Biochimica et Biophysica Acta 896 (1987) 305–310
Elsevier

BBA 73361

Voltage-activation of high-conductance K^+ channel in the insulin-secreting cell line RINm5F is dependent on local extracellular Ca^{2+} concentration

J.M. Velasco and O.H. Petersen

M.R.C. Secretory Control Research Group, The Physiological Laboratory, University of Liverpool, Liverpool (U.K.)

(Received 8 September 1986)

Key words: Potassium ion channel; Calcium ion activation; Voltage activation; (RINm5F cell)

Patch-clamp single-channel current recording experiments have been carried out on intact insulin-secreting RINm5F cells. Voltage-activation of high-conductance K^+ channels were studied by selectively depolarizing the electrically isolated patch membrane under conditions with normal Ca^{2+} concentration in the bath solution but with or without Ca^{2+} in the patch pipette solution. When Ca^{2+} was present in the pipette, 40 mV to 120 mV depolarizing pulses (100 ms) from the normal resting potential (−70 mV) regularly evoked tetraethylammonium-sensitive large outward single-channel currents and the average open state probability during the pulses varied from about 0.015 (40 mV pulses) to 0.1 (120 mV pulses). In the absence of Ca^{2+} in the pipette solution the same protocol resulted in fewer and shorter K^+ channel openings and the open-state probability varied from about 0.0015 (40 mV pulses) to about 0.03 (120 mV pulses). It is concluded that Ca^{2+} entering voltage-gated channels raises $[Ca^{2+}]_i$ locally and thereby markedly enhances the open-state probability of tetraethylammonium-sensitive voltage-gated high-conductance K^+ channels.

Introduction

There are several types of Ca^{2+}-activated K^+ channels [1] but the most widely studied type has a high unit conductance (100–300 pS), is very voltage sensitive and can be blocked by low concentrations (< 5 mM) of tetraethylammonium acting specifically from the outside of the cell membrane [1–4] although there are exceptions to this rule [5]. The high-conductance Ca^{2+}-activated K^+ channel has been characterized in single-channel current experiments on many different cell types [1–9] including insulin-secreting cells [10–12]. All reports concerning the properties of this channel in insulin-secreting cells deal with measurements carried out on excised membrane patches and there is no direct information from intact cells. From the excised patch experiments of Cook et al. [10] it might be predicted that the high-conductance channel is of little physiological significance as very high Ca^{2+} concentrations (> 1 μM) are needed on the membrane inside in order to evoke a substantial degree of channel opening whereas similar type of experiments carried out by Findlay et al. [11] indicate that submicromolar Ca^{2+} concentrations are sufficient to cause activation. From the results of whole-cell current experiments Rorsmann and Trube [13] suggest that the outward currents following voltage-gated inward Ca^{2+} currents are entirely due to delayed outward rectifier K^+ channels whereas Findlay and Dunne [14] conclude that a substantial fraction of this outward current is due to the Ca^{2+}- and voltage-activated high-conductance channel. In order to begin to resolve this controversy we have now

Correspondence: O.H. Petersen, M.R.C. Secretory Control Research Group, The Physiological Laboratory, University of Liverpool, P.O. Box 147, Brownlow Hill, Liverpool, L69 3BX, U.K.

0005-2736/87/$03.50 © 1987 Elsevier Science Publishers B.V. (Biomedical Division)

studied opening of the Ca^{2+}-activated K^+ channel in intact insulin-secreting cells. This study attempts to assess the importance of external Ca^{2+} entering the cell during depolarizing voltage pulses for the activation of the high-conductance K^+ channel. We demonstrate that voltage activation of the K^+ channel is significantly enhanced when external Ca^{2+} is present locally outside the isolated patch membrane.

Methods

All experiments were carried out on the insulin-secreting cell line RINm5F [15]. Cells were maintained in RPM1 1640 tissue culture media containing 11 mM glucose and supplemented with 10% (w/v) foetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 1 μg/ml fungizone. Cells were seeded out every two to three days onto Falcon style 3001 type petri dishes (35 × 10 mm) and kept in a humidified atmosphere of 95% air and 5% CO_2 at a temperature of 37°C. The cells were washed with a Na^+-rich Hepes-buffered saline solution before use in the patch-clamp experiments which were carried out at room temperature (20–23°C).

The patch-pipettes and the bath were filled with a Na^+-rich saline solution of the following composition (mM): 140 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.13 $MgCl_2$, 10 Hepes with a pH of 7.2, except in the experiments labelled Ca^{2+}-free where the pipette solution contained no added Ca^{2+} and EGTA (0.5 mM) was present. Atomic absorption spectroscopy indicates that our physiological saline solutions contain about 10 μM Ca^{2+} in the absence of any added Ca^{2+}. According to calculations previously described [11] this would give a free Ca^{2+} concentration of about $5 \cdot 10^{-9}$ M in the nominally Ca^{2+}-free pipette solutions. The bath solution always contained 2.5 mM Ca^{2+}.

Single-channel current recordings were carried out in the cell-attached configuration as described by Hamill et al. [16] using the List EPC 5 patch-clamp amplifier system. Glass patch pipettes were coated with a Sylgard resin (Corning) and fire polished so that they had a final resistance of between 5 and 10 MΩ when filled with the Na^+-rich saline solution. Membrane current records (filtered at 1 kHz low pass) were primarily stored on tape (Racal 4DS recorder) for subsequent replay and analysis. In all the single-channel current traces upward deflections represent current flow from the inside to the outside of the isolated membrane patch.

All experiments were carried out according to the following protocols. A high-resistance seal (20–50 GΩ) between glass pipette and cell membrane was established and the pipette potential was clamped at 0 mV (bath potential). Thereafter depolarizing voltage pulses of 100 ms duration were applied at a frequency of 0.6 Hz to pipette potentials of −40, −60, −80, −100 or −120 mV. A jump to a particular voltage value was always repeated at least 30 times, so that each experiment would consist of at least 150 voltage jumps.

Results

Fig. 1 shows selected sets of traces from an experiment with Ca^{2+} and one without Ca^{2+} in the pipette solution. In all experiments there were many blanks (i.e. voltage jumps during which no outward channel currents were seen), but there were many more blanks in the experiments with Ca^{2+}-free pipette solution. The traces shown in Fig. 1 for the Ca^{2+}-free series are far from typical, since blanks were most frequently encountered. The Ca^{2+}-free experiment from which the traces shown were obtained was in fact the one showing the highest degree of channel activity. Experiments in which no channel openings could be observed at any potential level were not included in the quantitative analysis. There were none in the series with Ca^{2+}-containing pipette solution, but four such experiments in the series with Ca^{2+}-free pipette solution. A total of 14 experiments with Ca^{2+}-containing and seven experiments with Ca^{2+}-free pipette solution were then used for the analysis. The average open-state probability at each potential level (30 pulses) was calculated for every experiment and the mean value ± S.E. thereafter obtained for the whole series. Fig. 2 summarizes the result. The mean open-state probability was always lower in the experiments with Ca^{2+}-free pipette solution than in the experiments with Ca^{2+}-containing pipette solution, but the differences were not statistically

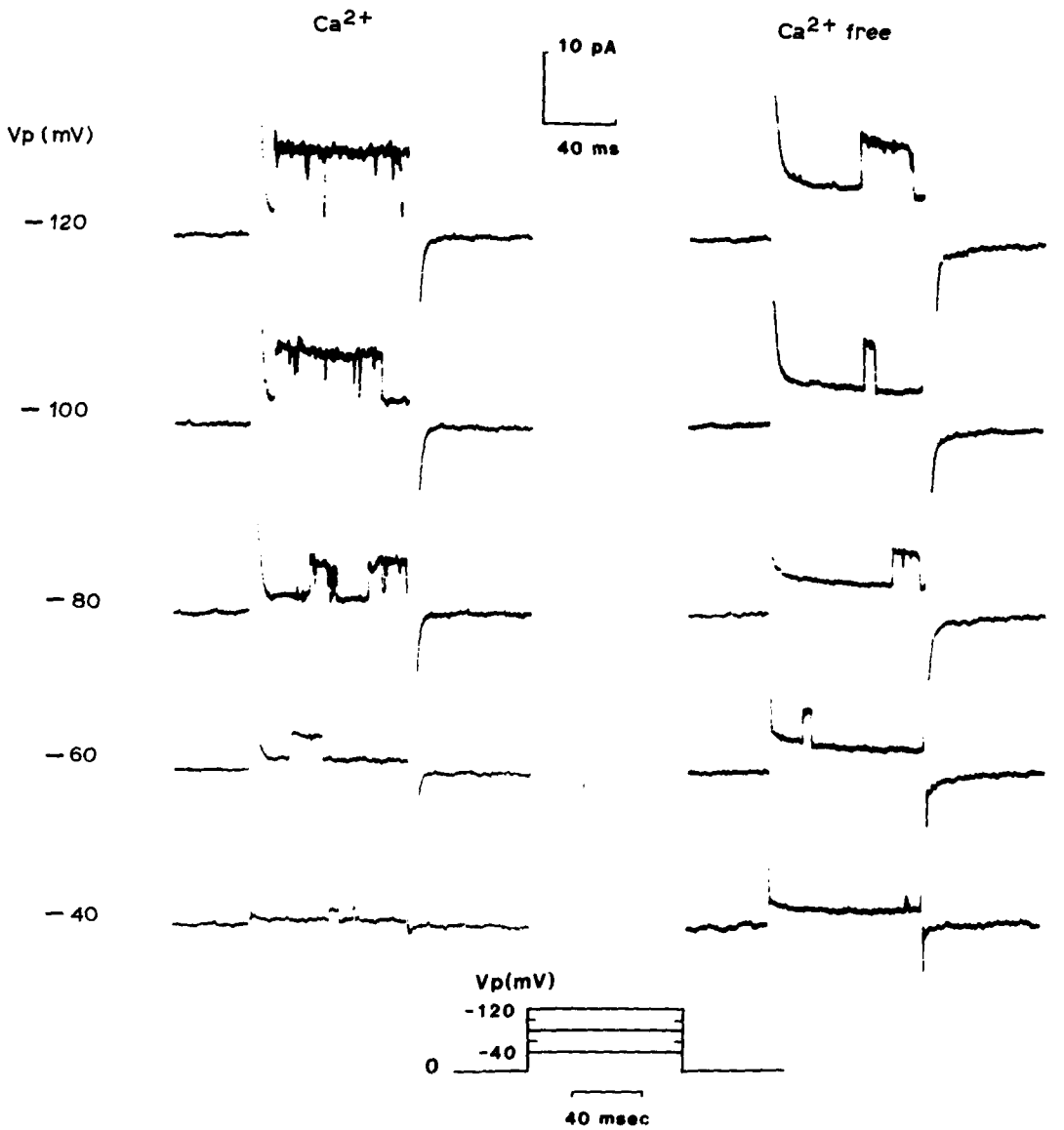

Fig. 1. Single-channel currents obtained in cell-attached recording configuration from two separate experiments, one with Ca^{2+}-containing solution in the patch pipette (Ca^{2+}) and one in which the pipette was filled with Ca^{2+}-free solution (Ca^{2+}-free). The pipette voltage (V_p) was held at 0 mV (holding potential) and 100 ms voltage jumps to V_p of −40, −60, −80, −100 and −120 mV (see bottom trace) were imposed. Each voltage step was repeated 30 times, but this figure only shows one selected example of the current pattern at each voltage.

significant at all potential levels. However, for both the voltage jumps of 80 and 100 mV magnitudes the *t*-test showed that the open-state probabilities in the experiments with Ca^{2+}-free solution were significantly lower than in the experiments with Ca^{2+}-containing pipette solution ($P <$ 0.05). That the actual transmembrane patch potentials in the two series of experiments were very similar was checked by measuring the average single-channel current amplitudes at the different pipette potentials. Fig. 3 summarizes the result of this analysis and shows that there was no dif-

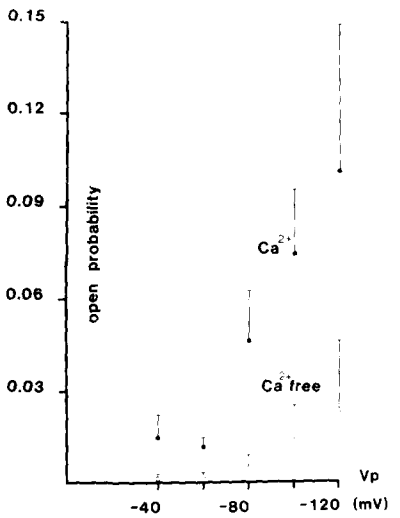

Fig. 2. The mean open-state probability ± S.E. from all the experiments (in which some channel openings occurred) in the two series (Ca^{2+} and Ca^{2+}-free solution in pipette) as a function of the pipette voltage (V_p).

ference between the average single-channel current amplitudes with or without Ca^{2+} in the pipette solution. The effect of tetraethylammonium was assessed in two experiments carried out using the same protocol, but with tetraethylammonium (2 mM) in the Ca^{2+}-containing pipette solution. In these experiments voltage-activated outward channel currents were never observed.

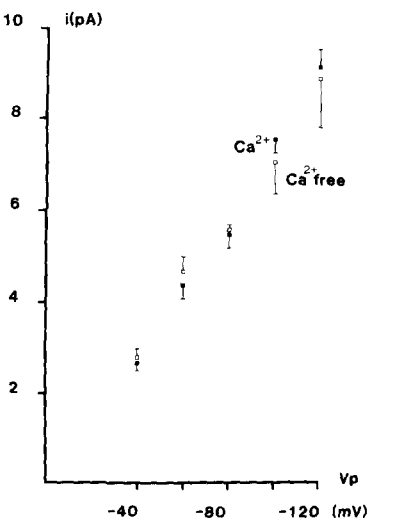

Fig. 3. The mean single-channel current amplitude (i) ± S.E. in the two series of experiments (Ca^{2+} and Ca^{2+}-free solution in the pipette) as a function of the pipette voltage (V_p).

Discussion

The experiments reported here deal with selective depolarization of the isolated patch membrane in intact insulin-secreting RINm5F cells and demonstrate that the presence of extracellular Ca^{2+} locally in the pipette solution markedly enhances voltage-activation of the high-conductance Ca^{2+}-dependent K^+ channel.

In the original study of the high-conductance K^+ channel in chromaffin cell membranes Marty [6] demonstrated voltage activation of the channel in intact cells under conditions where no Ca^{2+} was present in the pipette solution indicating directly that the channel is voltage sensitive. A later study by Marty and Neher [17] did, however, show that the presence of extracellular Ca^{2+} is significant for the degree of activation of the high-conductance channel in intact cells. Depolarizing the cell to about 0 mV by introducing a high-K^+ solution in the presence of Ca^{2+} into the bath was reported to activate high-conductance channels whereas depolarizing the patch membrane alone to 0 mV (in the absence of Ca^{2+} in the pipette solution) failed to cause activation. These experiments were interpreted to indicate activation of high-conductance K^+ channels by Ca^{2+} entry through voltage-gated Ca^{2+} channels [17].

In order to compare the curves representing the relationship between open-state probability and pipette voltage (Fig. 2) with similar curves obtained from work on excised membrane patches it is necessary to know the resting membrane potential of the RINm5F cells. This has recently been estimated by measuring the pipette voltage at which single K^+ channel currents (ATP-sensitive channel) reverse under conditions with a 150 mM K^+ concentration in the pipette solution and an ordinary Na^+-rich physiological saline solution (same as in present work) in the bath. The pipette voltage at which the polarity of the single K^+ channel currents reverses should correspond to the normal resting potential [8]. Using this approach the resting membrane potential in RINm5F cells has been estimated at −70 mV [18]. At the hold-

ing potential used in this work of $V_p = 0$ mV the patch membrane potential would therefore be about -70 mV and during voltage jumps to pipette voltages of -40, -60, -80 and -120 mV the patch membrane potential should be about -30, -10, $+10$, $+30$ and $+50$ mV assuming that the resistance of the patch membrane is large relative to the rest of the cell membrane [19]. The RINm5F cells are however, very small (about 10 μm in diameter) and the degree of coupling between neighbouring cells is unknown. It is therefore possible that the changes in V_p will cause some change in the cell membrane potential and that therefore the changes in patch membrane potential (ΔV_m) evoked by the voltage jumps are smaller than the changes in V_p (ΔV_p) [19]. This, however, does not endanger the comparison of results obtained with Ca^{2+} and Ca^{2+}-free pipette solutions. Since the average amplitudes of the single-channel currents at the different V_p levels were the same in the two series (Fig. 3) we can conclude that the patch membrane potentials were also the same. Neglecting at this point the possible complication that ΔV_m may be somewhat smaller than ΔV_p, the curves shown in Fig. 2 can be used to estimate roughly $[Ca^{2+}]_i$ by comparison with curves representing open-state probability of the same channel in excised inside-out membrane patches as a function of membrane potential with varying $[Ca^{2+}]$ in the bath solution. The only detailed data from insulin-secreting cells have been obtained from work on acutely dissociated rat pancreatic islet cells [11]. Our curve for Ca^{2+}-free pipette solution (Fig. 2) would seem to fit in reasonably well with the data in excised patches obtained at $[Ca^{2+}]_i \approx 10^{-7}$ M whereas the curve in Fig. 2 obtained from experiments with Ca^{2+}-containing pipette solution would seem to correspond to a level of $[Ca^{2+}]_i$ between $6 \cdot 10^{-7}$ and $8 \cdot 10^{-7}$ M. This type of argument depends of course on the assumption that no factors other than $[Ca^{2+}]_i$ and membrane potential determine the open-state probability of the high-conductance K^+ channel. However, it is now known that pH [10] and $[Mg^{2+}]$ [20] on the membrane inside are also of importance. In our previous excised patch experiments [11] the solution in contact with the membrane inside had a pH of 7.2 and $[Mg^{2+}]$ was close to 1 mM. These are reasonable intracellular values [21,22], but since the exact intracellular activities in the RINm5F cells under the conditions of our experiments are unknown the quantitative comparison of data from excised patches and intact cells should be interpreted with caution.

It would appear that Ca^{2+} entering the patch membrane during the depolarizing voltage pulses has caused a substantial elevation of $[Ca^{2+}]_i$ locally. The direct measurements of $[Ca^{2+}]_i$ in intact cells obtained with the help of quin2 fluorescence measurements indicate a resting level of $[Ca^{2+}]_i$ of about $5 \cdot 10^{-8}$ to 10^{-7} M [23,24] with an increase to about $3 \cdot 10^{-7}$ M during various types of maximal stimulation. However, the measurements of quin2 fluorescence can only give average values for the whole cytosol with a limited time resolution and it is known that quin2, because it is a Ca^{2+} buffer, attenuates transient changes in $[Ca^{2+}]_i$ [25].

The RINm5F cells display over-shooting action potentials during depolarizing stimuli [14]. The voltage range employed by us in this study is therefore physiologically relevant and our data suggest that activation of the high-conductance K^+ channel may play a role in spike repolarization in the insulin-secreting cells, in agreement with the conclusion from a detailed analysis carried out in frog sympathetic ganglia by Adams and his co-workers [26].

A recent study by Matteson and Matschinsky [27] on another pancreatic (HIT) cell line demonstrates in whole-cell current experiments voltage-activated inward Ca^{2+} currents followed by outward voltage-dependent K^+ currents which could be blocked by tetraethylammonium. These results, though less direct than ours, would also appear to be in agreement with the conclusion that Ca^{2+}- and voltage-activated K^+ channels could play a significant role in spike repolarization in insulin-secreting cells.

Acknowledgement

We thank Dr. I. Findlay for helpful discussions and reading of the manuscript. J.M.V. is supported by a 'Bolsa de Estudio' fellowship of the Spanish Government. This work was further supported by an MRC project grant to O.H.P.

References

1 Petersen, O.H. and Maruyama, Y. (1984) Nature 307, 693–696
2 Marty, A., Evans, M.G. and Trautmann, A. (1986) J. Exp. Biol. 124, 15–32
3 Petersen, O.H., Findlay, I., Suzuki, K. and Dunne, M.J. (1986) J. Exp. Biol. 124, 33–52
4 Iwatsuki, N. and Petersen, O.H. (1985) J. Membrane Biol. 86, 139–144
5 Wong, B.S. and Adler, M. (1986) Pflügers Arch. 407, 279–284
6 Marty, A. (1981) Nature 291, 497–500
7 Barrett, J.N., Magleby, K.L. and Pallotta, B.S. (1982) J. Physiol. (Lond.) 331, 211–230
8 Maruyama, Y., Petersen, O.H., Flanagan, P. and Pearson, G.T. (1983) Nature 305, 228–232
9 Tabares, L., Lopez-Barneo, J. and Demiguel, C. (1985) Biochim. Biophys. Acta 314, 96–102
10 Cook, D.L., Ikeuchi, M. and Fujimoto, W.Y. (1984) Nature 311, 271–273
11 Findlay, I., Dunne, M.J. and Petersen, O.H. (1985) J. Membrane Biol. 83, 169–175
12 Findlay, I., Dunne, M.J., Ullrich, S., Wollheim, C.B. and Petersen, O.H. (1985) FEBS Lett. 185, 4–8
13 Rorsmann, P. and Trube, G. (1986) J. Physiol. (Lond.) 374, 531–550
14 Findlay, I. and Dunne, M.J. (1985) FEBS Lett. 189, 281–285
15 Praz, G.A., Halban, P.A., Wollheim, C.B., Blondel, B., Strauss, A.J. and Renold, A.E. (1983) Biochem. J. 210, 345–352
16 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100
17 Marty, A. and Neher, E. (1985) J. Physiol. (Lond.) 367, 117–141
18 Dunne, M.J., Findlay, I., Petersen, O.H. and Wollheim, C.B. (1986) J. Membrane Biol. 93, 271–279
19 Fischmeister, R., Ayer, R.K. and DeHaan, R.L. (1986) Pflügers Arch. 406, 73–82
20 Golowasch, J., Kirkwood, A. and Miller, C. (1986) J. Exp. Biol. 124, 5–13
21 Lindstrom, P. and Sehlin, J. (1984) Biochem. J. 218, 887–892
22 Corkey, B.E., Duszynski, J., Rich, T.L., Matschinsky, B. and Williamson, J.R. (1986) J. Biol. Chem. 261, 2567–2574
23 Wollheim, C.B. and Pozzan, T. (1984) J. Biol. Chem. 259, 2262–2267
24 Boyd, A.E., Hill, R.T., Oberwetter, J.M. and Berg, M. (1986) J. Clin. Invest. 77, 774–781
25 Nemeth, E.F. and Scarpa, A. (1986) FEBS Lett. 203, 15–19, 1986
26 Adams, P.R., Jones, S.W., Pennefather, P., Brown, D.A., Koch, C. and Lancaster, B. (1986) J. Exp. Biol. 124, 259–285
27 Matteson, D.R. and Matschinsky, F.M. (1986) Biophys. J. 49, 432a