SOME PROPERTIES OF SINGLE-CHANNEL Ba CURRENTS IN INSULIN SECRETING CELLS

J. M. VELASCO*, O. H. PETERSEN** and D. PEREZ*
* Departamento de Psicología. Facultad de Psicología. Somosaguas. Madrid.
** Department of Physiology. University of Liverpool.

ABSTRACT

Single-channel Ba^{++} current recordings have been made from the insulin-secreting cell line RINm5F with the patch-clamp technique. We have found two different single-channel currents. Both were distinguished kinetically by applying depolarizing test pulses at various levels from different holding potentials (Hp). Jumps from −70 to −30 mV were insufficient to trigger channel openings, but larger depolarizations evoked L-type unitary inward current. Depolarizing voltage pulses from −90 to −30 or −20 mV activate a different Ca^{++}-channel current characterized by a smaller conductance. This T-type channel activity is not seen with large depolarizing voltage pulses. Glyceraldehyde, a substance evoking insulin secretion from the RINm5F cells, enhance the voltage-activated L-type Ca^{++} channel opening by increasing the mean open-time and also decreases the voltage threshold for channel opening. Stimulation of the cells with the membrane permeable diacilglycerol analogue didecanoylglycerol (DC-10, 5 Ug/ml) markedly enhance the open-time of channels during depolarizing voltage pulses. It seems possible that carbohydrate-evoked cellular Ca^{++} uptake is mediated via protein-kinase C activation.

Key words: Insulin-secreting cell. Channel Ba^{++}. Patch-clamp.

INTRODUCTION

Single-channel recordings by means of the patch-clamp technique provide a method for observing the kinetic properties of individual ion channels in cell membranes (Sackman 1983).

Calcium channels allow passage of Ca ions into the cytoplasm through a selective pore which is opened in response to depolarization of the cell membrane, generating both electrical and chemical signals (reviews by Tsien 1983, Reuter 1983, McCleskey 1986, Tsien 1988).

Ca influx carries depolarizing charge that can contribute to electrical activity such as pacemaker depolarizations or full-blown Ca spikes. Ca entry also leads to a rise in intracellular calcium concentration, a chemical message for calcium sensitive mechanisms that control ion channel gating, transmitter release, enzyme activation, metabilism, gene expression etc. (Tsien 1988).

Carbohydrate-evoked insulin secretion is dependent on extracellular Ca and

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is associated with an increase in the free intracellular Ca concentration, as well as with membrane depolarization and action potential generation. Entry of Ca through the beta-cell membrane it seems mediated by voltage-gated Ca currents (Matthews 1975, Satin 1985, Rorsman 1986).

The coexistence of multiple types of Ca-currents within a given type of cell has been demonstrated in many different vertebrate and invertebrate cell types.

The purpose of the present work is to characterize in the RINm5F cell line, the Ca-currents at the single-channel level and their possible regulation by carbohidrate secretagogues.

MATERIAL AND METHODS

All the experiments were carried out on the insulin-secreting cell line RINm5F (Halban 1983), maintained as described (Findlay 1985).

Single-channel current recording was carried out in the cell-attached configuration by standard patch-clamp methods (Hamil 1981). The taped current record was filtered at 1 kHz low pass and digitized at 8 kHz (CED, 1401 digitizer). A Tandom microcomputer in conjunction with a software package supplied by CED Cambridge England, was used for analysis.

The bath solutions 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 contai-

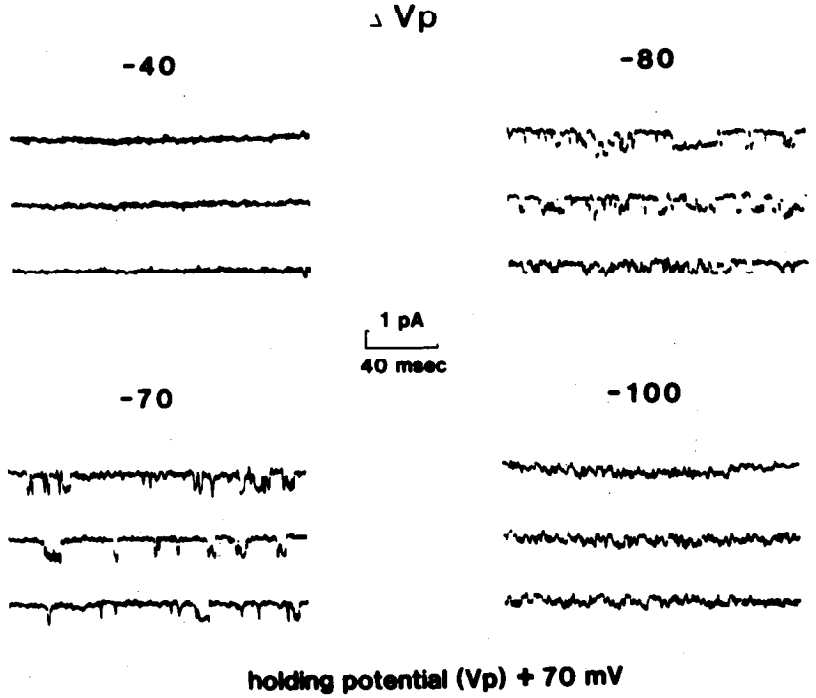

Figure 1.– Single-channel Ba^{++} current recordings from RINm5F cells. Each individual trace represents the membrane patch current during a 200 msec depolarizing voltage jump. Different voltage jumps were applied from a holding potential (Hp = 70 mV) corresponding to a potential across the patch membrane of about −70 mV.

ned 1 Um tetrodotoxin (TTX).

RESULTS

Fig. 1 illustrates the voltage-dependent activation of the unitary Ca channel activity corresponding to L-type Ca currents in single-channel recordings. Each column shows leak-substracted current traces, recorded consecutively during a series of 200 msec test depolarizations from a holding potential (Hp) = 70 mV imposing on the electrically isolated membrane patch a normal membrane potential of about −70 mV.

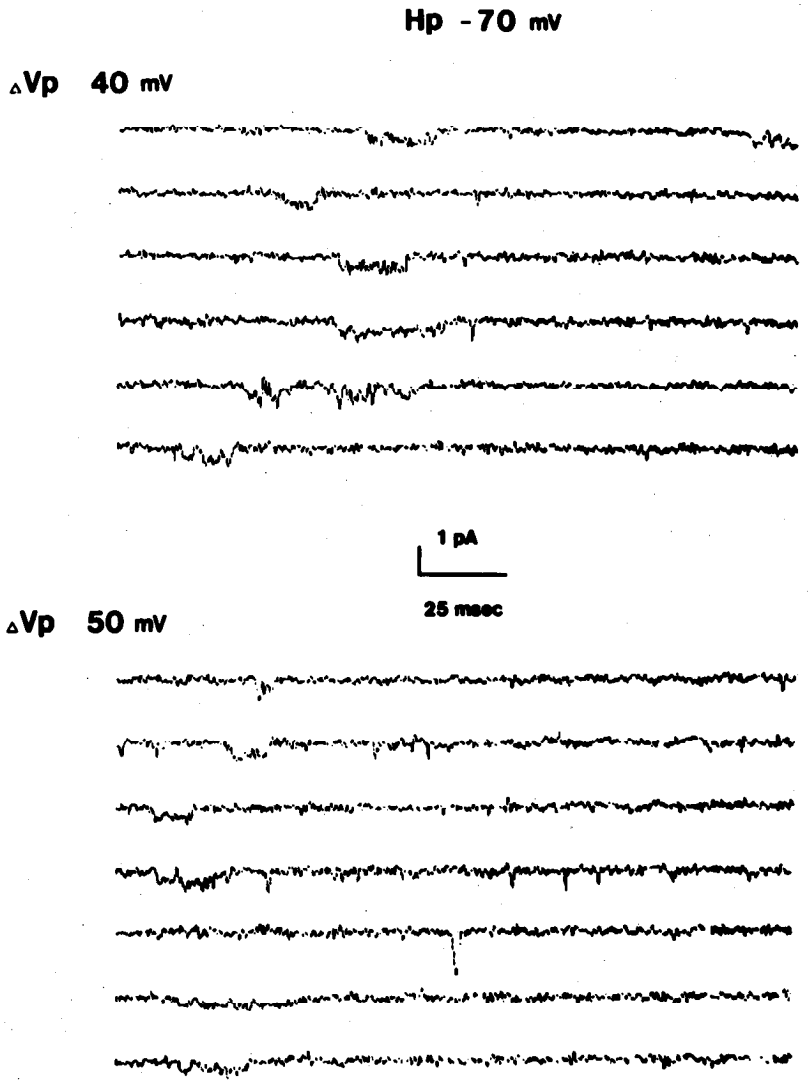

Figure 2.– Single-channel Ba^{++} currents associated with «T-type channels». In general, this kind of channels activate at more negative potentials than the L-type (Fig. 1) as corresponding to their different kinetic properties.

Depolarizing potential jumps were applied to the pipette. 40 mV depolarizing voltage jumps never evoked transient inward current steps, whereas larger steps of 70 mV often resulted in short lasting inward current steps (Fig. 1). Further depolarizations evoked a marked increase in the time the channels spent in the open state, showing so a increase open-state probability, whereas the single channel current amplitude decreased. The single-channel conductance was about 30 pS.

Several characteristic features of the L-type Ca channel activity are evident: 1.- the high-threshold of activation, 2.- channel activity is intense with depolarizations beyond 0 mV (Fig. 1), 3.- openings are fairly evenly distributed throughout the duration of the test pulse.

Fig. 2 shows a second type of bariun currents associated with T-type Ca-channels (Nowicky 1985). Patch recordings allowed each channel type to be studied. Distinctions between these channel types have been based on several criteria including, gating, ionic conductance and pharmacology. T-currents activate at much more negative potentials than the other channel type and can thus be studied in relative isolation.

Depolarizing jumps of 60 an 70 mV from a Hp = 90 mV elicited channel openings much smaller in amplitude than the L-type channels, and with a relatively high tendency to occur near the beginning of the pulse (Fig. 2). The single-channel conductance was about 8 pS.

T-channel activity disappeared when the Hp was shifted from 90 mV to 20, showing so steady-state inactivation, a property no seen in L-type channels for the same Hp.

Calcium channels carbohidrate modulation

In the upper part of Fig. 3, we can appreciate that depolarizing jumps of 50 mV a Hp = 70 mV are able to activate some L-type Ca-channel openings. In addition, is possible to see in some particular records T-type channel openings.

When glyceraldehyde (10 mM) was added to the bath, channel opening was stimulated, using the same depolarizing jump as before in control situation (Fig. 3, lower part). Effects were noticed about 2 minutes after star of glyceraldehyde exposure and were fully developped after 3-5 min of continuous stimulation. The effect of 50 mV depolarizing steps, it become clear that the open-state probability was markedly enhanced by glyceraldehyde, whithout afecting the current amplitude of the channel.

The same cualitative effect was possible to see using a 40 mV depolarizing jumps, but with larger jumps there was relatively little difference between control and glyceraldehyde records.

Glyceraldehyde increases the open-state probability by making the mean open-time longer and reducing the length of the two mean shut time (Velasco 1988).

Carbohidrate stimulation of insulin-secreting cells evokes rapid metabolic generation of diacilglycerol, and exogenous activators of protein-kinase C mimic the carbohydrate-evoked membrane depolarization, and specifically, the cell permeable diacilglycerol analogue di-decanoilglycerol (DC-10) besides evoke depolarization, also elicit the rapid appearence of action potentials (Wollhein 1988). About 1 minute after the start of cellular stimulation with 5 Ug/ml of DC-10, much more frequent and larger inward Ba currents became apparent for a depolarizing jump of 50 mV from a Hp = 70 mV (Fig. 4). In one experiment where 30-100 oscilloscope sweeps of the type shown in Fig. 4 were analysed, the effect of DC-10 was to increase the open-state probability from the

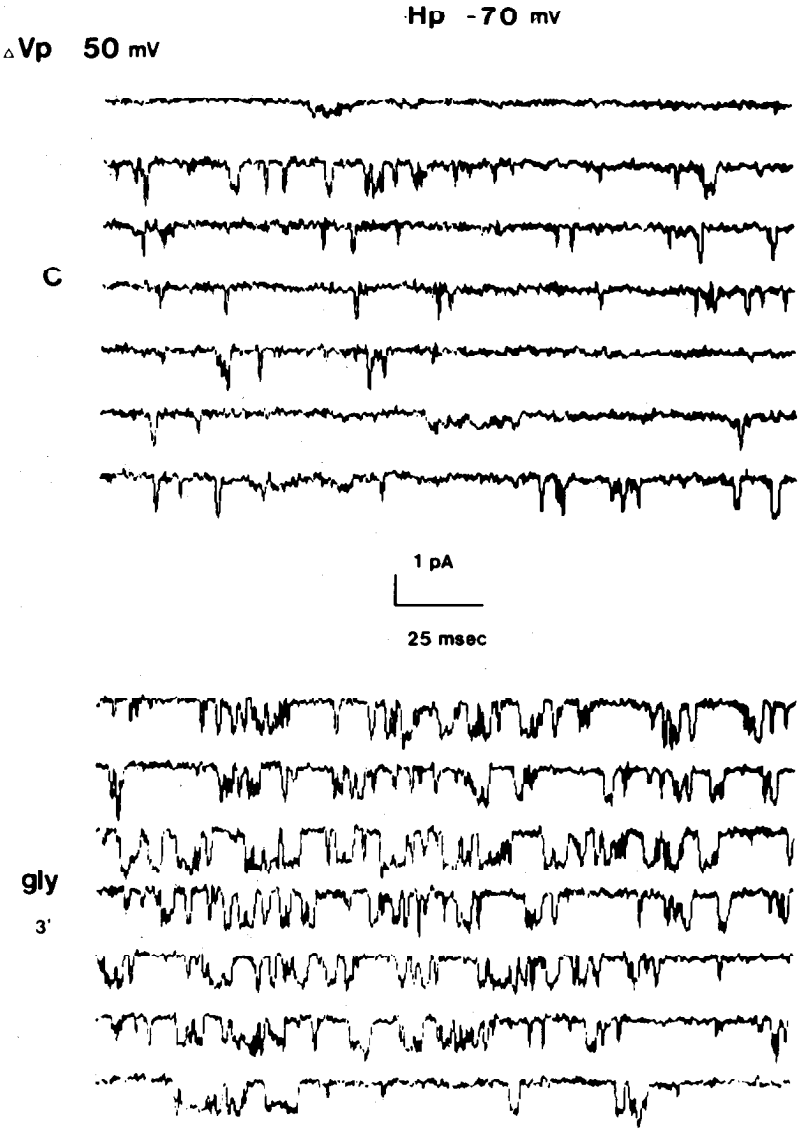

Figure 3.– L-type Ba^{++} currents during 50 mV depolarizing voltage jumps from a holding patch membrane potential of about −70 mV. The control traces (C) show a low level of channel opening. Glyceraldehyde stimulation (gly) increase the open-state probability of the channel, for the same voltage-jump.

control value of 0.0066 + 0.0004 (s.e.m.) to 0.0435 + 0.0014, for a membrane potential of −20 mV.

DISCUSSION

The past recent years have been very productive for the description of cells with

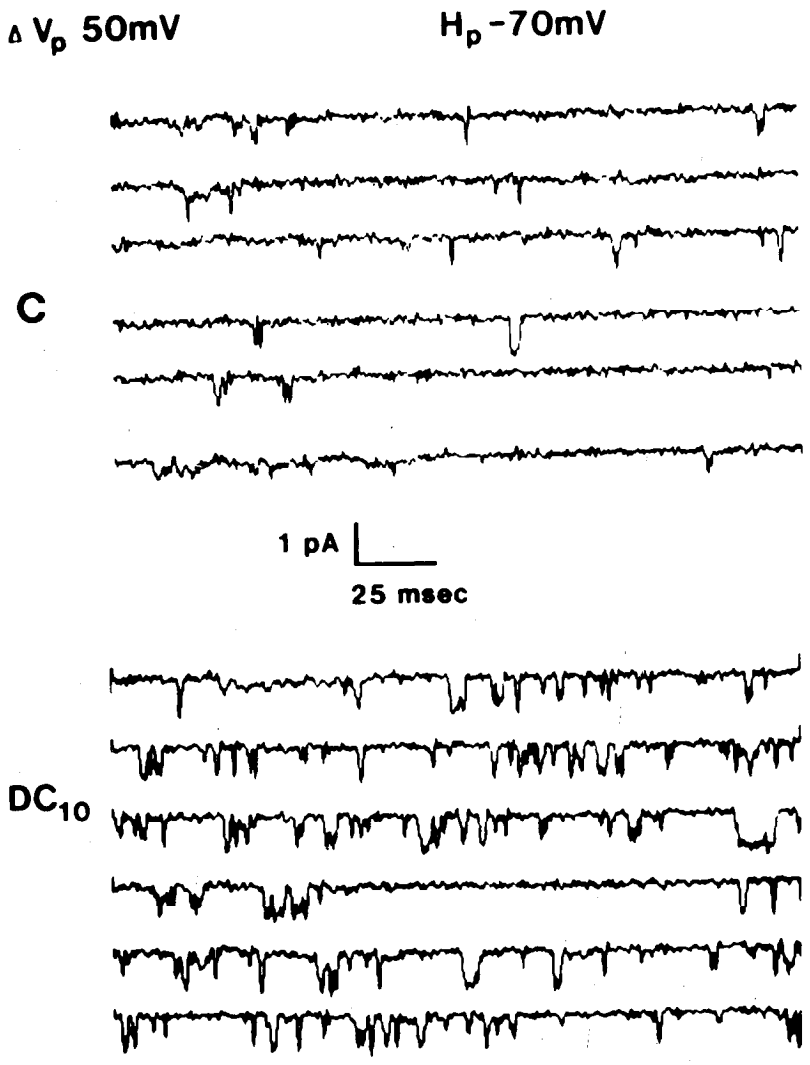

Figure 4.– 50 mV depolarizing voltage jumps from a Hp of 70 mV elicited a low level of L-type channel openings (control), whereas in the same conditions, stimulation with 5 Ug/ml of DC-10 evoked frequent and larger inward current steps.

multiple types of calcium channels, a wide variety of neuronal, muscle and endocrine cells have been shown to have at least two kinds of Ca channels.

Single-channel Ba currents with a unitary conductance of about 30 pS and 8 pS have been now demonstrated in the insulin-secreting RINm5F cell line. The characteristic of these channels are similar to those classified as «L- and T-channels» in neurons (Nowicky 1985). L-type channels are found in neurones, gland cells and muscle cells. Their high-conductance, high threshold and non-inactivating kinetic seem well-suited for converting membrane depolarization into an intracellular

Ca signal for triggering cellular responses. These channels seem to explain the properties of the whole-cell Ba currents described in pancreatic beta-cell (Satin 1985, Rorsman 1986).

T-type channels are found along with L-channels also in a wide variety of neurones and endocrine cells, as well as heart, smooth muscle and skeletal muscle. Their properties are in line with a dominant role in pacemaker activity or rebound excitation (Llinas 1981).

The most important finding in this study is that glyceraldehyde, a substrate 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. According to the results shown in Fig. 3 a 50 mV depolarization by itself only results in a very low level of Ca channel opening, but glyceraldehyde markedly increases the open-state probability obtained by such 50 mV depolarizing pulses. In order to activate the high threshold Ca 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.

The effects of DC-10 stimulation described in the present work and previously (Velasco 1988), are very similar to those found for glyceraldehyde in the same cells (Velasco 1988). It has been shown that DC-10 evokes a Ca-dependent increase in insulin secretion, an acute increase in intracellular calcium concentration, and the inmediate appearence of action potentials (Wollheim 1988). Our results indicate that the DC-10 evoked action potential generation is due to activation of Ca channels of the L-type and suggest that at least one type of protein kinase C activation is able to modulate the opening of such Ca-channels.

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