STUDIES ON GLUCOSE SYNTHESIS IN RAT KIDNEY CELL SUSPENSIONS

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1. Introduction

Glucose formation by rat kidney has been studied in tissue slices [1,2] the isolated perfused organ [3] and homogenate preparations [4]. In contrast to liver the rates of glucose synthesis from various precursors in slices of kidney cortex are similar or above those observed in kidney perfusion. However, after homogenization 90% of the glucogenic capacity of the slices is lost [4]. Most of the residual 10% of glucose formation in kidney homogenates was found to be due to contamination with nuclei surrounded by cytoplasmic residues [5]. On the basis of this observation the present system was developed which exhibits the metabolic properties of the cortex slices combined with the possibility of dispensing aliquots in large series.

2. Methodology and results

2.1. *Preparation of cell suspensions*

Before preparation of suspensions the rat kidneys were perfused with 20 ml of icecold medium (see section 2.4) *in situ*. The blood-free organs were decapsulated and the major part of the medulla was excized. The organs were then blotted and pressed through a nylon mesh with the aid of a pestle covered with Parafilm (Marathon Products, Neenah, Wisc., USA). In most cases a commercial tea sieve (pore diameter 500 μm) was used. Sieves with smaller pores resulted in less active cell preparations. The resulting cell brei was stirred into the icecold medium, and, after weighing, diluted 1:5 with the same medium. After the tissue was suspended by hand with a loosely fitting glas rod, the intact cells were separated from subcellular particles by a 5 min centrifugation at 50 *g*. After decanting the supernatant fresh medium was added up to the original volume and the sediment washed twice by resuspension and centrifugation at 50 *g*. The final sediment was taken up and suspended in the original volume (1:5 w/v) and the suspension was then pipetted into the incubation flasks. In order to obtain reproducible aliquots the suspension should be constantly stirred, and dispensed by a blow-out pipette with a large orifice. Table 1 demonstrates the reproducibility of the whole system by determination of glucose, formed from lactate during 30 min of incubation.

Table 1
Reproducibility of glucose synthesis in suspended rat kidney cells. Aliquots from a cell pool of 8 fed rats was dispended into 15 incubation vessels and incubated for 30 min at 37°C. Substrate: 10 mM sodium lactate. Endogenous glucose (0.256 μmoles) has been subtracted. Dry weight/flask: 16 mg.

Flask	Glucose formed (μmole/flask · 30 min)
n	15
Mean	0.4600
S.D.	0.0496
S.E.M.	0.0137

2.2. *Light microscopy*

Most of the final sediment consists of tissue particles with the size of the pores of the sieve used. These particles mainly consist of tubules and tubule fragments, including glomeruli, and some isolated tubule cells.

2.3. *Incubation procedure*

In most cases 0.5 ml of the cell suspension was pipetted into 50 ml (25 ml) flasks together with substrates (10 mM final concentration) and made up with incubation medium to 4 ml (2 ml) of total volume. The flasks were gassed with 95% O_2 – 5% O_2 while they stood in ice and then stoppered with serum stoppers. Incubation was carried out in a shaking water bath at 37°C. After 30 or 60 min of incubation the flasks were taken out, put on ice and the reaction stopped by addition of 0.2 ml of 30% perchloric acid. After neutralization of the supernatants with solid $KHCO_3$ the substrates and reaction products were determined enzymatically in the clear filtrates.

2.4. *Incubation conditions*

In all basic experiments glucose synthesis from pyruvate was measured. Generally the same conditions that were shown to be suitable for kidney cortex slices [2] gave similar results with the cell suspension system. Krebs-Henseleit bicarbonate medium was used in most experiments. Bicarbonate of this medium could be replaced by isotonic phosphate- or triethanolamine buffers of pH 7.4. This allowed measurement of O_2 consumption in Warburg vessels.

Sodium in the incubation mixture could not be replaced by potassium; calcium (2.4 mM) was an essential factor for maximal glucose synthesis as shown before by Krebs et al. [2] for kidney cortex slices.

Using O_2 (Tram-medium as fluid) or 95% O_2 – 5% CO_2 as gas phase was without major effect on metabolic activity, but the rate of glucose synthesis from pyruvate was markedly decreased when the flasks were incubated under air.

Increasing the incubation volume also decreased glucose synthesis. A ratio of fluid volume to gas volume of 1:12 was found to be most effective in our studies.

2.5. *Rate of glucose synthesis and the effect of starvation*

Glucose synthesis from 10 mM pyruvate was nearly

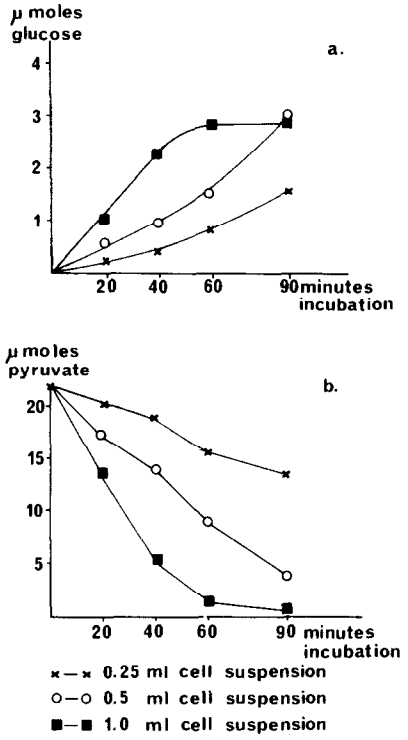

Fig. 1. Time dependency of glucose formation from pyruvate by different amounts of kidney cell suspension. 0.5 ml of a cell preparation from 24 hr starved rats was incubated with pyruvate (10 mM) in Krebs-Henseleit bicarbonate medium (2 ml final volume) at 37°C. 1 ml cell suspension corresponds to 25 mg dry weight. (a) Glucose in µmoles/flask (endogeneous glucose subtracted). (b) Pyruvate in µmoles/flask.

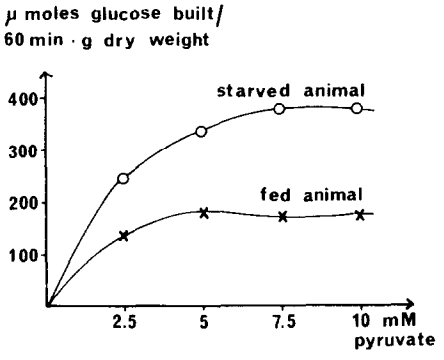

Fig. 2. Substrate dependency of glucose synthesis from pyruvate in kidney cell suspensions from fed (mixed standard diet) and 24 hr starved rats. Incubation conditions were as in fig. 1. Incubation time was 60 min.

Table 2
Glucose synthesis in kidney cell suspensions
(μMoles/g dry weight/hr).

Substrate used	Fed	Starved for 24–48 hr
Pyruvate	196.02 ± 19.67 (9)	316.71 ± 16.15 (7)
Lactate	76.43 ± 8.24 (5)	156.20 ± 9.13 (4)
Malate	202.61 ± 22.70 (6)	260.00 ± 23.00 (4)

Experimental conditions are as described in the text. Rats with a body weight of 140–160 g were used. Results are given as means ± s.e.m. with numbers of experiments in parentheses.

linear with time up to exhaustion of substrate. Fig. 1 also demonstrates the linear dependency of glucose formation on the amount of cells. Fig. 2 shows the dependency of glucose formation from different concentrations of pyruvate. Maximal glucose synthetase was achieved with 5 mM pyruvate in cells of fed animals and with 10 mM in those of starved ones. Because 10 mM pyruvate resulted in the same glucose formation as 5 mM in fed animals, all following experiments were performed with 10 mM substrate. Table 2 summarizes the results obtained with 3 representative substrates in cells from fed and starved animals. The rates are in the same range with those obtained with the slice technique by Krebs et al. [2] and above those shown in the isolated perfused kidney by Nishiitsutsuji-Uwo et al. [3]. Single experiments with other substrates such as oxaloacetate and α-ketoglutarate also corresponded to the finding in slices.

2.6. *Influence of acetate on glucose formation*

The findings of Krebs et al. [6] that gluconeogenesis in kidney from lactate and other precursors can be stimulated by acetate was also found in the present study with the suspension system. Acetate, in a concentration of 2.5 mM stimulated glucose formation from malate and lactate, but not from pyruvate. With 5 mM acetate this stimulation was not always observed.

3. Discussion

The method presented herein offers a simple model for metabolic studies in kidney cortex cells. Although the suspension obtained from a washed cell brei does not mainly contain isolated cells, the particles can be dispended by pipetting in reproducible aliquots. The results of the light microscopical and metabolic studies indicate that the tissue suspension mainly represents fragments of kidney cortex. The method can be applied for studies on the metabolism of kidney cortex *in vitro*, and its hormonal, nutritional and metabolic regulation.

References

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