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Single-channel Ba^{2+} currents in insulin-secreting cells are activated by glyceraldehyde stimulation

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Single-channel Ba^{2+} current recordings have been made from the insulin-secreting cell line RINm5F with the patch-clamp technique. Depolarization evokes opening of Ca^{2+} (Ba^{2+}) channels with a relatively high conductance (30 pS) and during the 200 ms depolarizing pulses there is no inactivation. The threshold is high as 50 mV depolarization from the resting membrane potential of −70 mV is required for any channel opening to occur. Glyceraldehyde, a substance evoking insulin secretion from the RINm5F cells, enhances the voltage-activated Ca^{2+} channel opening by increasing the mean open time and decreasing the longer of the two mean shut times and also decreases the voltage threshold for channel opening.

Patch-clamp; Ca^{2+} channel; Ba^{2+} current; Glyceraldehyde; (RINm5F cell)

1. INTRODUCTION

Ca^{2+} is a key intracellular regulator of insulin secretion. Stimulation of pancreatic B-cells with glucose evokes an increase in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and glucose-evoked insulin secretion is totally dependent on the presence of Ca^{2+} in the extracellular fluid [1]. Entry of Ca^{2+} through the B-cell membrane appears to be mediated by voltage-gated Ca^{2+} currents [2–4] and this is also the case in the insulin-secreting cell line RINm5F [5]. So far there have been no reports of single-channel Ca^{2+} currents in insulin-secreting cells and the purpose of this work is therefore to characterize at the single-channel level the Ca^{2+} currents and their possible regulation by carbohydrate secretagogues. The experiments were carried out on the clonal RINm5F cells that do not respond to glucose stimulation, but can be activated to secrete insulin by glyceraldehyde [6,7], using Ba^{2+} as the current carrier. This is common practice in the field of single-channel Ca^{2+} current recording [8] and has the advantage that K^+ channels are blocked, that the amplitude of single-channel currents is enhanced and that Ca^{2+}-dependent inactivation does not occur.

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2. MATERIALS AND METHODS

All the experiments were carried out on the insulin-secreting cell line RINm5F [6,7] maintained as described [9,10].

Singel-channel current recording was carried out in the cell-attached configuration by standard patch-clamp methods [11] as described for studies on RINm5F cells [10]. The taped current record was filtered at 1 kHz low pass and digitized at 8 kHz (CED, 1401 digitizer). A Tandon microcomputer in conjunction with a software package supplied by CED Cambridge, England, was used for analysis.

The bath solution contained (mM): 140 KCl, 1.13 $MgCl_2$, 2.5 glucose, 10 Hepes and 1 EGTA. The patch-clamp pipettes were filled with a solution containing (mM): 110 $BaCl_2$ and 10 Hepes and the pH of both solutions was adjusted to 7.2. The pipette solution always contained 1 μM tetrodotoxin (TTX).

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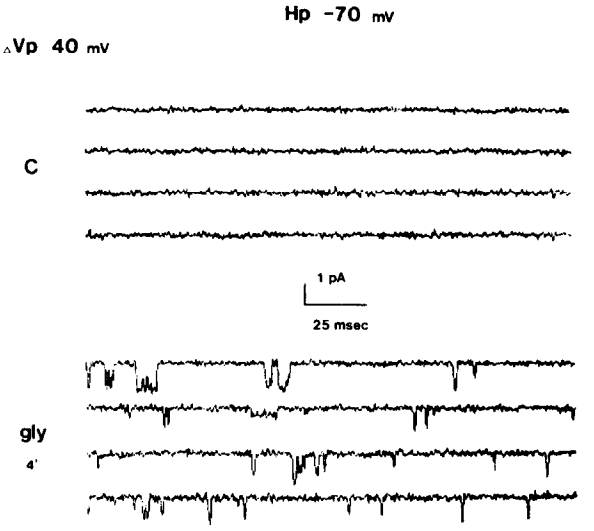


Fig.1. Single-channel Ba^{2+} current recordings from RINm5F cells. Each individual trace represents the membrane patch current during a 200 ms depolarizing voltage jump of 40 mV from the holding potential (V_p = +70 mV corresponding to a potential across the patch membrane of about −70 mV). In the 4 control traces (C) there are no signs of unitary inward current steps whereas in the four lower traces obtained from the same patch 4 min after start of exposing the cell to 10 mM glyceraldehyde (gly) inward (downwards) current steps of varying durations are observed.

3. RESULTS

The pipette voltage (V_p) was held at +70 mV imposing on the electrically isolated membrane patch a normal membrane potential of about −70 mV, since the membrane potential across the cell membrane outside the isolated patch area must be close to 0 mV (bath solution contains 140 mM K^+). Depolarizing potential jumps of 200 ms duration were applied to the pipette. 40 mV depolarizing voltage jumps never evoked transient inward current steps (fig.1) whereas slightly larger steps of 50 mV often resulted in short-lasting inward current steps with an amplitude of about 1.3 pA (fig.2). Further depolarization evoked a marked increase in the time the channels spent in the open state (fig.3). The open-state probability increased

Fig.3. The open-state probability (p) as a function of the magnitude of the depolarizing voltage jumps from the holding patch membrane potential of about −70 mV in the absence (control) and in the presence of 10 mM glyceraldehyde. Mean values ± SE are shown (n varied between 16 and 62).

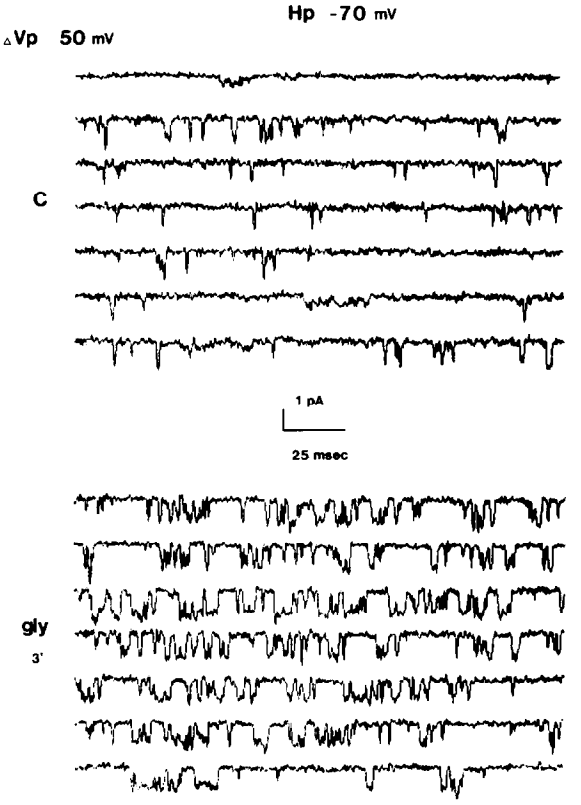
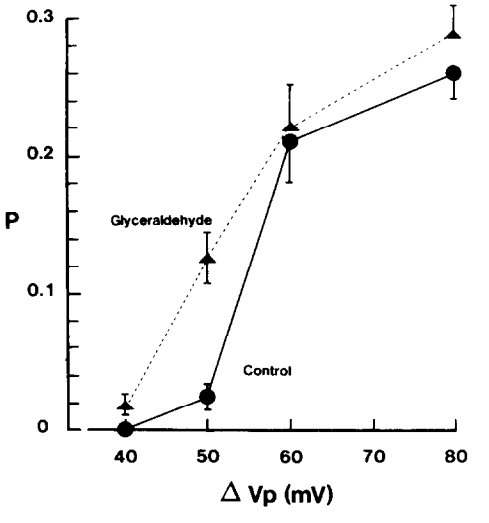


Fig.2. Single-channel Ba^{2+} currents during 50 mV depolarizing voltage jumps from the holding patch membrane potential of about −70 mV. The seven control traces (C) show a low level of channel opening whereas in the lower seven traces obtained from the same membrane patch 3 min after start of glyceraldehyde stimulation frequent and relatively longer inward current steps are seen.

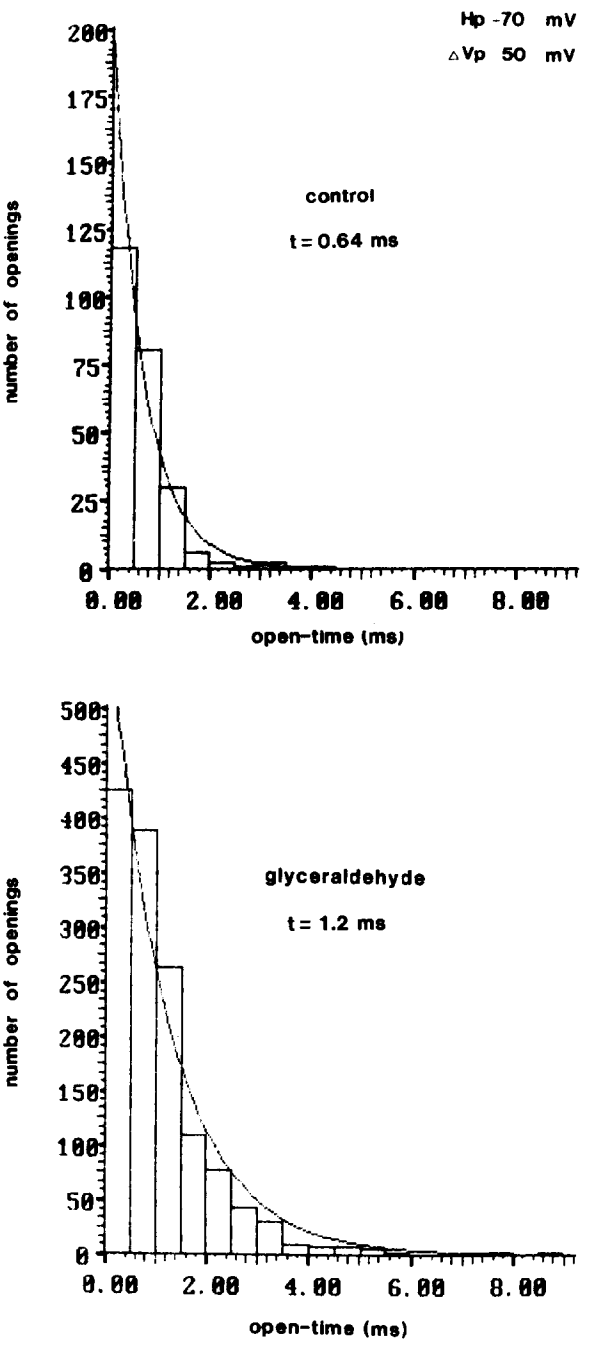


with increasing depolarization whereas the single-channel current amplitude (i) decreased. At ΔV_p = 50 mV i was about 1.3 pA, at 60 mV about 1 pA, at 70 mV about 0.8 pA and at 80 mV 0.55 pA. These values correspond to a single-channel conductance of about 30 pS. Channel openings seemed to occur randomly throughout the 200 ms pulses so that openings were also observed late in the pulse (figs 1,2).

When glyceraldehyde (10 mM) was added to the bath, channel opening was stimulated. Effects were noticed about 2 min after start of glyceraldehyde exposure and were fully developed after 3–5 min of continuous stimulation. Fig.1 shows that 4 min after start of glyceraldehyde stimulation 40 mV depolarizing jumps, which in the control period never caused channel openings, are associated with clear inward current steps with an amplitude of about 1.7 pA. Investigating the effects of 50 mV depolarizing steps, it became clear that the open-state probability (p) was markedly enhanced by glyceraldehyde (fig.2). For the larger voltage jumps there was relatively little difference between control and glyceraldehyde records.

There was no tendency for spontaneous increases in p with time in the absence of glyceraldehyde stimulation. Thus 40 mV depolarizing voltage jumps never resulted in channel openings early or late (after 10 min of recording) in the experiments. For larger voltage jumps p values stayed relatively constant (within ± 25% over periods of 5–20 min), but typically declined slightly.

Inspection of fig.2 gives the impression that glyceraldehyde not only increases the frequency of channel openings but also makes them longer. This can be assessed quantitatively by measuring the dwell times in the open and closed states. The kinetics of Ca^{2+} channels normally fits a 3-state model with one open and two closed states [12,13]. This is also the case in the RINm5F cells as seen in fig.4, which shows the distribution of open and shut times for one experiment (typical of 10). The mean open and close times (control) are very similar to those obtained in cultured heart cells [13]. Glyceraldehyde increases p by making the mean open time longer and reducing the longer of the two mean shut times. This result was obtained in 10 out of the 11 experiments carried out. In one experiment glyceraldehyde had no effect on the mean open time and only slightly reduced the mean second shut time.



4. DISCUSSION

Single-channel Ba^{2+} currents with a unit conduc-

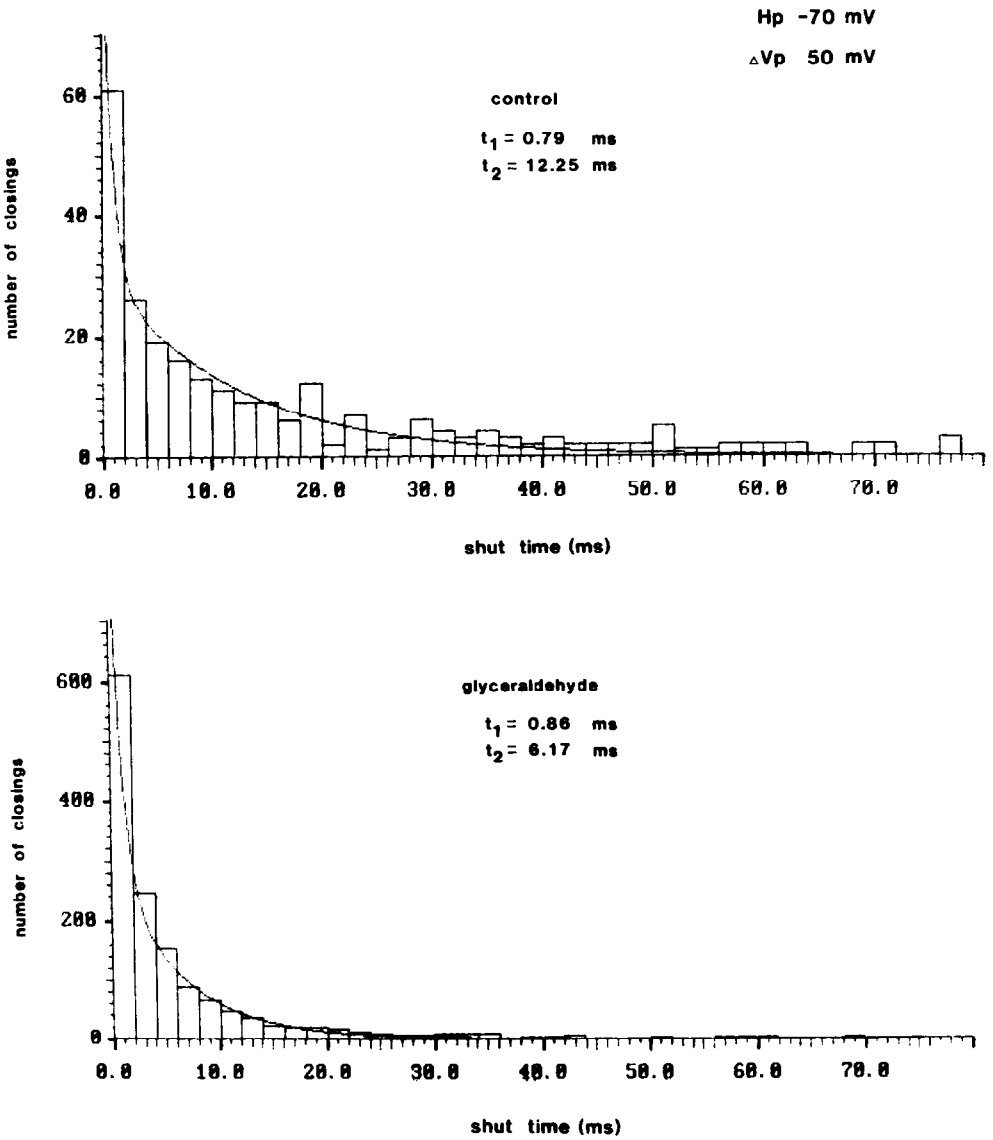


Fig.4. Frequency distribution of open and shut times, all obtained from the same patch before (control) and after start of stimulation with 10 mM glyceraldehyde.

tance of about 30 pS, that do not display inactivation during depolarization pulses of 200 ms duration and have a voltage threshold requiring 50 mV depolarization for channel opening to occur have been demonstrated in the insulin-secreting cell line RINm5F. The characteristics of these channels are similar to those originally described in cardiac cells by Reuter [8] and those later classified as L-channels in neurons [14]. These high-conductance, non-inactivating, high-threshold channels seem to explain the properties of the whole-cell Ba^{2+} currents described in pancreatic B-cells [3,4].

The most important finding in this study is that glyceraldehyde, a substance that stimulates insulin secretion in the RINm5F cells, decreases the voltage threshold for channel activation and increases the mean open time as well as decreasing

the longer of the two shut times. This effect may be of considerable importance. In the RINm5F cells, 10 mM glyceraldehyde depolarizes the cell membrane from about −70 mV to about −20 mV by closing the ATP-sensitive K^+ channels [10]. According to the results shown in fig.3 a 50 mV depolarization by itself only results in a very low level of Ca^{2+} channel opening, but glyceraldehyde markedly increases the open-state probability attained by such 50 mV depolarizing pulses. Glyceraldehyde therefore seems to have two distinct effects on membrane channel properties by (i) acting to close ATP-sensitive K^+ channels thereby promoting depolarization which tends to open Ca^{2+} channels, and (ii) markedly enhancing the voltage-gated Ca^{2+} channel opening. It is likely that glucose has similar effects on normal pancreatic B-cells since their action potentials are small and never overshoot 0 mV [15]. In order to activate the high threshold Ca^{2+} channels of the L-type it would therefore seem necessary to postulate that glucose enhanced the voltage-gated channel opening in a manner similar to that described here for the effect of glyceraldehyde on the RINm5F cells.

Cyclic AMP has been shown to increase the open-state probability of cardiac Ca^{2+} channels [13], but it is unlikely that cyclic AMP is mediating the effect of glyceraldehyde described here, since 10 mM glyceraldehyde failed to evoke any increase in the cyclic AMP level in RINm5F cells in experiments where forskolin (30 μM) caused a 3-fold increase in the cellular cyclic AMP concentration [16]. It would be interesting to investigate whether the glyceraldehyde effect could be mediated by protein kinase C activation via metabolically generated diacylglycerol.

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