Regulation of pathways of glucose metabolism in kidney

Specific linking of pentose phosphate pathway activity with kidney growth in experimental diabetes and unilateral nephrectomy

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The pentose phosphate pathway operates at an elevated level in rat kidney following induction of diabetes and in the compensatory hypertrophy following unilateral nephrectomy in control and alloxan-diabetic rats, as shown by the yields of $^{14}Co_2$ from [1-^{14}C]glucose, [6-^{14}C]glucose and ^3H_2O yields from [2-^{3}H]glucose. The elevated flux through the pentose phosphate pathway is correlated with the increased RNA content and weight of the kidney. The direct utilization of NADPH for reductive synthetic reactions and the potential for indirect utilization via the sorbitol route and the linked transhydrogenase reactions of the glucuronate-xylulose pathway, for NADH and ATP generation, are also discussed.

[^{14}C]Glucose utilization *Pentose phosphate pathway* *Unilateral nephrectomy* *Correlation with growth* *Rat kidney* *Experimental diabetes*

1. INTRODUCTION

There is an apparent anomaly in experimental diabetes in that there is a loss of body and liver weight concomitant with an accelerated rate of renal growth, this latter occurring shortly after the induction of diabetes [1–4] and it has been shown that treatment of diabetic rats with insulin will prevent this kidney growth [4]. There is evidence for a decrease in nucleic acid and protein synthesis in liver and muscle in diabetes but an increase in these biosynthetic routes in the kidney under similar conditions [5–9]. Even more striking, perhaps, is the observation [4] that the already-considerable kidney growth that follows unilateral nephrectomy (UN) is enhanced even further when the operation is performed on diabetic animals.

A relationship exists between the blood glucose level and the rate of kidney growth [10]; this study attempts to establish whether the increased kidney growth in diabetic animals is related to multiple changes in the pattern of carbohydrate metabolism or whether any single pathway is particularly involved.

An increase in the flux of glucose through the pentose phosphate pathway (PPP) in the kidney has been reported to occur following the induction of diabetes [11,12] and in the compensatory hypertrophy following UN [13] possibly related to the increased requirement for ribose 5-phosphate and NADPH in biosynthetic reactions and, in the case of diabetes, to the changes in acid–base and electrolyte balance [10–16].

There is a direct correlation between the activity of the PPP and the rate of kidney growth while no such correlation was observed between kidney growth and the flux of glucose through the glycolytic–tricarboxylic acid cycle route.

2. MATERIALS AND METHODS

2.1. *Animals*

Diabetes was induced in adult male Wistar rats by intravenous injection of alloxan (50 mg/kg

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Table 1

Activity of enzymes of the pentose phosphate pathway and key enzymes of the glycolytic route in kidney from control and alloxan-diabetic rats with and without unilateral nephrectomy

Enzyme	Control	UN-Control	UN-C /C (%)	Diabetic	D/C (%)	UN-Diabetic	UN-D /C (%)	UN-D /D (%)
Units.g kidney^{-1}								
Glucose phosphorylation								
Hexokinase								
Soluble	0.720 ± 0.051	0.685 ± 0.029	95	0.807 ± 0.059	112	0.615 ± 0.031	85	76[a]
Particulate	0.754 ± 0.060	0.753 ± 0.081	99	0.730 ± 0.080	96	0.717 ± 0.112	95	98
Pentose phosphate pathway								
G6P dehydrogenase	1.34 ± 0.13	1.30 ± 0.10	97	1.32 ± 0.12	98	1.31 ± 0.09	97	100
6GP dehydrogenase	0.880 ± 0.03	0.96 ± 0.08	109	0.99 ± 0.04	112	1.15 ± 0.09	130[a]	116
Transketolase	0.990 ± 0.10	1.06 ± 0.17	107	1.09 ± 0.15	110	1.01 ± 0.12	102	92
Transaldolase	1.45 ± 0.15	1.30 ± 0.12	89	1.42 ± 0.11	97	1.32 ± 0.09	91	93
Glycolytic route								
Phosphofructokinase	0.838 ± 0.111	0.996 ± 0.095	119	1.39 ± 0.23	165[a]	1.39 ± 0.22	165[a]	100
Pyruvate kinase	19.3 ± 1.70	17.5 ± 1.94	91	21.6 ± 1.94	111	17.6 ± 1.90	91	81
Units.kidney^{-1}.100 g body wt^{-1}								
Glucose phosphorylation								
Hexokinase								
Soluble	0.229 ± 0.016	0.327 ± 0.014	143[b]	0.646 ± 0.047	282[c]	0.579 ± 0.029	252[c]	89
Particulate	0.240 ± 0.019	0.360 ± 0.039	150[a]	0.584 ± 0.064	243[c]	0.675 ± 0.105	281[b]	115
Pentose phosphate pathway								
G6P dehydrogenase	0.426 ± 0.041	0.621 ± 0.046	146[b]	1.05 ± 0.10	246[c]	1.23 ± 0.08	288[b]	117
6GP dehydrogenase	0.280 ± 0.009	0.459 ± 0.038	164[b]	0.79 ± 0.03	282[c]	1.08 ± 0.08	385[c]	137[a]
Transketolase	0.315 ± 0.032	0.507 ± 0.081	161[a]	0.87 ± 0.12	276[c]	0.95 ± 0.11	301[c]	109
Transaldolase	0.461 ± 0.048	0.621 ± 0.050	135[a]	1.14 ± 0.08	247[c]	1.24 ± 0.08	268[c]	109
Glycolytic route								
Phosphofructokinase	0.266 ± 0.035	0.476 ± 0.045	179[b]	1.11 ± 0.19	417[b]	1.31 ± 0.22	492[b]	118
Pyruvate kinase	6.14 ± 0.53 (6)	8.36 ± 0.93 (6)	136 (6)	17.2 ± 1.5	280[c]	16.6 ± 1.79	270[c]	96

Fisher's p-values: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$

The values are given as means ± SEM; (no. obs.); a unit of enzyme activity is defined as μmol substrate converted/min at 25°C. All enzyme activities are for the dialysed high speed supernatant fraction with the exception of hexokinase for which activities of soluble and particulate (mitochondrial + microsomal) fractions are given. Kidney weight and enzyme activity.kidney^{-1}.100 g body wt^{-1} refer to a single kidney. The initial body weight was 302 ± 14 g and kidney weight was 1.26 ± 0.08 g (17 values). Rats were unilaterally nephrectomized (UN) 5 days after administration of alloxan and used 6–7 weeks later (see section 2)

Abbreviations: C, control; UN-C, UN-control; D, diabetic; UN-D, UN-diabetic

body wt). Two units of insulin (Monotard MC, Novo) were injected daily for the following 5 days at which time half the control and diabetic animals underwent unilateral nephrectomy, the diabetic groups continuing the insulin treatment for another 5 days. The animals were killed 6–7 weeks after alloxan treatment. The HbA_1 was determined as in [17].

2.2. *Flux of glucose through alternative pathways*

The conversion of specifically labeled glucose to $^{14}CO_2$ and $^{3}H_2O$ by kidney cortex slices incubated for 1 h in Krebs-Ringer bicarbonate medium containing 5 mM glucose (control and UN-control groups) and 20 mM glucose (diabetic and UN-diabetic groups) and 0.5 μCi [^{14}C]glucose or 1 μCi [2-^{3}H]glucose was measured as in [11]. The artificial electron acceptor phenazine methosulphate (PMS) was present at 0.1 mM final conc.

3. RESULTS AND DISCUSSION

Table 1 shows the yield of $^{14}Co_2$ from specifically labelled glucose. The following pattern of change is apparent:

(i) The rate of glucose phosphorylation ([2-^{3}H]glucose → $^{3}H_2O$) is decreased relative to the control group in UN-control, diabetic and UN-diabetic groups;

(ii) The glycolytic pathway and pyruvate dehydrogenase ([3,4-^{14}C]glucose → $^{14}CO_2$) is also significantly lower in these groups;

(iii) The flux of glucose through the tricarboxylic acid cycle ([6-^{14}C]glucose → $^{14}CO_2$) is unchanged;

(iv) In sharp contrast to the other metabolic routes, the activity of the PPP (C1–C6) increases dramatically in UN-controls, diabetic and UN-diabetic kidney.

Examination of the 'proportionate yields' of $^{14}CO_2$ from labelled glucose, which indicates the amount of glucose utilized via an oxidative pathway relative to the rate of glucose phosphorylation, again reveals the highly significant increase of the PPP in the kidney in diabetes and following unilateral nephrectomy. In the double-lesioned animal there is a 12-fold increase in the proportion of glucose oxidized via the PPP.

The close correlation between the extent of PPP activity and kidney hypertrophy is further illustrated in fig. 1 which draws on these data and [11]. The specific relationship is emphasized by the fact that no correlation exists between kidney hypertrophy and the flux of glucose through the tricarboxylic acid cycle, despite the expected increased demand of ATP. This finding raises the question of the significance of the PPP in kidney hypertrophy and of the mechanism by which this pathway is stimulated.

The significance of the increase in the PPP centres upon the supply of two important intermediates, ribose-5-phosphate, used for nucleotide and nucleic acid synthesis, and NADPH which is used for reductive biosynthetic reactions including lipogenesis, conversion of GSSG → GSH, deoxyribonucleotide synthesis and in transhydrogenase reactions via linked $NADPH:NADP^+$ and $NAD^+:NADH$ steps in the sorbitol route and glucuronate xylulose pathway. The function of the supply of ribose 5-phosphate may be of major quantitative importance, particularly in view of the rapid rise in the RNA content of kidney in uncontrolled diabetes and in UN-diabetic rats [15,18].

The mechanism of the increased flux of glucose through the PPP may rest, in part, on the increased blood glucose in the diabetic state and the free permeability of the kidney to glucose in the absence of insulin. Thus, with normal rat kidney cortex slices there is a 5-fold increase in the flow of glucose through the PPP when medium [glucose] is raised from 5–20 mM [11], the higher concentration paralleling the glycaemic condition of the diabetic state; in [19] sorbitol and fructose accumulated in kidney in experimental diabetes. It is proposed that in kidney, as in lens, an increased flux of glucose through the sorbitol route could act as a 'drive' to the PPP by reoxidation of NADPH, the latter being a powerful inhibitor of the oxidative reactions of this route [20–22]. The interrelationship between the oxidative reactions of the PPP and the glycolytic route via the activation of phosphofructokinase by 6-phosphogluconate [23] may also lead to a coordinated increase in intermediates of the glycolytic route, in particular of fructose bisphosphate.

There is increasing interest in the role of sugar phosphates and NADPH as regulatory factors in protein synthesis, in particular that of glucose 6-phosphate and fructose bisphosphate play such a role [24,25]. The increased availability of glucose

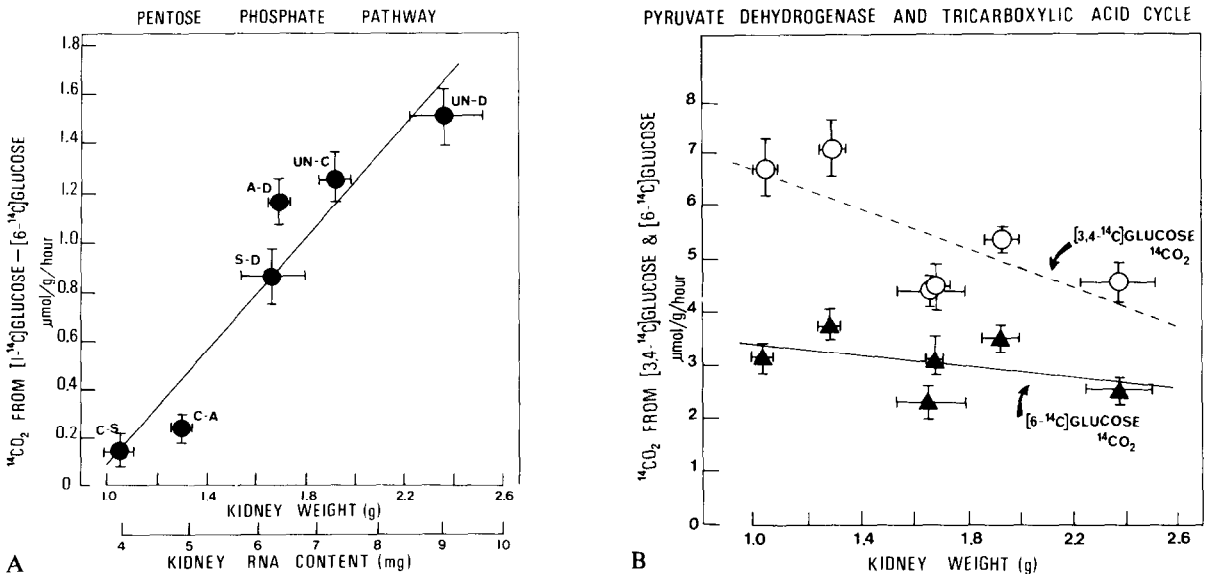

Fig. 1. The relationship between flux of glucose through the PPP, pyruvate dehydrogenase and tricarboxylic acid cycle and the growth of the kidney in experimental diabetes and unilateral nephrectomy. The pathways and correlation coefficients with kidney weights are: (●) PPP, $^{14}CO_2$ yield C1–C6, $r = 0.95$, $P < 0.001$; (○) glycolysis and pyruvate dehydrogenase, $^{14}CO_2$ yield [3,4-^{14}C]glucose, $r = 0.74$, $P = 0.06$; (▲) tricarboxylic acid cycle, $^{14}CO_2$ yield [6-^{14}C]glucose, $r = 0.39$, NS; the number of observations are as in table 1, the streptozotocin diabetic group (SD) and controls (C-S) each contain 6 values and are from [11]. The horizontal and vertical lines represent the SEM of the kidney weights and $^{14}CO_2$ yields, respectively. *Abbreviations*: AD, alloxan-diabetic; UN-D and UN-C, unilaterally nephrectomized diabetic and control groups; C-A, control group for alloxan-diabetic rats

in the diabetic state and the consequent increase in kidney content of glucose and glucose 6-phosphate [26] might therefore be key factors influencing protein synthesis, as well as RNA synthesis, UDP glucose and glycogen synthesis, the stimulation of these pathways providing a biochemical network linking hyperglycaemia to kidney hypertrophy.

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