Originals

Glucose and α -ketoisocaproate induce transient inward currents in rat pancreatic beta cells

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Summary The perforated patch technique was used to study changes in membrane potential and whole-cell currents in single isolated rat pancreatic beta-cells during stimulation with glucose or α -ketoisocaproate. Increasing the glucose concentration from 4 to 20 mmol/l, or addition of 15 mmol/l α -ketoisocaproate, caused depolarization and, in most cases, initiation of action potentials. Under voltage-clamp conditions close to a potassium equilibrium potential (E_K) (–60 to –70 mV) these effects were accompanied by the appearance of transient inward currents. These transient currents resembled those elicited during cell swelling in response to a 10% hypotonic bath solution, a manoeuvre which also caused beta-cell depolarization and electrical activity. Tolbutamide (0.2 mmol/l), in the absence of glucose depolarized beta-cells but did not induce transient inward

currents. Nutrient-induced electrical activity and inward currents were abolished by the anion channel inhibitors 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid and 5-nitro-2-(3-phenylpropylamino) benzoic acid, compounds which also inhibited glucose-induced insulin release. It is concluded that nutrient secretagogues induce transient inward currents in isolated rat beta-cells, possibly by activating a volume-sensitive anion conductance. These inward currents could enhance the intensity of electrical, and hence secretory, activity in the beta-cell during nutrient stimulation. [Diabetologia (1997) 40: 1–6]

Keywords Pancreatic beta-cell, glucose, insulin release, electrical activity, whole cell current, anion channel inhibitors.

Glucose-induced insulin release from pancreatic islets is accompanied by depolarization of the beta-cells and a distinctive 'bursting' pattern of electrical activity, consisting of Ca^{2+} -dependent action potentials (see [1] for review). A similar response can be elicited by the insulinotropic nutrient α -ketoisocaproate [2]. The depolarization of the beta-cell to threshold potential is brought about by a rise in

glucose concentration within the range 0–7 mmol/l [3]. At higher concentrations of the sugar effective in stimulating insulin release (7–20 mmol/l), bursts of action potentials occur, the duration of the bursts being progressively prolonged with increasing glucose concentration [3, 4, 5]. All of these effects of glucose require its metabolism within the beta-cell [1, 4]. It is generally agreed that the initial depolarization of the beta-cell involves a reduction of K^+ permeability of the plasma membrane, due to closure of ATP-sensitive K^+ (K_{ATP}) channels [1, 3, 6]. However, the ionic mechanisms which couple glucose metabolism to the modulation of the intensity of electrical activity are not fully understood. Although agents, such as sulphonylureas and diazoxide, which affect K_{ATP} channel opening [7], clearly influence beta-cell electrical and secretory activity [8], the simultaneous measurement of glucose-induced electrical activity and K_{ATP}

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Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid; NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid.

currents in mouse beta-cells suggest that changes in activity of these channels are unlikely to regulate the intensity of electrical activity [5]. Thus, it is probable that at least one other conductance exists which is responsible for modulating beta-cell electrical activity within the effective range of glucose concentrations [9].

The present study utilized the 'perforated patch' technique to investigate whole cell currents in single rat beta-cells. Upon exposure to stimulatory concentrations of the nutrient stimuli glucose or α -ketoisocaproate, transient inward currents were observed. It is suggested that these currents could be carried by anion channels, which may play an important role in coupling nutrient metabolism to electrical activity in the beta-cell.

Materials and methods

Rat pancreatic islets were prepared from male or female rats (weighing 350–450 g) by collagenase digestion and dispersed into single cells and cell aggregates by incubating islets for 5 min in Ca^{2+} -free medium supplemented with 0.5 mmol/l EGTA, followed by centrifugation and resuspension in RPMI medium. The cells were subsequently plated out in small (diameter 30 mm) polystyrene dishes, allowed to adhere and used within 3–9 days of culture, the period during which the cells were found to be optimally responsive to nutrient stimuli. beta-cells were identified by their larger size and granular appearance combined with their electrical response to glucose or α -ketoisocaproate.

Patch-clamp recording was performed on single isolated beta-cells adhering to culture dishes, forming a bath chamber with an approximate volume of 200 μl . Single cells were used in order to eliminate possible 'contaminating' currents (e.g. action currents) arising from adjacent, electrically coupled cells. The cells were continuously superfused at a rate of 4 ml/min with a solution consisting of: (in mmol/l) 135 NaCl, 5 KCl, 1.2 MgCl_2 , 1 NaH_2PO_4 , 1.2 CaCl_2 , 10 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) (pH 7.35) and 4 glucose. In experiments designed to cause cell swelling, 15 mmol/l NaCl was replaced by 30 mmol/l mannitol and a 10% hypotonic solution obtained by withdrawal of mannitol. Recordings of membrane potential and whole cell currents in beta-cells were performed using the 'perforated patch' configuration of the patch-clamp technique [10]. The pipette solution contained: (in mmol/l) 60 K_2SO_4 , 20 KCl, 10 NaCl, 1 MgCl_2 , 10 HEPES (pH 7.2) supplemented with amphotericin B (240 $\mu\text{g/ml}$). Patch pipettes were pulled from soda glass and tip- and back-filled with the amphotericin-containing solution. This procedure greatly accelerated the perforation process which was monitored by nulling the cell capacitance and by the appearance of a membrane potential of -50 to -65mV (in 4 mmol/l glucose). Access resistance was 8–20 $\text{M}\Omega$ whilst whole cell capacitance was $9.4 \pm 0.4\text{ pF}$ ($n = 30$). Although this capacitance value is greater than that reported for single mouse beta-cells (5–7 pF; [5]), it has been previously documented that rat beta-cells can be significantly larger, particularly under the medium-term culture conditions used in the present study [11]. Voltage and current signals were recorded using a List EPC-7 patch-clamp amplifier (List Electronic, Darmstadt, Germany) and filtered at 3 kHz. All experiments were performed at 28–31 °C.

Fig. 1. Perforated patch recording of electrical activity (upper trace) and whole-cell current at a holding potential of -65 mV (lower trace) in a single isolated rat pancreatic beta-cell in response to an increase in glucose concentration from 4 to 20 mmol/l. The amplifier was switched between current-clamp and voltage-clamp during the recording as indicated. The horizontal dotted line on the lower trace represents zero current

Insulin secretion was measured by radioimmunoassay, using groups of 10 islets incubated for 90 min at 37 °C in 1 ml incubation medium (as above).

All biochemicals were obtained from the Sigma Chemical Co., Poole, Dorset, UK (unless otherwise stated). The 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) was a generous gift from Professor R. Greger, University of Freiburg, Germany. Collagenase (Worthington) was obtained from Cambridge Biosciences, Cambridge, UK.

Statistical analysis

Statistical significance was ascribed using Student's *t*-test.

Results

An increase in glucose concentration from 4 to 20 mmol/l caused depolarization of rat beta-cells and, in most cases, led to the generation of action potentials (Figs. 1 and 2). In a minority of cases, glucose induced irregular oscillations in membrane potential in the absence of clear action potentials. Figure 1 also shows voltage-clamp recordings taken from the same cell held at -65 mV . In the presence of 4 mmol/l glucose, whole-cell current was close to the zero current level. Raising the concentration of glucose to 20 mmol/l appeared to have two distinct effects. First, there was usually a small net inward shift in standing current, possibly reflecting a further diminution in membrane K^+ permeability. More strikingly, however, rapid inward transient currents appeared superimposed upon this inward current shift. Two distinct patterns of inward transient currents were observed, with approximately equal frequency, in beta-cells in

Fig. 2A, B. Examples of different patterns of glucose-induced electrical activity and inward currents in two single rat beta-cells. In both cases, the voltage-clamp recordings (lower traces) were made at points corresponding to the breaks in the current-clamp recordings (upper traces). The holding potential was -65 mV. These recordings are representative of those made from a total of 54 of 67 cells which responded to glucose (see text)

response to 20 mmol/l glucose. Representative examples of such recordings are shown in Figure 2. Thus, the transient currents could be rapid and 'spiking' (Figs. 1 and 2A), or of a longer, slower oscillatory nature (Fig. 2B). In a minority of cases a combination of these two patterns was apparent. Of the 67 islet cells examined, 54 responded to glucose in terms of depolarization and induction of transient inward currents. Of the 13 cells which did not respond to glucose, 5 of 6 depolarized upon application of tolbutamide (100 μ mol/l), although it is uncertain whether this represents true beta-cell heterogeneity or a degree of damage to some cells. As shown in Figure 3, addition of α -ketoisocaproate induced electrical activity and transient inward currents in a manner essentially indistinguishable from glucose (15 of 18 cells). The pattern of electrical activity and inward currents elicited by α -ketoisocaproate persisted even when all the glucose was removed from the medium (3 of 3 cells, results not shown). Again, both patterns of transient current were apparent with this stimulus. The amplitude of the inward currents was also found to vary from one cell to another, usually within the range 10–60 pA, giving an approximate whole-cell conductance of 170–1000 pS.

In order to establish whether the induction of transient inward currents was a specific response to nutrient stimuli, beta-cells were exposed to the

non-nutrient tolbutamide. In the absence of glucose, the sulphonylurea caused a marked depolarization and electrical activity but no transient current activity (Fig. 4). Neither membrane potential nor whole-cell current were affected by the subsequent addition of 4 mmol/l glucose (not shown). Thus, the inward currents were not merely a consequence of beta-cell depolarization and electrical activity.

Maximum current amplitudes were measured at negative holding potentials (-60 to -70 mV); close to the predicted value for potassium equilibrium potential (E_K) (approximately -80 mV). Thus, it is unlikely that these transient currents arise from glucose-induced changes in K^+ permeability. Attempts were made to measure glucose-induced transient currents in the absence of K^+ conductances by replacing K_2SO_4 in the pipette solution with Cs_2SO_4 . However, transient currents in response to glucose or α -ketoisocaproate were small under such conditions and were only observed in 6 of 13 cells. It is possible that the metabolism of these nutrients in the beta-cell is perturbed in the presence of Cs^+ . For example, at least one glycolytic enzyme is reported to be K^+ -dependent [12].

Nutrient-induced transient currents were apparent at -60 to -70 mV, potentials at which voltage-sensitive Ca^{2+} channels are inactive [1]. It is therefore improbable that these currents are due to activation of a Ca^{2+} conductance. Rat beta-cells are also reported to have Na^+ channels, although these would also be inactive at the negative potentials employed here [13].

The exposure of beta-cells to a 10% hypotonic solution was also found to cause depolarization and electrical activity accompanied by a similar pattern of transient inward currents to that observed with nutrient stimuli (Fig. 5). This current pattern contrasts with the more sustained inward current observed upon exposure to a larger (30%) hypotonic shock

Fig. 3. Perforated patch recording of electrical activity (upper trace) and whole-cell current at a holding potential of -65 mV (lower trace) in a single rat pancreatic beta-cell in response to 15 mmol/l α -ketoisocaproate (KIC). 4 mmol/l glucose was present throughout. Similar transient inward currents, or in some cases resembling those in Figure 2B, were observed in 15 of 18 cells

Fig. 4. Perforated patch recording of electrical activity (upper trace) and whole-cell current (lower traces) in a single rat pancreatic beta-cell in response to 0.2 mmol/l tolbutamide (absence of glucose). The voltage-clamp recordings **a**, **b** and **c** were made at points corresponding to the breaks in the membrane potential recordings. The holding potential was -70 mV. These recordings are representative of those made from a total of 6 of 6 cells which responded to tolbutamide (see text)

[14] and suggests that transient currents could be carried by a swelling-activated anion conductance, recently described in insulin-secreting cells [14–16]. In order to further investigate this possibility, nutrient-stimulated beta-cells were exposed to two inhibitors of this conductance, $4,4'$ -diisothiocyanatostilbene- $2,2'$ -disulphonic acid (DIDS, 100 μ mol/l) and NPPB, 50 μ mol/l [15, 17]. As shown in Figure 6, both compounds caused repolarization of beta-cells and

Fig. 5. Perforated patch recording of electrical activity (upper trace) and whole-cell current (lower traces) in a single rat pancreatic beta-cell in response to a 10% hypotonic solution (withdrawal of 30 mmol/l mannitol). The voltage-clamp recordings **a** and **b** were made at points corresponding to the breaks in the membrane potential recordings. The holding potential was -65 mV. These recordings are representative of those made from four other cells

abolished the transient inward currents. The stimulatory effects of α -ketoisocaproate were similarly inhibited by these compounds (data not shown). The inhibitory effects of these blockers were partly reversible, although in cells displaying action potentials, the latter were reduced or abolished following exposure to either compound (e.g. Fig. 6A). Both DIDS and NPPB, at the concentrations which inhibited electrical activity, were found to inhibit glucose-induced insulin release, although basal secretion was not significantly affected (Table 1).

Discussion

The aim of the present study was to investigate changes in membrane potential and whole-cell current in metabolically intact single rat beta-cells upon raising the glucose concentration from a physiologically 'resting', or sub-threshold, level to a near-maximal concentration. Most studies of this kind [5, 18, 19] have investigated the effects of adding glucose to cells previously incubated in its absence. It is likely that cells incubated in the presence of sub-threshold levels of glucose differ metabolically, and perhaps functionally, from cells deprived of the sugar. For example, it would be predicted that K_{ATP} channel activity would be reduced by up to 90% at 4 mmol/l glucose compared to 50% at zero glucose [1, 20]. This could account for the slightly less negative 'resting' membrane potential of -50 to -65 mV in the present

Fig. 6 (A, B). Inhibition of glucose-induced electrical activity and transient inward currents by the anion channel blockers DIDS (A) and NPPB (B). The holding potential in both cases was -60 mV. Time calibration bar: 3 min (current clamp recordings) or 30 s (voltage-clamp recordings). Each experiment is representative of two others which showed similar results

study compared with previous reports (-60 to -70 mV) [5, 18, 19]. Nevertheless, in the vast majority of cells studied, raising the glucose concentration from 4 to 20 mmol/l, or the addition of 15 mmol/l α -ketoisocaproate, caused a further depolarization and, most frequently, the appearance of action potentials. These action potentials invariably consisted of irregular or continuous bursts, as described previously in isolated rat beta-cells [19]. The 'slow wave' bursting pattern observed in intact mouse islets [1, 3, 4] and also reported using small clusters and even single mouse beta-cells [5] was not observed in the present study, probably because of the near-maximal concentration of nutrient used and possible species differences.

The principal finding of the present study, which has not been previously reported, was that stimulatory concentrations of glucose or α -ketoisocaproate

Table 1. Effects of DIDS (100 μ mol/l) and NPPB (50 μ mol/l) on glucose-induced insulin release

Condition	Insulin (μ U/islet in 90 min)	<i>p</i>
1. 4 mmol/l glucose	15.3 ± 1.0 (9)	
2. 4 mmol/l glucose + DIDS	20.4 ± 3.1 (4)	NS vs 1
3. 4 mmol/l glucose + NPPB	15.4 ± 2.3 (4)	NS vs 1
4. 20 mmol/l glucose	81.3 ± 8.8 (9)	< 0.001 vs 1
5. 20 mmol/l glucose + DIDS	32.0 ± 1.7 (4)	< 0.001 vs 4
6. 20 mmol/l glucose + NPPB	14.7 ± 1.7 (6)	< 0.001 vs 4

The figures in parentheses refer to the number of separate determinations. Statistical significance was ascribed using Student's unpaired *t*-test. NS, Not significant

induced transient inward currents which were apparent at negative holding potentials close to E_K . The ionic nature of these currents is uncertain at present. Investigation of the identity of the charge-carrying ion(s) cannot be achieved unambiguously using the perforated patch recording technique since the intracellular ionic composition is unknown and cannot be precisely controlled. In addition, it is likely that altering the composition of the extracellular medium will affect that of the cytosol in a manner not readily predictable. A further problem would be measuring a relatively small conductance against the background of the very high K^+ permeability characteristic of beta-cells. On the other hand, the conventional whole-cell recording technique, which does allow control of the intracellular medium, does not permit studies of the effects of nutrient stimuli, due to wash-out of the metabolic 'machinery' of the cell. However, as discussed above, it is unlikely that these inward currents are the result of changes in K^+ , Ca^{2+} or Na^+ permeability of the beta-cell membrane.

The finding that the nutrient-induced transient inward currents closely resembled currents elicited during hypotonically induced cell swelling raises the possibility that these currents arise from activation of a volume-sensitive conductance, possibly the anion conductance recently described in beta-cells [14–16]. This possibility is further supported by the finding that the effects of nutrients on electrical activity and whole cell current were inhibited by DIDS and NPPB, inhibitors of this conductance [15, 17], although the selectivity of these compounds is at present uncertain. In view of this suggestion, it is of interest that glucose has been reported to stimulate the efflux of $^{36}Cl^-$ from prelabelled islets in a manner sensitive to anion channel inhibition [21]. Activation of a volume-sensitive Cl^- conductance would be predicted to generate an inward current, since E_{Cl^-} has been reported to be positive to the membrane potential [21].

The mechanism by which nutrients might activate a volume-sensitive conductance is unknown. However, it has been reported that glucose causes beta-cell swelling [22], a finding recently confirmed in this laboratory [Miley et al. unpublished data]. An additional possibility is that the formation of anionic metabolites of glucose and α -ketoisocaproate, such as lactate, bicarbonate and acetoacetate, causes an increased outward electrochemical anion gradient, and the efflux of these species via an anion conductance could also generate an inward current. Indeed, lactate-induced electrical activity in insulin-secreting cells is also accompanied by inward transient currents resembling those reported in the present study [23].

The induction of an inward current during glucose stimulation could contribute to the increased intensity of beta-cell electrical activity. The finding that the anion channel blockers DIDS and NPPB had a marked inhibitory effect on glucose-induced insulin release implies that the generation of these inward transient currents could also be an important determinant in the secretory response to nutrients, although it should be borne in mind that the selectivity of these blockers remains to be established.

In summary, the present study describes transient inward currents in rat pancreatic beta-cells induced by stimulatory concentrations of glucose and α -ketoisocaproate. It is suggested that these currents could be carried by a volume-sensitive anion conductance and could play a role in enhanced electrical and secretory activity during glucose stimulation.

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References

1. Ashcroft FM, Rorsman P (1989) Electrophysiology of the pancreatic beta-cell. *Prog Biophys Mol Biol* 54: 87–143
2. Hutton JC, Sener A, Herchuelz A et al. (1980) Similarities in the stimulus-secretion coupling mechanism of glucose- and 2-keto acid-induced insulin release. *Endocrinology* 106: 203–219
3. Henquin JC (1987) Regulation of insulin release by ionic and electrical events in B-cells. *Horm Res* 27: 168–178
4. Meissner HP, Schmelz H (1974) Membrane potential of beta cells in pancreatic islets. *Pflugers Arch* 351: 195–206
5. Smith P, Ashcroft FM, Rorsman P (1990) Simultaneous recordings of glucose dependent electrical activity and ATP-regulated K^+ currents in isolated mouse pancreatic beta-cells. *FEBS Lett* 261: 187–190
6. Rorsman P, Trube G (1985) Glucose dependent K^+ channels in pancreatic beta-cells are regulated by intracellular ATP. *Pflugers Arch* 405: 305–309
7. Trube G, Rorsman P, and Ohno-Shosaku T (1986) Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in pancreatic beta-cells. *Pflugers Arch* 407: 493–499
8. Henquin JC (1988) ATP-sensitive K^+ channels may control glucose-induced electrical activity in pancreatic beta-cells. *Biochem Biophys Res Commun* 156: 769–775
9. Henquin JC (1992) ATP-sensitive K^+ channels may not be the sole regulators of glucose-induced electrical activity in pancreatic β -cells. *Endocrinology* 131: 127–131
10. Rae J, Cooper K, Gates P, Watsky M (1991) Low access resistance perforated patch recordings using amphotericin B. *J Neurosci Methods* 37: 15–26
11. Mathers DA, Buchen AMJ, Brown JC, Otte SC, Sikora LKJ (1985) Rat pancreatic islet cells in primary culture: occurrence of giant cells amenable to patch clamping. *Experientia* 41: 116–118
12. Bloxham D, Lardy H (1972) Phosphofructokinase. In: Boyer PD (ed) *The Enzymes*, vol 8. Academic Press, New York London, pp 239–278
13. Hiriart M, Matteson DR (1988) Na channels and two types of Ca channels in rat pancreatic beta-cells identified with the reverse hemolytic plaque assay. *J Gen Physiol* 91: 617–639
14. Best L, Miley H, Yates AP (1996) Activation of an anion conductance and beta-cell depolarization during hypotonically-induced insulin release. *Exp Physiol* 81: 927–933
15. Best L, Sheader EA, Brown PD (1996) A volume-activated anion conductance in insulin-secreting cells. *Pflugers Arch* 431: 363–370
16. Kinard TA, Satin LS (1995) An ATP-sensitive Cl^- channel current that is activated by cell swelling, cAMP and glyburide in insulin-secreting cells. *Diabetes* 44: 1461–1466
17. Paulmichl M, Gschwentner M, Woll E et al. (1993) Insight into the structure-function relation of chloride channels. *Cell Physiol Biochem* 3: 374–387
18. Chow RH, Lund P-E, Loser S, Panten U, Gylfe E (1995) Coincidence of early glucose-induced depolarization with lowering of cytoplasmic Ca^{2+} in mouse pancreatic beta-cells. *J Physiol* 485: 607–617
19. Falke LC, Gillis KD, Pressel DM, Misler S (1989) 'Perforated patch recording' allows long-term monitoring of metabolite-induced electrical activity and voltage-dependent Ca^{2+} currents in pancreatic islet beta-cells. *FEBS Lett* 251: 167–172
20. Ashcroft FM, Ashcroft SJH, Harrison DE (1988) Properties of single potassium channels modulated by glucose in rat pancreatic beta-cells. *J Physiol* 400: 501–527
21. Sehlin J (1978) Interrelationship between chloride fluxes in pancreatic islets and insulin release. *Am J Physiol* 235:E501–E508
22. Semino C, Gagliardino AM, Bianchi C, Rebolledo OR, Gagliardino JJ (1990) Early changes in the rat pancreatic beta-cell size induced by glucose. *Acta Anat* 138: 293–296
23. Best L, Ammala C, Rorsman P, Tomlinson S (1994) Intracellular pH, cytosolic calcium concentration and electrical activity in RINm5F insulinoma cells. *Biochim Biophys Acta* 1192: 107–111