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'Perforated patch recording' allows long-term monitoring of metabolite-induced electrical activity and voltage-dependent Ca^{2+} currents in pancreatic islet B cells

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We describe the application of 'perforated patch recording' using the pore-forming antibiotic nystatin, to monitor the electrical activity and underlying ionic currents of rat and human pancreatic islet B cells. We demonstrate that glucose-induced electrical activity is seen even in single B cells during current-clamp recordings lasting hours 'L-type' Ca^{2+}-channel currents can also be monitored over this period of time. This technique may prove useful in examining hormone and neurotransmitter modulation of electrical activity in B cells, while minimizing the effects of cytoplasmic 'wash-out'.

Patch recording, permeabilized; Nystatin; Electrical activity; Ca^{2+} current; (Pancreatic B cell)

1. INTRODUCTION

During metabolic stimulation by glucose, insulin-secreting B cells of pancreatic islets initially depolarize and then display complex Ca^{2+}-dependent bursting pacemaker-like electrical activity; the intensity of the electrical activity is modulated by a variety of hormones and neurotransmitters [1]. In principle, the modulation of excitation by products of cell metabolism or by second messengers could be studied by monitoring the time course of the underlying ionic currents. Unfortunately, B cells rapidly lose their responsiveness to glucose during the first several minutes of conventional 'whole-cell' patch-clamp recording, and critical components of excitability, such as Ca^{2+} channels rapidly 'run-down' [2], a phenomenon commonly referred to as 'wash-out'.

Recently, a 'perforated' or permeabilized cell-attached patch recording technique has been described [3]. Low resistance electrical contact between the pipette solution and the cytosol is achieved via multiple small diameter pores induced by intramembrane aggregation of the polyene antibiotic nystatin. This technique has been reported to reduce the 'wash-out' of cytoplasmic factors and 'run-down' of membrane excitability usually seen with conventional whole-cell recording. Using this technique, we now demonstrate that metabolite-induced electrical activity can be recorded from single rat B cells and surface B cells of intact human islets and islet fragments for up to several hours after achieving sufficiently low resistance contact with the cytosol to permit high quality recording of whole-cell membrane current. In this recording configuration, otherwise labile Ca^{2+} currents also persist for several hours.

2. MATERIALS AND METHODS

Purified human islets isolated by collagenase digestion and Ficoll gradient separation techniques from pancreases newly

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harvested from life-supported cadavers were a generous gift of the Islet Transplantation Laboratory, Washington University (David Scharp, director) [4]. Dispersed rat islets, prepared by previously described methods [5] were a gift of the laboratory of Dr Michael McDaniel (Dept of Pathology, Washington University). Islets were plated on sterile glass coverslips and cultured for 3–15 days at 37°C in Hepes-buffered CMRL-1066 tissue culture medium (Gibco), enriched with 10% heat-inactivated fetal calf serum, 0.5% penicillin and 0.5% streptomycin, in 5% CO_2/95% air. Coverslips seeded with islets were transferred to a recording chamber containing an extracellular-like solution (ES) containing (in mM): 144 NaCl; 5.5 KCl; 2.5 $CaCl_2$; 1 $MgCl_2$; and 20 Hepes titrated to pH 7.3 with NaOH. The composition of ES was altered by iso-osmotically substituting a portion of the NaCl content with the test substance, or with an iso-osmotic NaCl solution containing the test substance.

Pipette-to-membrane seals of resistance >10 GΩ were formed on surface cells of 50–150 μm diameter human islets, islet fragments, or single rat islet cells using 5–10 MΩ pipettes fabricated from Boralex 100λ micropipettes. Our nystatin stock solution consisted of 50 mg/ml nystatin dissolved in DMSO. Pipettes were filled in a two stage process: the tip of the pipette was initially filled with nystatin free high K^+ solution containing (in mM): 1 $MgCl_2$; 0.5 EGTA; 11.8 NaCl; 63.7 KCl; 28.35 K_2SO_4; 47.2 sucrose; and 20.75 Hepes titrated to pH 7.37 with KOH. The remainder of the pipette was then backfilled with the high K^+ solution also containing 150–300 μg/ml nystatin (Sigma, St. Louis, MO). The pipette solution was designed to minimize changes in intracellular solute content due to electroneutral diffusion from the patch pipette (see [3]). No cell swelling or shrinking was observed during the course of these experiments. Cell-attached patch recording and analysis of single-channel currents were done at room temperature with techniques previously described for our laboratory [4,5]. Cells were presumed to be B cells and selected for further study if they displayed inward unitary channel currents of 3.5–4.5 pA in amplitude at 0 mV clamping potential in 0 glucose ES, with subsequent reduction in frequency on application of glucose; or if such single-channel currents appeared after brief bath application of the oxidative inhibitor sodium azide (3 mM). These are convenient identifying characteristics of the ATP-sensitive K^+ channel [6] in the cell-attached patch [4,5,7]. This channel has not been found in glucagon-secreting A cells, which are the next most prevalent cell in the islet [8].

After formation of the cell-attached patch, membrane potential (V_m) was monitored in the current-clamp mode. The patch clamp amplifier (List EPC-7, List Electronics, Darmstadt, FRG) was occasionally switched to voltage-clamp mode to monitor the progress of membrane permeabilization. The capacitative transients elicited in response to steps in voltage were nulled with the capacitative compensation circuitry of the amplifier, whose settings indicated both the cell's capacitance (C_m), and access resistance (R_a = 1/G-series) to the cell's interior. When R_a was <100 MΩ, the patch was considered 'perforated' enough to start current-clamp recording; when R_a was <30 MΩ voltage-clamp recording was attempted. About 50% of cell-attached patches formed in this manner 'perforate' sufficiently, after initial seal formation, to yield acceptable values of V_m and R_a after 20–60 min.

3. RESULTS

Fig.1 (left) demonstrates metabolically regulated electrical activity of a human islet cell recorded with the perforated patch technique. K^+(ATP) channels were clearly seen in the cell-attached patch immediately after gigaseal formation (a). After 35 min, V_m stabilized at −60 mV and R_a was ~75 MΩ (b). 5 min after the addition of 15 mM glucose to the ES bath, the cell depolarized and discrete 'slow waves' of oscillating membrane potential were seen, with plateau levels of V_m reaching −35 mV, and troughs descending to ~−50 mV. Trains of small spikes, riding on the plateau, reached −25 to −22 mV (c). Within 5 min after removal of glucose, V_m returned to ~−60 mV. Application of a small depolarizing current pulse (10 pA) was still able to evoke a train of 'spikes' (d). Within a minute of addition of 3 mM sodium azide (NaN_3) to the bath, V_m hyperpolarized to −69 mV. Repeat application of the current pulse now produced a smaller depolarization than in the absence of NaN_3, suggesting a larger resting conductance (e). V_m returned within 2–3 min to −62 mV on washout of NaN_3 (not shown).

Fig.1 (right) demonstrates that pharmacological modulation of metabolically induced electrical activity is also maintained in the perforated patch recording mode. In this cell, raising the bath glucose concentration from 3 mM to 10 mM depolarized the cell from −60 to −43 mV but evoked only rare spikes (a and b_1). Cyclical electrical activity with coarse spiking commenced seconds after addition of BAY K8644 (5 μM), an 'L-type' Ca^{2+}-channel agonist [9] (b_2). The cell hyperpolarized to −67 mV on addition of diazoxide (200 μM), a specific activator of K^+(ATP) channels and inhibitor of glucose-induced insulin secretion [10] (b_3). Washout of diazoxide in the presence of 3 mM glucose and BAY K8644 returned V_m to a near control value of −57 mV (c_1). Further addition of the oral hypoglycemic sulfonylurea tolbutamide (20 μM), a specific blocker of K^+(ATP) channels and clinically useful enhancer of glucose-induced insulin secretion [10], depolarized the cell and restored the pattern of electrical activity to one similar to that seen in 10 mM glucose (c_2).

Human islet cells shown to have K^+(ATP) chan-

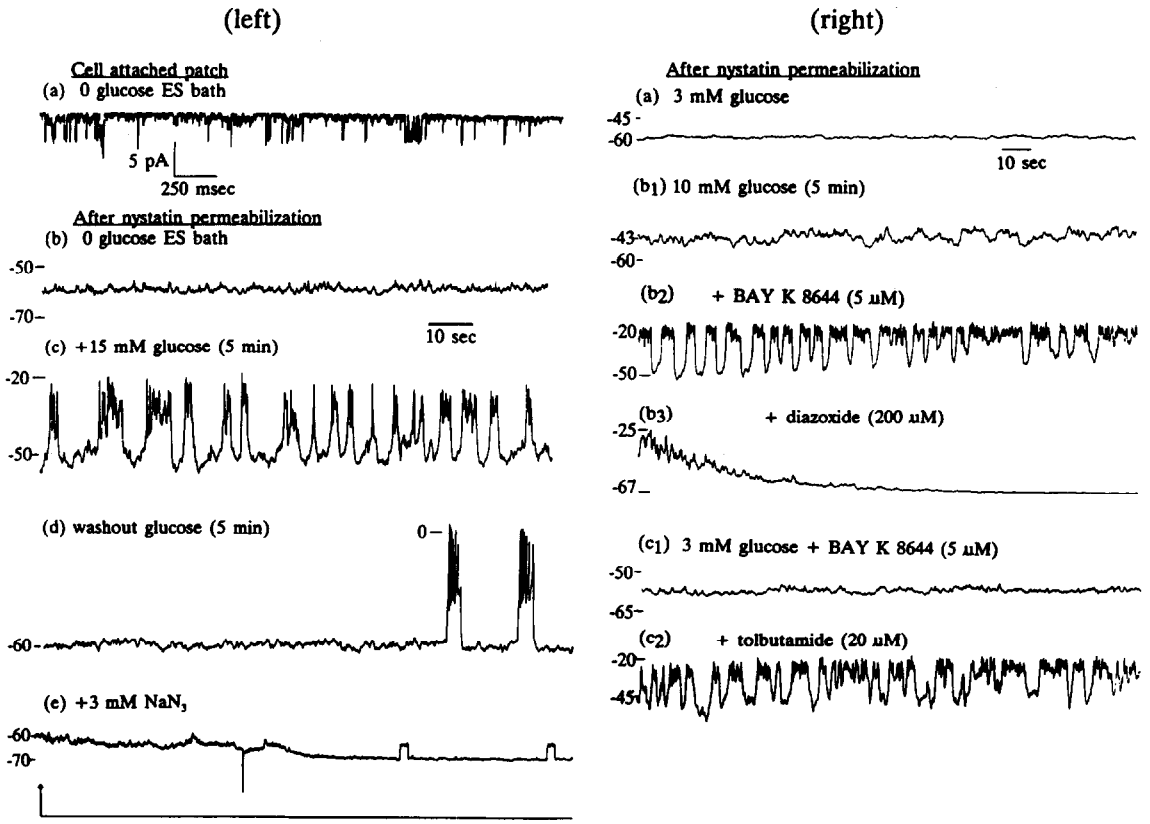

Fig.1. (Left) Metabolically regulated electrical activity recorded from a cell at the surface of human pancreatic islets using the nystatin permeabilized patch technique. The cell was operationally identified as a B cell by the presence of K^+(ATP) channels (trace a). Metabolic regulation of V_m is illustrated in traces b–e (see text for full description). (Right) Pharmacological modulation of metabolically regulated electrical activity from another surface human islet cell. (See text for description.)

nels in the cell-attached patch demonstrated a range of electrical responsiveness to glucose. While all cells rapidly hyperpolarized to −65 to −75 mV in response to 3 mM NaN_3 and depolarized to −35 to −30 mV in response to 20 μM tolbutamide, only 4 out of 11 consecutively tested cells from a single islet preparation demonstrated vigorous spiking in response to 10 mM glucose. Three depolarized to −45 mV and showed only rare spike activity, while two only depolarized to −50 mV; the remaining two were depolarized at −40 mV in the absence of glucose and spiked vigorously after washout of NaN_3 in 0 mM glucose.

In order to investigate whether the electrical responses we recorded from whole islets and islet fragments were generated by adjacent cells, coupled to the permeabilized cells via gap junctions, we performed a series of experiments on single isolated cells. Fig.2 demonstrates metabolically induced electrical activity recorded from one of several single rat B cells (C_m = 3–5 pF). Glucose-induced channel closure was seen in the cell-attached patch prior to patch perforation (a). 25 min later, when R_a had fallen to < 50 MΩ, addition of 16 mM glucose rapidly and reversibly depolarized the cell from −63 mV to a plateau of ~ −45 mV, decreased its membrane conductance from g_m > 3500 pS to <300 pS, and evoked high frequency spike activity (b). Subsequent addition of 8 mM glucose produced slower depolarization and less vigorous spike activity. Spike frequency and amplitude were rapidly enhanced by addition of BAY K8644 (5 μM) and were then rapidly suppressed after further addition of nifedipine (15 μM), a dihydropyridine L-type Ca^{2+}-channel

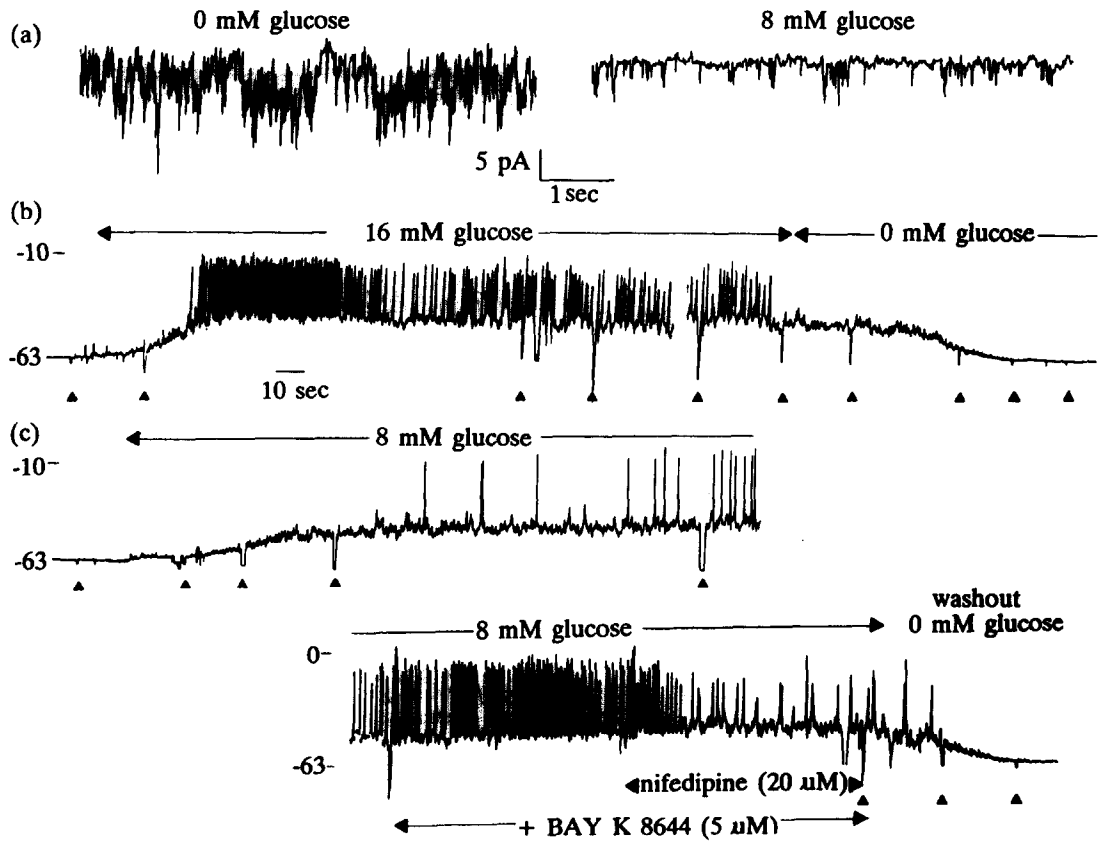

Fig.2. Metabolic regulation of electrical activity recorded from a single rat B cell. Experiment described in the text. Arrowheads under segments of the record indicate the application of a 5 pA hyperpolarizing current pulse used for cell conductance measurements.

antagonist (c).

In single rat B cells bath application of 3 mM NaN_3 hyperpolarized the membrane to −75 to −80 mV. In the presence of NaN_3, the cell behaved as a nearly perfect K^+ electrode, with V_m decreasing 58–62 mV per decade increase in $[K^+]_o$ (not shown).

Often we interrupted our current-clamp recordings to examine membrane currents. Fig.3 presents voltage-clamp records obtained from a single B cell after nearly 1 and 1/2 h of current-clamp recording during which the cell appropriately responded to repeated applications of glucose and NaN_3. When the cell was bathed in ES, only small inward currents were observed. After addition of 15 mM $BaCl_2$ and 20 mM tetraethylammonium chloride to the ES bath to enhance inward Ca^{2+} current and suppress outward K^+ current, inward currents consisted of a transient component activated at voltages greater than −40 mV and a steady-state component seen at voltages greater than or equal to −20 mV (left column). The steady-state component was reversibly increased after addition of BAY K8644 (5 μM) (middle column) and was then suppressed by further addition of nifedipine (20 μM) (right column). Similar results were obtained while recording from human islet cells in clumps. These currents resemble whole-cell Ca^{2+} currents previously reported in rodent B cells [2,8]. Single L-type Ca^{2+}-channel currents have been observed and partially characterized in rodent [11] and human islet B cells [12], which display voltage-dependent activation resembling the steady-state component of the

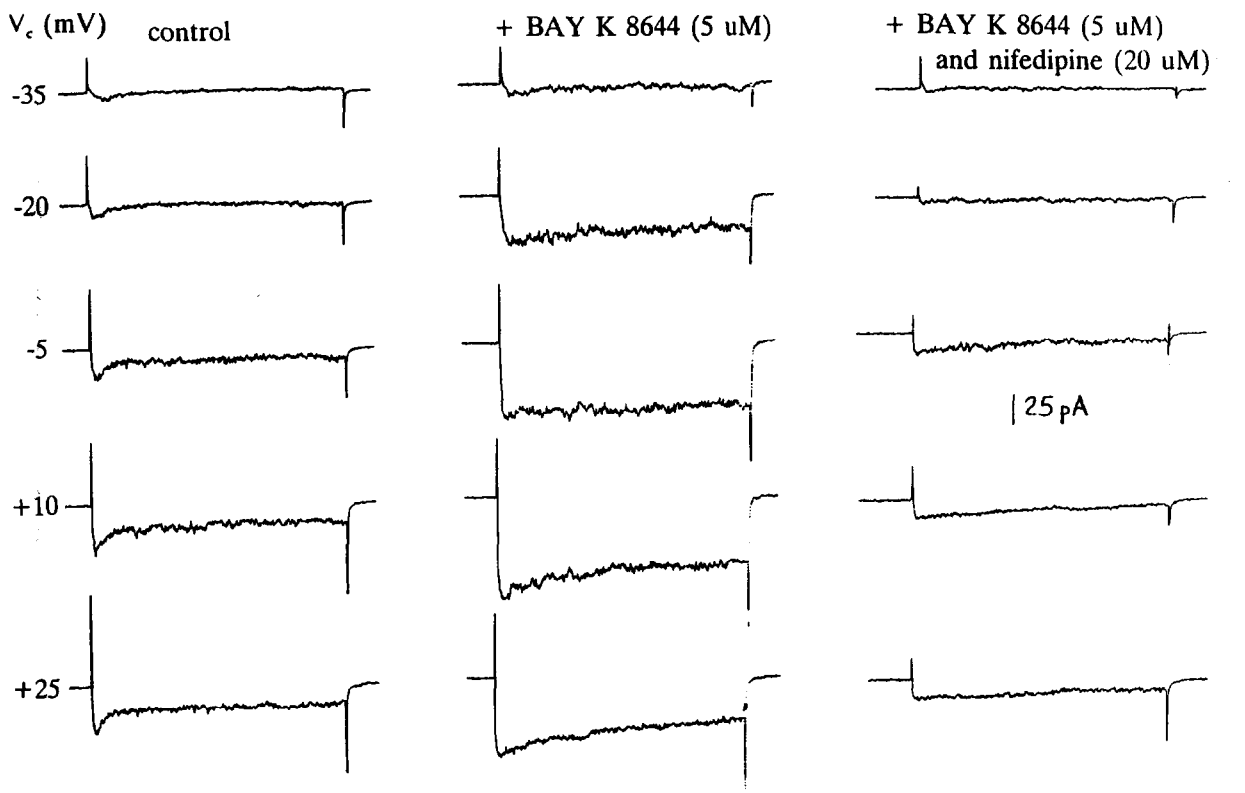

Fig.3. Dihydropyridine-sensitive inward Ca^{2+}-channel currents recorded on pulsing for 400 ms from a holding potential of −90 mV to indicated clamping potentials. Recording conditions described in the text.

whole-cell current. A more complete characterization of these currents will be presented elsewhere.

4. DISCUSSION

These experiments demonstrate that perforated or permeabilized cell-attached patch recording can be used to monitor metabolically induced electrical activity and even evanescent Ca^{2+} currents from pancreatic islet B cells for hours. This technique combines the best features of whole-cell patch-clamp recording and intracellular microelectrode recording (i.e. ease of obtainment of stable, low resistance electrical access to the cytoplasm and limited perturbation of the intracellular milieu). Single electrode voltage clamping has not proven practical in single B cells, while conventional whole-cell recording from isolated cells has been constrained by the inability to obtain electrical responses to glucose as well as the rundown of Ca^{2+} channels (even when pipettes contained Mg_2ATP). Our results suggest that the perforated patch technique may be useful for combined voltage and current clamp analysis of the electrical response to hormones and neurotransmitters operating through second messenger systems which otherwise deteriorate during conventional whole-cell recording.

These experiments further demonstrate that human islet cells are a reliable and easy-to-use tissue for cellular electrophysiology. They possess a similar repertoire of electrical activity as rodent, and in particular mouse, islets which have become the 'model' system for analysis and modelling of complex islet electrogenesis. Recently we have had similar success applying perforated patch recording to cultured canine islets (Pressel, D. and Misler, S., unpublished data). The apparent ease of recording from islets obtained from several species should provide a wider range of tissue

sources for the exploration of defects in stimulus-secretion coupling in B cells.

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