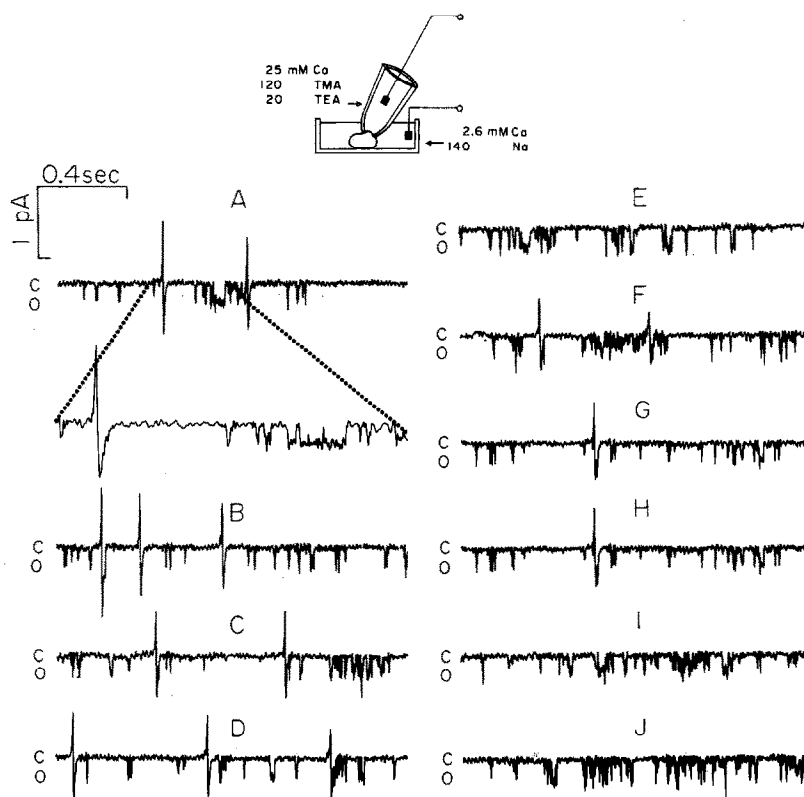


Fig.3. Effects of mannoheptulose on Ca^{2+} -channel activity and the generation of action potentials induced by glucose. Cell-attached patch on a cell forming part of a cluster of ~ 8 cells. Time elapsed between the segments shown varied for different records and included long lasting portions (3–12 s) in which the patch of membrane was silent. Records A–D were made 20, 80, 260 and 440 s after the application of glucose (11 mM). The mean amplitude of the single Ca^{2+} -channel current was measured as -0.45 ± 0.05 pA ($n = 56$ events). Records E–J were made in the continued presence of glucose and 20, 80, 120, 200, 320 and 600 s after the addition of mannoheptulose (11 mM) to the dish. Mean value for the single Ca^{2+} -channel was -0.43 ± 0.05 pA ($n = 112$ events). Time calibration for segment under record A was expanded 4-fold.

was estimated as 14 mV. We may use this value of V_{rev} to estimate the relative permeability P_{Ca}/P_K as follows:

$$V_{rev} = RT/F \ln\{(4P_X[X^{2+}]/P_K[K^+])\},$$

and

$$P_X = P_K / \{1 + \exp(VF/RT)\},$$

where X^{2+} represents the divalent cation in the pipet; R , T and F have their usual meanings and RT/F equals 25.2 mV at 20°C. Taking $[K^+]$ as 120 mM, we estimate P_{Ca}/P_K as 6.

Exposure of the inner aspect of the plasmalemma to Mg^{2+} (in mM: 50 $MgCl_2$, 10 TMA-Hepes, pH 7.4) always resulted in single channel events corresponding to the movement of positive charge from the pipet to the bath ($P_{Ca}/P_{Mg} > 50$). Subsequent exposure of the internal side of the membrane to either Ca^{2+} (in mM: 50 $CaCl_2$, 10 TMA-Hepes, pH 7.4), Ba^{2+} (in mM: 50 $BaCl_2$, 10 TMA-Hepes, pH 7.4) or Sr^{2+} (in mM: 50 $SrCl_2$, 10 TMA-Hepes, pH 7.4) caused the appearance of channel events, the direction of which depended on the potential across the patch of membrane ($n = 3$ cells). While the reversal pipet potential with high Ca^{2+} (50 mM) was 6.2 mV, that for Ba^{2+} (or Sr^{2+}) was ~ 20 mV indicating that both Ba^{2+} and Sr^{2+} are better charge carriers than Ca^{2+} across the open channel. Similar experiments showed that the Ca_G -channels were also permeant to the monovalent cations Li^+ and Cs^+ ($P_{Li} > P_{Ca} > P_{Cs}$). Thus, the results suggest that the channel activity observed in the excised patch corresponds to that of the Ca_G -channel.

4. DISCUSSION

Taken together, our data indicate that modulation of Ca^{2+} entry by glucose may play a crucial role both in

glucose sensing and insulin release in human pancreatic β -cells. We propose that the depolarization of the β -cell membrane evoked by glucose (>6 mM) or KIC (>6 mM) during the onset of electrical activity is due, at least in part, to the activation of this new class of channel. An attractive hypothesis is that the gating of the Ca_G -channel depends on the mitochondrial control of the reduced state of the pyridine nucleotides of the human pancreatic β -cell. We also propose that the activation of this Ca^{2+} -channel may impart a voltage-independent glucose sensing mechanism to the β -cell. As we always detected at least one Ca_G -channel per patch of membrane ($\sim 5 \mu m^2$), we estimate that roughly 110 Ca_G -channels are present in the plasmalemma of a human pancreatic β -cell ($\sim 550 \mu m^2$). Thus, a substantial fraction of the Ca^{2+} required for insulin secretion might enter the β -cell through the Ca_G -channel.

Although the cell cultures used for our studies were rich in insulin producing cells, it was necessary to identify the type of cell used in our studies. This was done in 38 out of the 49 cells examined by adopting the following electrophysiological criteria. Since the electrical response to glucose constitutes a characterizing trait of islet β -cells [11,12], we selected those cells which responded electrically to the addition of glucose (>10 mM). Another criterion used was to check for the presence of both the $[Ca^{2+}]_i$ -sensitive and the glucose-blockable K^+ -channels [3–6]. The presence of the glucose-blockable channel was determined using a patch pipet filled with either the solution used for single Ca^{2+} -channel recordings or with a solution containing no TEA. Under our recording conditions, the conductance of the glucose-blockable K^+ -channel for outward K^+ current was 28 ± 2 pS. Finally, in 3 experiments carried out in the absence of glucose, the hypoglycemic drug glyburide (50 μM) induced continuous electrical

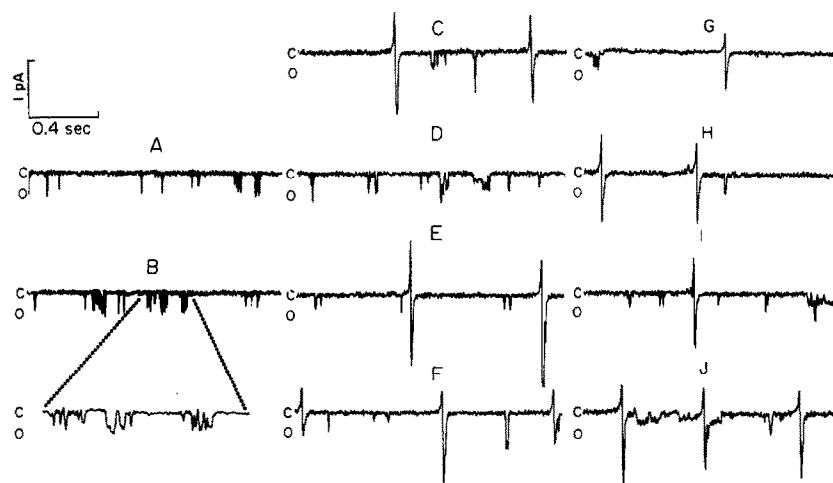


Fig.4. Effects of KIC on Ca^{2+} -channel and electrical activity. Cell-attached patch on a cell forming part of a cluster of ~ 5 cells. Time elapsed between the records shown varied from record to record and included segments in which no electrical or single-channel activity could be detected. Record A was 15 s prior to the application of KIC. Records B–J were made 20, 65, 70, 95, 120, 140, 155, 185 and 210 s after the addition of KIC (11 mM) to the bath. Time for the segment below record B was expanded 4-fold. Mean amplitude from record A is -0.48 ± 0.03 pA ($n = 13$ events). Mean amplitude from records B–J is -0.44 ± 0.08 pA ($n = 35$ events).

activity. The cells responded to the stimulation by glucose of the Ca_G -channel.

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