Voltage-activated Ca^{2+} currents in insulin-secreting cells

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Membrane voltage and voltage-clamped membrane currents have been investigated with the whole-cell patch clamp method in the insulin-secreting cell line RINm5F. The mean resting membrane potential of RINm5F cells was found to be −52 mV. Overshooting spike potentials could be evoked by membrane depolarisation in the absence of a secretagogue. Inward membrane currents evoked by depolarising voltage steps were dependent upon extracellular Ca^{2+} and blocked by Co^{2+}, nifedipine and verapamil. Outward membrane currents which were evoked by depolarising voltage steps to positive membrane potentials were reduced when Ca^{2+} entry was prevented. It is concluded that the voltage-activated Ca^{2+} currents underlie the voltage-activated spike potentials recorded from insulin-secreting cells.

RINm5F cell *Whole-cell patch clamp* *Ca^{2+} current* *Spike potential*

1. INTRODUCTION

The secretion of insulin from pancreatic islet cells is dependent upon extracellular calcium [1] which is thought to enter the cells during voltage-activated action potential-like spikes [2]. We have applied the whole-cell patch clamp method [3] to individual cells of the insulin-secreting cell line RINm5F [4,5] to directly examine their Ca^{2+} conductance and here demonstrate for the first time the inward membrane current carried by Ca^{2+} which underlies the spike potential recorded in insulin-secreting cells.

2. MATERIALS AND METHODS

All experiments were performed on the insulin-secreting cell line RINm5F [4,5]. Cells were maintained as described [6]. Patch clamp experiments were performed upon single cells in the 'whole-cell' configuration [3]. The series resistance and linear capacitance of the patch pipettes (resistances between 2 and 5 MΩ) and the cells were compensated with a List EPC-7 patch clamp amplifier. Once the whole-cell recording configuration was achieved the cell membrane potential was clamped at −50 mV and 30 ms duration depolarising or hyperpolarising voltage pulses were given at a frequency of 0.2 Hz. The mean resting cell resistance measured with voltage pulses to between −60 and −100 mV was 9.1 GΩ ($n = 20$). Whole cell currents were recorded on tape and later replayed for analysis. Current records have not been corrected for leakage conductance or residual capacitive transients. Current-voltage *(I-V)* curves have been corrected for leakage conductance.

The standard extracellular solution contained (mM): 140 NaCl, 4.7 KCl, 1.13 $MgCl_2$, 2 $CaCl_2$, 2.5 glucose, 10 Hepes, pH 7.2. Cobalt ($CoCl_2$), additional Ca or EGTA were added directly to the bath solution when required. Stock solutions of nifedipine and verapamil were prepared in dimethyl sulfoxide (DMSO) and small aliquots were added to standard extracellular solution just prior to use. The application of similar concentrations of DMSO alone was without effect (5 experiments). The standard 'intracellular' solution contained in the pipette consisted of (mM): 140 KCl, 10 NaCl, 1.13 $MgCl_2$, 10 glucose, 1 ATP, 10 Hepes and 1 EGTA. No Ca^{2+} was added and the pH was 7.2.

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3. RESULTS

The membrane potential of RINm5F cells was measured by clamping the membrane current once the whole-cell recording configuration had been achieved. The mean resting potential recorded from unstimulated cells was -52 ± 1 mV ($n = 109$). Fig.1A shows a typical voltage recording made from a RINm5F cell. In the absence of any secretagogue a positive (depolarising) current step evoked a number of spike-like potentials. Fig.1A shows that each spike had 3 distinct phases. Initially the spike potential rose slowly, above approx. -30 mV the rate of depolarisation of the spike then increased dramatically, overshooting 0 mV to peak at approx. 20 mV and then rapidly repolarised. This result is typical of 18 experiments in which positive current pulses were used to evoke spike-potentials from RINm5F cells in the absence of any secretagogue. Fig.1B shows another record obtained from the same cell as fig.1A, in this record the cell had depolarised sufficiently to be spontaneously firing spike-potentials and clearly shows the afterhyperpolarisation that follows each spike.

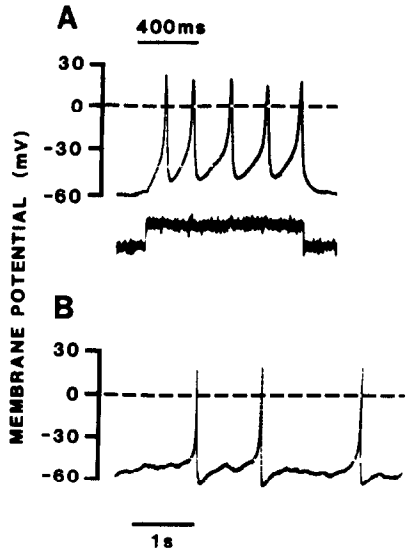

Fig.1. Whole-cell membrane voltage recorded by clamping the membrane current at 0 pA. (A) A positive current pulse (lower trace) evokes repetitive overshooting spike potentials in the membrane voltage record (upper trace). (B) Spontaneous overshooting spike potentials in the membrane voltage record.

Fig.2A illustrates the membrane currents which were evoked by the application of depolarising voltage steps from a clamped potential of -50 mV. Stepping the voltage to -40 mV resulted in no change in membrane current while depolarisation to -30 mV evoked a small inward membrane current which gradually declined during the 30 ms voltage step. However, when the membrane voltage was stepped to either -20 or -10 mV much larger inward currents were evoked which then declined rapidly, within 10 ms of the onset of the voltage step (fig.2A). The amplitude of these voltage-activated inward currents increased from the activation threshold of approx. -30 mV to maximum values at -10 and 0 mV (fig.2B). Stepping the membrane voltage to positive membrane potentials resulted in both a decline in amplitude of the initial inward membrane current (fig.2) and the appearance of an outward membrane current. The amplitude of the outward current increased with further positive depolarisation of the cell (fig.2A).

Although reversal of the inward currents has not been observed it would clearly be at a value more positive than 55 mV which is the equilibrium potential for Na^+ under these experimental conditions. Ca^{2+} was the only other ion with a positive equilibrium potential under the quasi-physiological cation gradients utilised in these experiments and evidence that the voltage-activated inward currents were carried by Ca^{2+} is provided in fig.3.

Fig.3A illustrates that chelation of the Ca^{2+} bathing the cell by the addition of an excess of EGTA abolished the voltage-activated inward current. This result was typical of 9 experiments where either excess EGTA was used to chelate Ca^{2+} in the bathing solution or where Ca^{2+} was added to an initially nominally Ca^{2+}-free bathing solution. Under both protocols no inward currents were observed in the absence of extracellular Ca^{2+}. Fig.3B illustrates that when the concentration of Ca^{2+} bathing the cell was increased from 1 to 10 mM the amplitude of the voltage-activated inward current was increased and the *I-V* curve for inward current shifted approx. 20 mV towards more positive potentials (not shown) (3 experiments).

In 7 experiments the inorganic Ca^{2+}-channel blocker Co^{2+} (5 or 10 mM) was added to the solution bathing the cells and caused both a shift of the *I-V* curve for inward currents towards positive

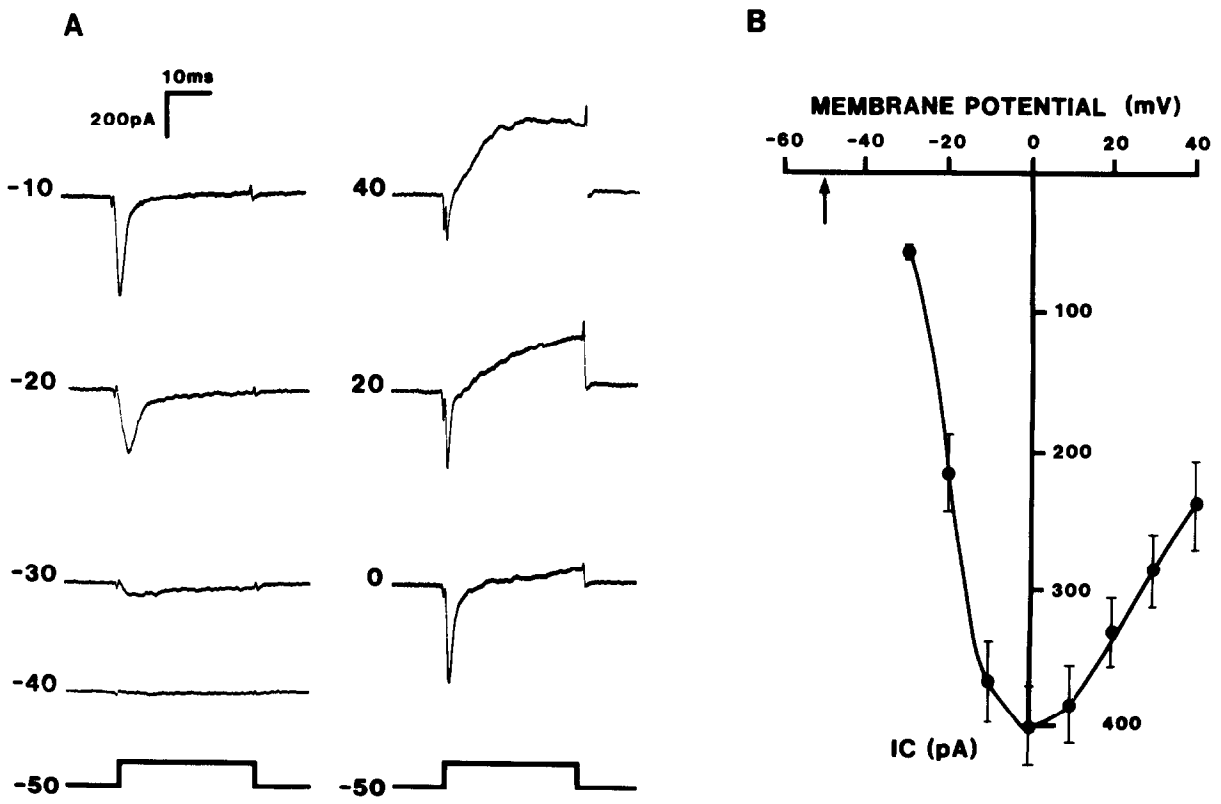

Fig.2. Membrane currents evoked by voltage steps from a holding potential of −50 mV. (A) Individual membrane current traces recorded during voltage steps from −50 mV to the potential indicated next to each trace. Downward deflections represent current moving into the cell, upward deflections represent current moving out of the cell. (B) Current-voltage *(I-V)* relationship of the maximum inward current recorded from experiments such as that illustrated in (A). Mean and SE values were obtained from 20 experiments. The arrow indicates the holding potential of −50 mV. IC = inward current.

potentials of approx. 40 mV and also a reduction in their amplitude. In each case of which fig.3C is a typical example inward currents could no longer be evoked at negative membrane potentials in the presence of Co^{2+} . The organic Ca^{2+}-channel blocker nifedipine on the other hand reduced voltage-activated inward currents in a dose-dependent manner without shifting the *I-V* curve. 1 and 10 μM nifedipine reduced inward currents by 30–40% (4 experiments); 50 μM nifedipine reduced inward currents by 85% (3 experiments); and 100 μM nifedipine completely abolished inward currents at all voltages (2 experiments) (fig.3D). 100 μM verapamil also completely abolished inward currents at all applied voltages (fig.3E) in 5 experiments.

In control experiments outward membrane current could be observed when the voltage was stepped to membrane potentials more positive than −10 mV (fig.2A). In the presence of each of the Ca^{2+}-channel blockers however, when the inward Ca^{2+} current was reduced or abolished, the outward currents were severely reduced. This was a consistent observation made during each experiment which involved the reduction of the voltage-activated Ca^{2+} inward current and is illustrated in fig.4.

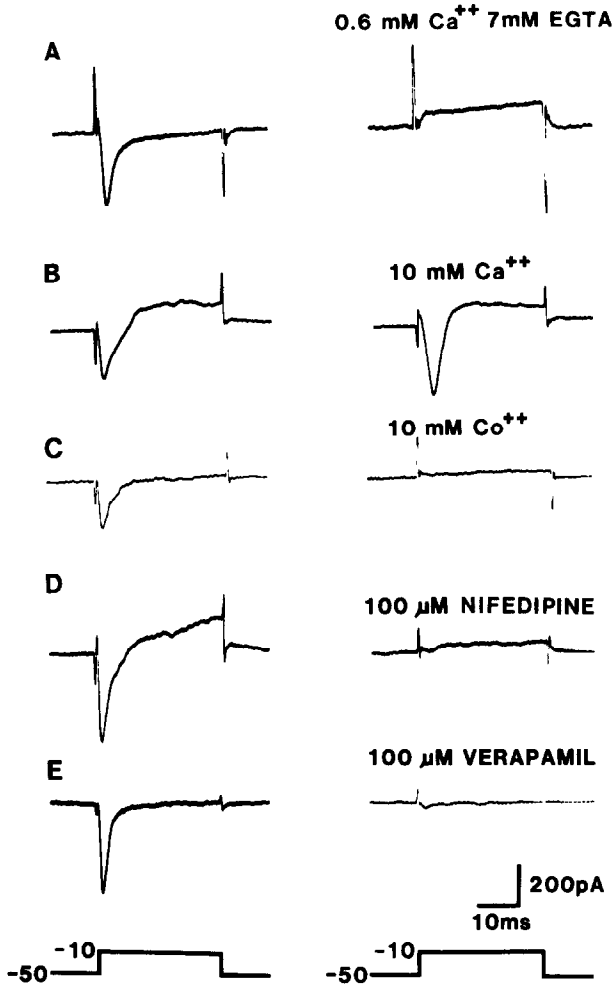

Fig.3. Membrane currents evoked by voltage steps from a holding potential of −50 to −10 mV. The left-hand column illustrates individual records obtained under control conditions with 2 mM $[Ca^{2+}]_o$ (except in B where $[Ca^{2+}]_o$ = 1 mM). The right-hand column illustrates records obtained from the same cells but now recorded under the test conditions which are identified by each individual trace.

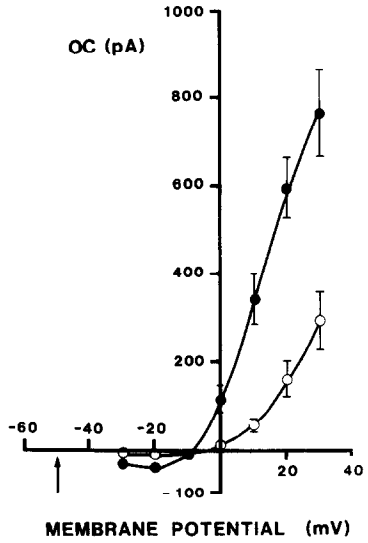

Fig.4. Current-voltage (*I-V*) relationship of membrane currents recorded at the end of each 30 ms duration depolarising voltage step from a holding potential of −50 mV (arrow). (●) Control experiments with 2 mM $[Ca^{2+}]_o$ ($n=20$). (○) Combined results of experiments which utilised Co^{2+} (5 and 10 mM), nifedipine (50 and 100 μM) and verapamil (100 μM) to block inward currents carried by Ca^{2+} ($n=11$). OC = outward current.

4. DISCUSSION

This report provides the first demonstration of membrane currents carried by Ca^{2+} in an insulin-secreting cell (fig.2). There can be little doubt that Ca^{2+} is the cation responsible for the inward membrane currents evoked by membrane depolarisation. The inward currents are not seen in the absence of extracellular Ca^{2+} (fig.3A), they are enhanced when the extracellular Ca^{2+} concentration is increased (fig.3B), and they are blocked by both inorganic (fig.3C) and organic Ca^{2+}-channel blockers (fig.3D,E). Insulin secretion from RINm5F cells stimulated by secretagogues such as glyceraldehyde and alanine and by K^+ depolarisation [4,7] is associated with an increase in intracellular Ca^{2+} as measured with quin2 fluorescence [8,9]. Both insulin secretion and the increase in $[Ca^{2+}]_i$ can be blocked by verapamil [9] and nifedipine [10]. The voltage-activated Ca^{2+} conductance which is described here is also blocked by these drugs (fig. 3D,E) and must therefore be a prime candidate for the mechanism of Ca^{2+} entry associated with insulin secretion.

RINm5F cells possess two types of K^+ channel, a large unit conductance voltage- and calcium-activated K^+ channel (K^+_{max}) and smaller unit conductance K^+ channels with inwardly rectifying properties which are unaffected by either membrane potential or intracellular Ca^{2+} [6]. The

voltage-activated outward current (fig.2A) therefore most probably represents activation of K^+_{max} channels by both membrane depolarisation and an increase in intracellular [Ca^{2+}] since when the entry of Ca^{2+} was prevented the outward current was reduced (fig.4).

This paper provides the first evidence that RINm5F cells can support spike potentials. These spike potentials can be directly evoked by membrane depolarisation in the absence of any secretagogue (fig.1A) as has been shown for pancreatic islet cells [2]. The characteristics of the spike-potentials provoke direct comparison with the membrane currents observed under voltage-clamp conditions (fig.2). The rising phase of the spike potential consists initially of a slow depolarisation which accelerates when the potential reaches -30 to $\div 25$ mV (fig.1A). Comparison with the voltage-clamp record shows that membrane depolarisation to about -30 mV evoked a small, sustained inward current which as the cell potential was stepped to more positive voltages was seen as a large and rapidly activating current (fig.2A). Repolarisation of the spike potential undershoots the previous resting membrane potential (fig.1B) which probably reflects the increase in K^+ conductance evoked by both depolarisation and Ca^{2+} entry (figs 2A,4).

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REFERENCES

[1] Wollheim C.B. and Sharp, G.W.G. (1981) Physiol. Rev. 61, 914–973.
[2] Matthews, E.K. and Sakamoto, Y. (1975) J. Physiol. 246, 421–437.
[3] Sakmann, B. and Neher, E. (1983) Single-Channel Recording, Plenum, New York.
[4] Praz, G.A., Halban, P.A., Wollheim, C.B., Blondel, B., Strauss, A.J. and Renold, A.E. (1983) Biochem. J. 210, 345–352.
[5] Halban, P.A., Praz, G.A. and Wollheim, C.B. (1983) Biochem. J. 212, 439–443.
[6] Findlay, I., Dunne, M.J., Ullrich, S., Wollheim, C.B. and Petersen, O.H. (1985) FEBS Lett. 185, 4–8.
[7] Wollheim, C.B., Ullrich, S. and Pozzan, T. (1984) FEBS Lett. 177, 17–22.
[8] Rorsman, P., Berggren, P.-O., Gylfe, E. and Hellman, B. (1983) Biosci. Rep. 3, 939–946.
[9] Wollheim, C.B. and Pozzan, T. (1984) J. Biol. Chem. 259, 2262–2267.
[10] Wollheim, C.B. Personal communication.