

Effects of lactate on pancreatic islets

Lactate efflux as a possible determinant of islet-cell depolarization by glucose

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The secretion of insulin from perfused rat pancreatic islets was stimulated by raising the glucose concentration from 5.6 to 20 mM or by exposure to tolbutamide. The addition of sodium lactate (40 mM) to islets perfused in the presence of glucose (5.6 mM) resulted in a small, transient, rise in the rate of secretion. The subsequent removal of lactate, but not glucose or tolbutamide, from the perfusate produced a dramatic potentiation of insulin release. The rate of efflux of $^{45}\text{Ca}^{2+}$ was also increased when islets were exposed to a high concentration of glucose or lactate or to tolbutamide, and again subsequently upon withdrawal of lactate. Efflux of $^{86}\text{Rb}^{+}$ was modestly inhibited upon addition of lactate and markedly enhanced by the subsequent withdrawal of lactate from islets. The output of [^{14}C]lactate from islets incubated in the presence of [^{14}C]glucose increased linearly with increasing concentrations of glucose (1–25 mM). It is proposed that the activation of islets by the addition or withdrawal of lactate is not due to increased oxidative flux, but occurs as a result of the electrogenic passage of lactate ions across the plasma membrane, resulting in islet-cell depolarization, Ca^{2+} entry and insulin secretion. The production of lactate via the glycolytic pathway, and the subsequent efflux of lactate from the islet cells with concomitant exchange of H^{+} for Na^{+} , could be a major determinant of depolarization and hence insulin secretion, in response to glucose.

INTRODUCTION

The stimulation of insulin secretion by glucose is thought to result primarily from a depolarization of the plasma membrane (Meissner & Schmelz, 1974) and the consequent entry of Ca^{2+} into the β -cell via voltage-sensitive Ca^{2+} channels (Satin & Cook, 1985; Findlay & Dunne, 1985). The latter response can be detected as an increase in $^{45}\text{Ca}^{2+}$ uptake (Malaisse-Lagae & Malaisse, 1971), a stimulation of $^{45}\text{Ca}^{2+}$ efflux from pre-loaded islets (Malaisse *et al.*, 1973), or a rise in cytosolic Ca^{2+} concentration (Deleers *et al.*, 1985; Grappengeisser *et al.*, 1988). The metabolism of glucose is a prerequisite for these effects, although the mechanism coupling glucose metabolism to islet-cell depolarization is not fully understood.

It has been suggested that a lowering of cytosolic pH, owing to glucose oxidation, could provide a link between metabolic and ionic events in islet cells (Malaisse *et al.*, 1980; Pace, 1984). However, intracellular acidification does not mimic the actions of glucose on $^{45}\text{Ca}^{2+}$ handling, and glucose does not appear to decrease cytosolic pH in islet cells (Best *et al.*, 1988a).

More recently, considerable attention has focused on a glucose-regulated K^{+} channel in the islet-cell plasma membrane. It is thought that a rise in cytosolic ATP concentration, generated by increased glucose oxidation, depolarizes the plasma membrane by inhibiting this channel and decreasing K^{+} permeability (Cook & Hales, 1984; Rorsman & Trube, 1985). However, a number of observations are not consistent with this as the sole mechanism regulating islet-cell membrane potential.

First, there is a poor correlation between glucose-induced insulin secretion and islet ATP concentrations, there being no, or only modest, changes in the latter over the range of glucose concentrations effective in stimu-

lating insulin release (Hellman *et al.*, 1969; Ashcroft *et al.*, 1973; Malaisse & Sener, 1987). Second, the nucleotide-sensitive K^{+} channel is maximally inhibited by 100 μM -ATP (Sturgess *et al.*, 1987) and is also blocked by HCO_3^{-} (Carroll *et al.*, 1988) suggesting that the channel is predominantly closed under physiological conditions. Although it appears that the channel can also be modulated by ADP (Takei *et al.*, 1986) and a high sensitivity of the channel to ATP may be consistent with modulation of membrane potential (Cook *et al.*, 1988), it remains to be established whether the changes that occur in adenine nucleotide concentrations during glucose stimulation are sufficient to influence K^{+} permeability of the β -cell plasma membrane.

Furthermore, some doubt exists concerning the exact role of K^{+} permeability in determining the changes in membrane potential brought about by high concentrations of glucose. A maximal effect of glucose on islet-cell K^{+} permeability is observed at approx. 7–8 mM of the sugar (Boschero & Malaisse, 1981), whereas islet-cell depolarization and secretion are maximal in the region of 20 mM-glucose (Meissner & Schmelz, 1974). In addition, raising the glucose concentration from 8.3 mM to higher values results in a transient increase, rather than a decrease, in K^{+} ($^{86}\text{Rb}^{+}$) permeability (Boschero & Malaisse, 1981), possibly owing to activation of Ca^{2+} -sensitive K^{+} channels. Last, the prevention by valinomycin of glucose-induced changes in K^{+} permeability has no effect on mobilization of Ca^{2+} in response to the sugar (Boschero *et al.*, 1979).

Taken together, the above observations indicate that an additional, or alternative, mechanism exists which couples glucose metabolism to islet-cell depolarization. The results of the present study suggest that the formation of lactate, and subsequent efflux of this anion from the islet cell, could constitute such a mechanism.

EXPERIMENTAL

Materials

^{125}I -insulin, $^{45}\text{CaCl}_2$, $^{86}\text{RbCl}$, D-[U- ^{14}C]glucose, [U- ^{14}C]-lactate and [1- ^{14}C]pyruvate were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Collagenase (*Clostridium histolyticum*) was supplied by Cambridge Biosciences, Cambridge, U.K., and L-lactic acid by Sigma Chemical Co., Poole, Dorset, U.K.

Insulin secretion

Pancreatic islets were isolated from 250–300 g male or female Wistar rats by collagenase digestion (Lacy & Kostianovsky, 1967). Groups of 25 islets were perfused at a rate of 1 ml/min with Hepes-buffered bicarbonate medium containing bovine serum albumin (0.5%, w/v) (Yates & Gordon, 1982), and the insulin content of the perfusate was measured by radioimmunoassay (Gordon *et al.*, 1985). Where additions were made to the incubation medium (sodium lactate, KCl), these replaced NaCl.

Radioisotope-efflux experiments

Groups of 150 islets were incubated for 120 min in 200 μl of medium containing 2.5 mM-glucose and either $^{45}\text{CaCl}_2$ (30 μCi) or $^{86}\text{RbCl}$ (15 μCi). The islets were then washed with 1 ml of unlabelled medium, placed in perfusion chambers and perfused at a rate of 1 ml/min. After 20 min, samples were collected at 1 min intervals, 4 ml of Aquasol scintillant was added to each, and the radioactivity was measured by liquid-scintillation counting. Results were expressed as fractional outflow rates (i.e. the fraction of total radioactivity lost from the islets per min).

[^{14}C]Lactate production

Groups of 50 islets were incubated in 100 μl of medium containing 10 μCi of D-[U- ^{14}C]glucose and appropriate

concentrations of unlabelled sugar. After 90 min, a 25 μl sample was taken, diluted with 1 ml of water and applied to a column containing 0.5 ml Dowex AG1X8 (formate form). The glucose was washed from the column with 20 ml of water, and the lactate was then eluted with 3×2 ml of 0.6 M-formic acid (Schadewaldt *et al.*, 1984). The ^{14}C content of the eluate was measured after the addition of 10 ml of Aquasol scintillation fluid. Approx. 95% of the radioactivity of the samples was eluted in this fraction, which corresponded to standard [U- ^{14}C]-lactate, [1- ^{14}C]pyruvate being retained on the column.

RESULTS

Raising the concentration of glucose from 5.6 to 20 mM caused a pronounced stimulation of insulin secretion (Fig. 1). Similarly, addition of tolbutamide to the perfusate resulted in a potentiation of insulin release. Addition of 40 mM-lactate produced a small, transient, stimulation of secretion. However, the subsequent removal of lactate from the perfusate resulted in a dramatic, biphasic, increase in the rate of insulin release. Such an effect was not observed upon withdrawal of glucose or tolbutamide from the medium. More modest effects of lactate addition/withdrawal were observed with a lower concentration (2.5 mM) of glucose in the medium (results not shown).

An increase in glucose concentration to 20 mM resulted in an initial fall, followed by a rise, in $^{45}\text{Ca}^{2+}$ efflux rate (Fig. 2a). Tolbutamide produced an immediate increase in $^{45}\text{Ca}^{2+}$ efflux (Fig. 2b). Addition of lactate to perfused islets elicited a transient rise in $^{45}\text{Ca}^{2+}$ fractional-outflow rate (Fig. 2c). A pronounced stimulation of $^{45}\text{Ca}^{2+}$ efflux was observed upon subsequent removal of lactate from the perfusion medium. The effect of lactate withdrawal on the efflux of $^{45}\text{Ca}^{2+}$ persisted in the absence of

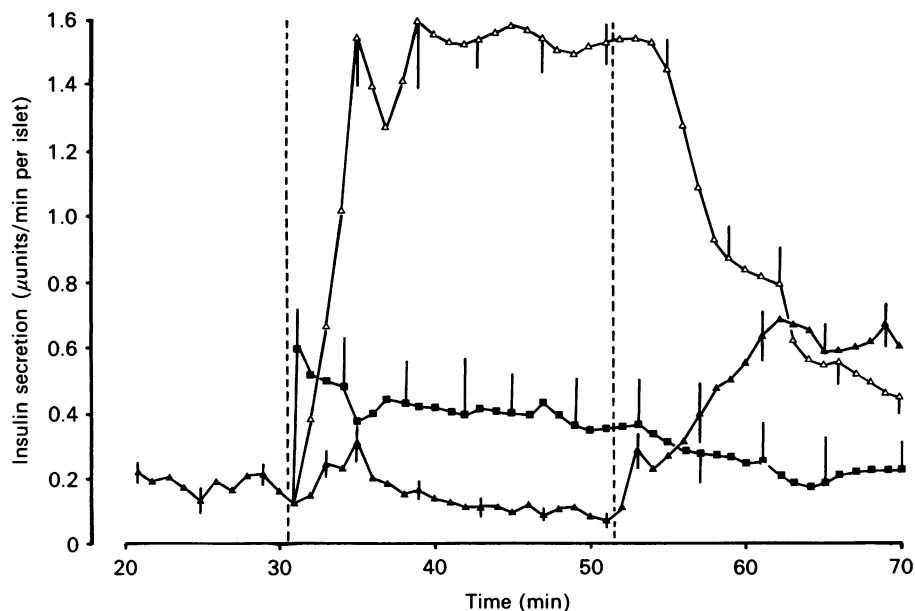


Fig. 1. Effects of glucose, tolbutamide and lactate on insulin secretion from perfused rat islets

Groups of 25 islets were perfused in the presence of 5.6 mM-glucose and exposed to 20 mM-glucose (Δ), 0.5 mM-tolbutamide (\blacksquare) or 40 mM-L-lactate (\blacktriangle) during the period designated between the vertical broken lines. Each point represents the mean \pm S.E.M. for three to six determinations.

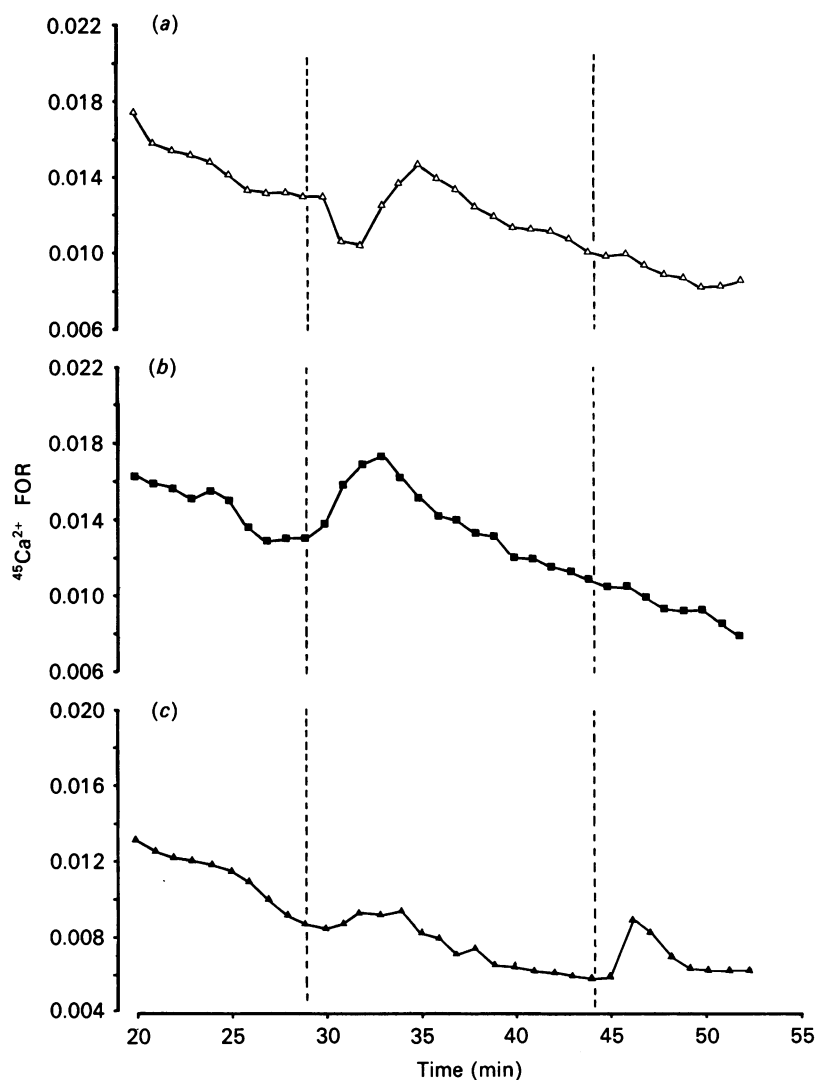


Fig. 2. Fractional outflow rates (FOR) of $^{45}\text{Ca}^{2+}$ from preloaded perfused rat islets

Groups of 150 islets were perfused in the presence of 5.6 mM-glucose and exposed to (a) 20 mM-glucose (Δ), (b) 0.5 mM-tolbutamide (\blacksquare) or (c) 40 mM-L-lactate (\blacktriangle) during the period designated by the vertical lines. The results shown are representative of two to five similar experiments.

glucose, but was greatly decreased in medium containing 0.5 mM-EGTA but no added Ca^{2+} (Fig. 3).

Exposure of islets loaded with $^{86}\text{Rb}^{+}$ to lactate resulted in a modest decrease in the rate of efflux of this radioisotope, particularly at a lower concentration of glucose (Fig. 4). The subsequent removal of lactate from the perfusate was associated with an increase in $^{86}\text{Rb}^{+}$ outflow in both cases.

The output of ^{14}C lactate, formed from $[\text{U-}^{14}\text{C}]$ -glucose, was found to be linear during a 120 min incubation (results not shown). The amount of ^{14}C lactate extruded into the incubation medium was related in a linear fashion to the concentration of $[\text{U-}^{14}\text{C}]$ glucose within the range 1–25 mM (Fig. 5).

DISCUSSION

It is generally accepted that the secretion of insulin in response to glucose is dependent upon metabolism of the sugar. However, the nature of the signal elaborated during glucose metabolism, which is responsible for

increased electrical, ionic and secretory activity in islets, is uncertain. Although it has been suggested that the mitochondrial oxidation of nutrient stimuli and generation of reduced nucleotides and ATP (Malaisse *et al.*, 1979a; Ozawa & Sand, 1986) may be involved in the generation of an intracellular 'metabolic signal', it has also been demonstrated that glucose-induced insulin secretion and $^{45}\text{Ca}^{2+}$ uptake correlate very closely with the rate of glycolysis in islet cells (Sener *et al.*, 1976).

The results of the present study suggest that the formation of lactate from glucose and the efflux of lactate from the islet cell could be a key determinant in the activation of islets by the hexose. It has previously been noted that lactate is readily metabolized by islet cells, but is a poor secretagogue (Malaisse *et al.*, 1979b; Lenzen & Panten, 1981). The transient stimulation of $^{45}\text{Ca}^{2+}$ efflux and insulin secretion observed upon addition of lactate could be the result of lactate oxidation, or of the actual transport of lactate across the plasma membrane. This latter process is thought to be H^{+} -dependent in a number of tissues (Spencer & Lehninger, 1976;

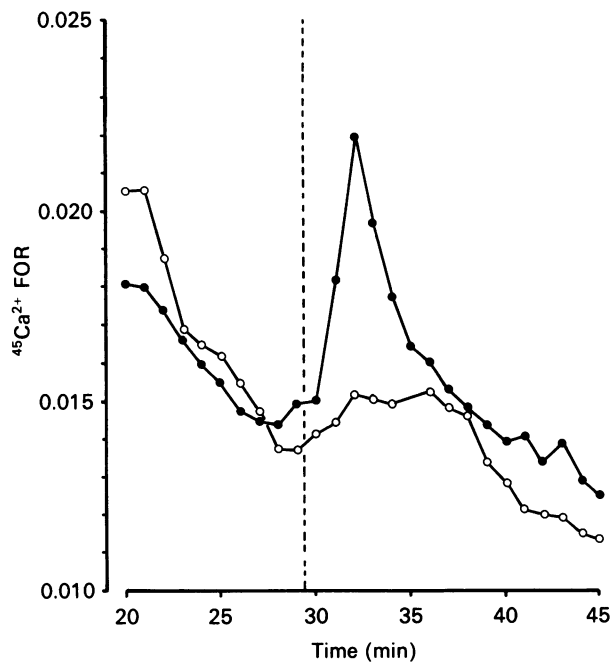


Fig. 3. Fractional outflow rates (FOR) of $^{45}\text{Ca}^{2+}$ from preloaded perfused rat islets in the absence of glucose

Groups of 150 islets were perfused in the absence of glucose and in the presence of 40 mM-lactate, in the presence of either 1 mM- Ca^{2+} (●) or zero Ca^{2+} , 0.5 mM-EGTA (○). Lactate was withdrawn from the perfusate at 29 min.

Jennings & Adams-Lackey, 1982; Fafournoux *et al.*, 1985), and we have observed a fall in cytosolic pH (pH_i) of insulin-secreting cells upon addition of lactate (J. E. Meats, unpublished work), which would be consistent with lactate-proton co-transport. A fall in pH_i could account for enhanced insulin secretion and a decreased rate of $^{86}\text{Rb}^+$ efflux (Best *et al.*, 1988b), but not the increased rate of $^{45}\text{Ca}^{2+}$ efflux observed in response to lactate (Best *et al.*, 1988a,b). An alternative possibility is that lactate enters the islet cells by an electroneutral mechanism such as proton co-transport, and that a

subsequent depolarization occurs as a result of lactate exit from the cell via a putative channel. Such a mechanism is thought to explain the progressive depolarization of Lettre cells incubated in increasing concentrations of lactate (Bashford & Pasternak, 1984), and is substantiated by our recent findings that insulin-secreting HIT cells are transiently depolarized by lactate, as assessed by oxonol-V fluorescence and direct measurement of plasma-membrane potential (J. E. Meats & M. D. Tuersley, unpublished work).

The possible importance of lactate efflux in regulating islet-cell plasma-membrane potential is emphasized by the observation that withdrawal of lactate from perfused islets causes an activation qualitatively similar to that elicited by raising the concentration of glucose. Clearly, these effects of lactate withdrawal are highly unlikely to be secondary to increased metabolic flux or decreased cytosolic pH. A depolarization of the islet cells via an effect on K^+ permeability is also excluded by the observation that lactate withdrawal increased, rather than decreased, $^{86}\text{Rb}^+$ permeability. This latter observation could possibly be the result of the opening of Ca^{2+} - or voltage-dependent K^+ channels (Matthews & Shotton, 1984). The simplest explanation for the activation of islets by lactate withdrawal is that the removal of lactate from the incubation medium permits a rapid, pronounced, increase in the rate of efflux of this anion from the islet cell, again perhaps via a putative channel. The efflux of lactate from the β -cell could, furthermore, be an important determinant of activation by glucose, a mechanism which would account for the close correlation between the rate of glycolysis and insulin release (Sener *et al.*, 1976).

It is proposed that the accumulation of protons in the cell that would otherwise occur is counteracted by the extrusion of protons from the cell in exchange for Na^+ ions. We have found evidence for an amiloride-sensitive Na^+/H^+ exchange system in islet cells (Best *et al.*, 1988a), and Malaisse *et al.* (1979c) have demonstrated that glucose-induced insulin secretion is accompanied by the production of H^+ in the incubation medium.

The efflux of lactate via such a mechanism would result in the net loss of negative charge from the inside of the cell, which could, in theory, cause a depolarization of

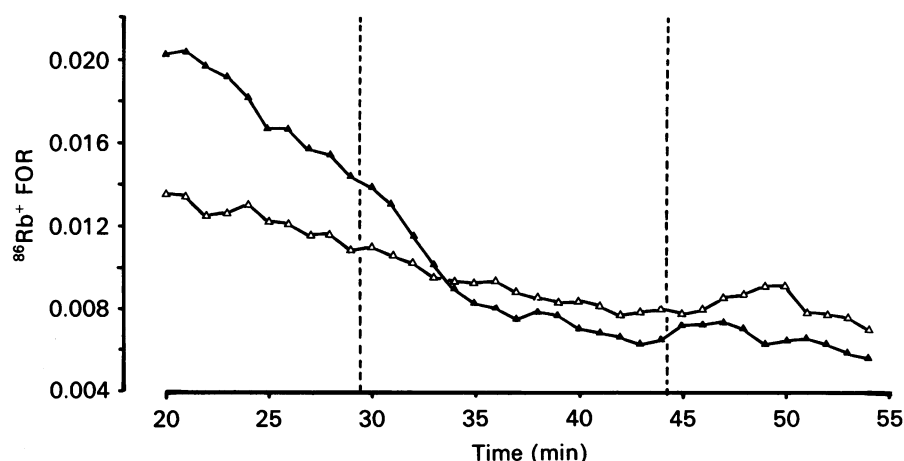


Fig. 4. Fractional outflow rates of $^{86}\text{Rb}^+$ from preloaded perfused rat islets

Groups of 150 islets were perfused in the presence of (▲) 2.5 mM or (△) 10 mM-glucose and exposed to 40 mM-L-lactate during the period designated by the vertical broken lines. The results shown are representative of three similar experiments.

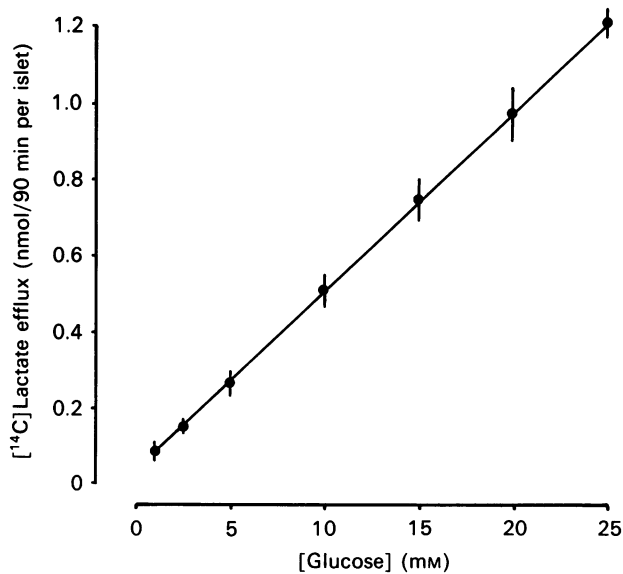


Fig. 5. Production of [¹⁴C]lactate from [U-¹⁴C]glucose by rat islets

Groups of 50 islets were incubated for 90 min in the presence of [U-¹⁴C]glucose together with appropriate concentrations of unlabelled glucose. Samples of incubation medium were then analysed for [¹⁴C]lactate content. Each point represents the mean \pm S.E.M. for five determinations.

the plasma membrane, leading to opening of voltage-sensitive Ca²⁺ channels and Ca²⁺ entry into the islet cell. It is noteworthy that the formation and extrusion of lactate from islets into the incubation medium occurred in a linear fashion with increasing concentrations of glucose over the range 1–25 mM. In this respect, the efflux of lactate from the islet β -cell is qualified as a potential coupling mechanism in the activation of islets by increasing glucose concentrations. This proposal does not deny a possible role for an ATP-regulated K⁺ channel in the modulation of β -cell membrane potential (Cook *et al.*, 1988). It is feasible that separate systems exist which regulate membrane potential over different ranges of glucose concentration, thus providing a multifactorial integrated control of islet function.

In conclusion, we have demonstrated novel actions of lactate upon pancreatic islets, consistent with the hypothesis that the electrogenic flux of lactate across the plasma membrane is a determinant of membrane potential. Whether such a mechanism is involved in glucose-induced depolarization of islet cells will await investigations into the modification of lactate production and efflux in islets, and the effects of such manipulations upon the activation of islets by glucose.

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