Insulin Biosynthesis in a Broken-Cell Preparation of Islets of Langerhans

DAVID G. PARRY and KEITH W. TAYLOR

Biochemistry Group, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, U.K.

There have been numerous attempts to study insulin biosynthesis by perfused pancreas slices and isolated islets of Langerhans, but so far insulin synthesis has rarely been successfully carried out with broken-cell preparation of islets or other subcellular fractions. Nevertheless, Wagle (1965) showed that a pH 5 enzyme-microsomal fraction obtained from foetal dog pancreas incorporated $^{14}$C-labelled amino acids into insulin, which could be subsequently precipitated by an anti-insulin serum. However, in these experiments the labelled insulin was not further characterized. In the experiments described below with rabbit islets of Langerhans, labelled proinsulin was obtained in a broken-cell preparation in the presence of a ribonuclease inhibitor.

Islets were prepared from male New Zealand White rabbits (2.5 kg) by collagenase digestion (Howell & Taylor, 1968). For each determination 100 islets were preincubated at 37°C for 20 min in Gey & Gey bicarbonate-buffered medium, pH 7.4, containing 2 mM glucose. After gentle centrifugation the medium was removed from the islets and replaced with 0.2 ml of a maintenance medium containing 80 mM KCl, 25 mM Tris, 4 mM magnesium acetate, 5 μM ATP, 1 μM GTP, 3.3 μM creatine phosphate, 1 unit of creatine phosphokinase and amino acids at a concentration of 1 μM for each of the amino acids present in porcine proinsulin but minus leucine. A ribonuclease inhibitor was added to the medium at a concentration of 2 Searle units/ml. The islets were then subjected to ultrasonic disruption for 2 s (this was found to be sufficient time to disrupt the cell membrane in this preparation). Then 10 μCi of L-[1$^{14}$]leucine and either 2 mM glucose, 16 mM glucose or 16 mM glucose and cycloheximide (250 μg/ml) were added to the islet preparation. An incubation for 1 h was carried out at 37°C and stopped by the addition of acetic acid (pH 3). The samples were then applied to a Sephadex G-50 (fine grade) column (1 cm x 55 cm) equilibrated with 1 M acetic acid. Peaks of radioactivity appearing in the proinsulin region of the elution profile were taken and freeze-dried in plastic tubes in the presence of 500 μg of albumin. The dried preparation was then taken up in 0.95% NaCl, and the proinsulin was purified by a double-antibody precipitation method previously described (Parry & Taylor, 1974). After extraction with acid ethanol the proinsulin was subjected to electrophoresis on paper at pH 2. At this stage a sample was taken for assay purposes in an insulin assay system (Hales & Randle, 1963). Radioactivity on the electrophoretogram was assayed by cutting 1 cm strips and counting their radioactivity in a liquid-scintillation spectrometer. The material was further characterized as proinsulin by gel electrophoresis at pH 8.9 and paper electrophoresis at pH 8.6.

Incorporation of L-[1$^{14}$]leucine into rabbit proinsulin was significantly increased when the glucose concentration was raised from 2 mM to 16 mM. Further, the presence of cycloheximide significantly lowered the rate of incorporation into proinsulin.

The results shown in the present communication suggest that cellular integrity of the β-cell is not essential for proinsulin synthesis to occur, provided that a ribonuclease inhibitor is present. Under conditions where glucose is present in the medium only proinsulin is produced, indicating that when cellular organization has been disrupted the conversion process to insulin can only occur at a specific locus within the β-cell, probably in the Golgi region (Howell et al., 1969).

Raising the glucose concentration of the medium from 2 to 16 mM increased leucine incorporation 3-fold, which compares favourably with values obtained for whole islets. A further indication that labelled proinsulin was being synthesized in a broken-cell preparation was obtained when it was shown that cycloheximide abolished the stimulatory effect of 16 mM glucose on incorporation.

These experiments suggest that glucose transport is unlikely to be involved in triggering the biosynthetic response to glucose in islets. Equally well, it seems unlikely that a mem-
brane receptor is involved unless it is assumed that cell-membrane fragments are functionally operative in a broken-cell preparation. The synthetic response obtained in a broken-cell preparation to the presence of glucose in the medium could suggest that glucose itself or a glucose metabolite is accelerating insulin biosynthesis.

Hales, C. N. & Randle, P. J. (1963) Biochem. J. 88, 137–144

The Electrogenericity of Amino Acid Absorption in Mouse Ascites-Tumour Cells
ROGER D. PHILO and A. ALAN EDDY

Department of Biochemistry, University of Manchester Institute of Science and Technology, Manchester M60 1QD, U.K.

A number of members of the merocyanine class of dyes have been investigated by Sims et al. (1974) as fluorescent probes of membrane potential. Dyes of this class have been used to investigate membrane potentials in human and amphiuma erythrocytes (Hoffman & Laris, 1974) and in a squid giant axon (Davila et al., 1973). In all cases an increase in fluorescence correlated with a decrease in membrane potential (cell interior going less negative) and vice versa.

We report here the use of one of these dyes, 3,3′-dipropyl-oxadicarbocyanine iodide, with mouse ascites-tumour cells to investigate changes in membrane potential in relation to Na⁺-dependent amino acid transport in these cells.

According to the ion-gradient hypothesis the energy for uphill transport of certain amino acids in these ascites cells is obtained from the gradients of Na⁺, and possibly K⁺, acting across the plasmalemma. However, it has been shown (Schafer & Heinz, 1971) that accumulation can occur even when the concentration gradients of Na⁺ and K⁺ are both very small and might lead to the actual expulsion of amino acid when this is already present in the tumour cells. Schafer & Heinz (1971) suggested that some at least of the energy required for amino acid accumulation was directly derived from cellular metabolism. This interpretation has been questioned by Gibb & Eddy (1972) and Reid et al. (1974), who proposed that the energy available from the Na⁺ gradient alone might be sufficient if the magnitude of the membrane potential was larger than had hitherto been supposed. The results presented here lend some support to this view.

Ascites cells (Reid et al., 1974) were incubated at 37°C for 30 min in 25 vol. of Ringer solution of composition 155 mequiv. of Na⁺/litre, 8 mequiv. of K⁺/litre, 1.2 mequiv. of MgSO₄ and 16 mM-sodium phosphate (termed 'standard Ringer' below). The cells (approx. 2 mg dry wt.) were then suspended in 4 ml of the same Ringer and 5 μg of 3,3′-dipropyl-oxadicarbocyanine iodide was added. Addition of glucose to this cell suspension caused the fluorescence to decrease after a lag of about 1 min (Fig. 1a). This effect also occurred in the presence of 2.5 μg of antimycin/ml, though not when K⁺ was omitted (Fig. 1b). The fluorescence decrease was blocked by prior addition of ouabain, and a fluorescence increase resulted if ouabain was added after glucose (Fig. 1a). Glucose and ouabain had no effect on the fluorescence of the dye in suspensions of frozen–thawed cells or in the absence of cells. Sucrose, which is non-permeant, and L-arabinose, which is taken up but probably not metabolized rapidly, did not affect the fluorescence.

Addition of glycine or methionine to the cell suspension, either before or after the addition of glucose or ouabain, caused a rapid fluorescence increase followed by a slower decrease (Fig. 1c). Addition of glycine to cells in a Ringer solution lacking Na⁺