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REGULATION OF ALTERNATIVE PATHWAYS OF GLUCOSE METABOLISM IN RAT HEART IN ALLOXAN DIABETES: CHANGES IN THE PENTOSE PHOSPHATE PATHWAY

Milena Sochor, Ana-Maria Gonzalez and Patricia McLean

Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W1P 7PN, Great Britain

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SUMMARY: The flux of glucose through the pentose phosphate pathway, important in relation to the provision of ribose 5-phosphate for nucleotide and RNA synthesis, was decreased by 70% in the diabetic rat heart in parallel with a similar decreased flux through the glycolytic route. A common factor linking the decreased flux through these alternative routes is the known fall in cardiac hexokinase; in these experiments there is a 50% decrease in Type II hexokinase (EC 2.7.1.1.) in both soluble and particulate fractions. The level of fructose 2,6-bisphosphate, a regulator of phosphofructokinase activity, is decreased by 20% in the alloxan diabetic rat heart, this may be a significant additional factor in the marked decrease in the flux of glucose through the glycolytic route in the myocardium in diabetes.

The disturbances of carbohydrate and lipid metabolism in the heart in diabetes have been widely studied [see 1-3]. Points of regulation of particular significance to glucose metabolism are those located at glucose transport, glucose phosphorylation, with changes reported in the intracellular distribution and relative contents of isoenzymic forms of hexokinase, phosphofructokinase and pyruvate dehydrogenase [4-7]. The changes in the metabolite content of the heart in diabetes, the rise in hexosemonophosphate and the fall in fructose 1,6-bisphosphate, point to regulation of phosphofructokinase (PFK 1 EC.2.7.1.11.) as a critical feature of the depressed glycolytic flux in the myocardium in diabetes [1,4]. Studies on the regulation of cardiac PFK have highlighted the importance of adenine nucleotides and citrate in its regulation [see 1-3]. A novel metabolite, fructose 2,6-bisphosphate (Fru-2,6-P_2) has been reported to be a potent activator of phosphofructokinase and its concentration has been shown to be regulated by hormonal and dietary factors, in particular, it has been shown to decrease dramatically in diabetes [8-10]. It therefore seemed of importance to gain further information as to whether Fru-2,6-P_2 was involved

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in the regulation of glycolytic flux in the heart in diabetes by measuring the concentration in cardiac tissue from alloxan-diabetic rats.

The regulation of the pentose phosphate pathway (PPP), an alternative route of glucose metabolism in the heart, has received less attention than the glycolytic route. While the PPP has a relatively low activity in the normal heart [11], it may be active in myocardium in certain patho-physiological states including experimentally induced hypertrophy and during repair processes after a myocardial infarction [12-16], two conditions where there is an increased requirement for ribonucleotides [15,16].

In diabetes there is a decrease in the weight of the heart relative to the age-matched control groups, and it has been shown that there is a reduced RNA content and depressed rate of protein synthesis [17]. In view of recent studies highlighting the importance of the PPP in phosphoribosylpyrophosphate formation and thus of nucleotide and nucleic acid synthesis in tissues such as heart and fibroblasts [15,16,18] studies have been made of the flux of glucose via the PPP in the diabetic rat heart and an assessment made of the relative effects of diabetes on the alternative routes of glucose metabolism.

METHODS

Animals. Adult male albino rats of the Wistar strain were used, the initial body weight was 220-250g. Diabetes was induced by the subcutaneous injection of alloxan-monohydrate (20 mg/100 g body weight) into rats previously starved for 24h; thereafter insulin was administered (2 units protamine zinc insulin daily for one week) and standard laboratory cube diet and water were allowed ad lib. The rats were used six weeks later.

Evaluation of alternative pathways. The conversion of ^{14}C-labelled glucose to $^{14}CO_2$ by heart slices was estimated as described previously [19,20]. Heart slices were incubated for 1h in 5ml of Krebs-Ringer bicarbonate medium containing 20mM glucose and 0.5 µCi of specifically labelled glucose. Phenazine methosulphate was added to the incubation medium to give a final concentration of 0.1mM, I unit of Novo insulin was added in vitro to the incubation medium. The calculation of the approximate contribution of the PPP, in the basal and in the activated state in the presence of phenazine methosulphate, was as previously described for kidney and brain [20,21].

Hexokinase activity. Hexokinase (EC. 2.7.1.1.) was estimated spectrophotometrically as previously described [22] using the method of Grossbard & Schimke [23] to determine the relative amounts of the types I and II isoenzymes. Homogenates were prepared using a Potter homogeniser with a Teflon plunger, the finely chopped tissue was homogenized in 10 volumes of 0.25M sucrose containing 20mM triethanolamine buffer pH7.4 and 0.1mM dithiothreitol. Hexokinase was estimated in the dialysed high speed supernatent fraction (105,000g for 45 min) and in the pellet fraction, the latter was resuspended in the homogenising medium. A unit of enzyme converts 1 µmol substrate/min at 25°C.

Fructose 2,6-bisphosphate. Fru-2,6-P_2 was determined by the method of Van Shaftingen et al [24]. The rats were anaesthetized with sodium pentobarbital (60 mg/kg body weight, administered i.p.) and the hearts quickly removed and freeze-clamped using liquid nitrogen. Fru-2,6-P_2 was assayed by its stimulatory action on potato tuber PPi: fructose 6-phosphate 1-phosphotransferase prepared by the method of Van Shaftingen et al [24].

RESULTS AND DISCUSSION

The rate of $^{14}CO_2$ production from 14C-labelled glucose by heart slices is shown in Table 1. There is an approximately 60% decrease in the rate of $^{14}CO_2$ yield from [U-14C] glucose and [3,4-14C] glucose by heart slices from alloxan-diabetic rats and a 35% decrease in the yield from [6-14C] glucose indicating a marked fall in the flux of glucose through the glycolytic pathway and pyruvate dehydrogenase reaction and via the tricarboxylic acid cycle respectively. These observations are in accord with the known decrease in glycolytic flux, and the decrease in the active form of pyruvate dehydrogenase in the rat heart in diabetes [4-7].

The activity of the PPP, as shown by the difference in yields of $^{14}CO_2$ from carbons 1 and 6 of glucose (C_1-C_6), is markedly decreased in the diabetic rat heart, falling to 31% of the control value (Table 1). The potential activity of this route, as measured in the presence of the artificial electron acceptor phenazine methosulphate, suggests that the heart has a large reserve capacity to metabolize glucose via the PPP in both normal and diabetic states. It may be noted that the degree of stimulation by phenazine methosulphate is greater in the diabetic rat heart (Table 1) indicative of a greater restriction by the NADPH/NADP+ couple in this condition [25]. These data are in accord with evidence that the PPP has a low activity in the normal heart but can exhibit a greater activity in experimentally induced hypertrophy and possibly during repair process after myocardial infarction [12-16]. Studies by Zimmer et al [15,16] have indicated that in the heart the PPP may play a rate-limiting role in the supply of ribose 5-phosphate for phosphoribosylpyrophosphate formation and thus for nucleotide and nucleic acid synthesis. Their observations, together with the present data on the decreased activity of the PPP in diabetes, could form the basis of one mechanism relating to the known decrease in cardiac RNA

Table 1. Conversion of ^{14}C-labelled glucose to $^{14}4CO_2$ by heart slices from control and alloxan diabetic rats.

	CONTROL	DIABETIC	Diabetic x 100 / Control %	P
$^{14}CO_2$ YIELDS FROM ^{14}C GLUCOSE (μmol/g/h)				
[1-14C] glucose	5.21 ± 0.60	3.07 ± 0.40	58	**
[1-14C] glucose + PMS	9.86 ± 0.80	6.13 ± 0.72	62	**
[1-14C] glucose + insulin	6.01 ± 0.59	3.21 ± 0.41	53	**
[3,4-14C] glucose	6.40 ± 0.48	2.57 ± 0.40	40	***
[6-14C] glucose	4.23 ± 0.37	2.76 ± 0.34	65	**
[U-14C] glucose	6.43 ± 0.55	2.42 ± 0.40	38	***
Approximate PPP (C_1-C_6)	0.98 ± 0.09	0.31 ± 0.06	31	***
Approximate PPP fully activated (C_1 + PMS-C_6)	5.63 ± 0.50	3.37 ± 0.34	60	**
PMS Stimulation	5.7-fold	10.9-fold		
Blood glucose (mM)	5.6 ± 0.5	26.1 ± 1.0	464	***
Body weight (g)	306 ± 18	188 ± 11	61	***
Heart weight (g)	0.95 ± 0.04	0.65 ± 0.02	68	***
Heart weight per 100g body weight	0.31 ± 0.01	0.35 ± 0.01	112	

The results are given as means ± SEM of 8 observations. The glucose concentration in the medium was 20 mM, additions in the medium were phenazine methosulphate (PMS) present in a final concentration of 0.1mM, and insulin (Novo) 1iU/5ml medium. For details see Methods. Fisher's P values are given by asterisks: ** $P<0.01$; *** $P<0.001$.

in diabetes. Williams et al [17] have reported that 30% of cardiac RNA is lost 10 days after induction of diabetes; they also observed a reduction in protein synthesis shortly after induction of diabetes.

One common factor in the regulation of the glycolytic route and PPP is the rate of phosphorylation of glucose and, in line with observations by Katzen et al [5], it was found in the present experiments that there was a decrease in hexokinase activity, the most significant change being the fall in the type II isoenzyme, of both soluble and particulate fractions, to less than half the control values (Table 2). Additionally there was an apparent redistribution of hexokinase type I, the total activity remained constant at 1.36 and 1.40 units/g tissue for control and diabetic rats respectively. However, the soluble/bound quotient changed from 0.9 to 1.4 respectively. This pattern of shift in content and distribution of isoenzymic form of hexokinase is characteristic of the

Table 2. Hexokinase activity and distribution in control and alloxan-diabetic rat hearts

	CONTROL	DIABETIC	Diabetic x 100 / Control %	P
HEXOKINASE ACTIVITY (units/g)				
Soluble fraction				
Type I	0.65 ± 0.07	0.82 ± 0.03	126	
Type II	1.26 ± 0.26	0.61 ± 0.12	48	**
Total	1.86 ± 0.33	1.46 ± 0.11	78	
Pellet fraction				
Type I	0.71 ± 0.07	0.58 ± 0.02	82	
Type II	0.43 ± 0.06	0.19 ± 0.02	44	***
Total	1.13 ± 0.08	0.75 ± 0.05	66	**

The values given are the means ± SEM of 8 observations. Separation of fractions and assay of hexokinase are as described in Methods. Fisher's P values are given by asterisks: **P < 0.01; *** P<0.001

response of insulin dependent tissues to diabetes eg: lactating mammary gland and adipose tissue [26,27] and contrasts with the increased hexokinase in tissues permeable to glucose and not requiring insulin for glucose uptake eg: kidney and lens [20,28,29].

An additional factor which could be involved in the regulation of the glycolytic route in heart muscle in diabetes is Fru-2,6-P_2, an allosteric modifier of PFK [8-10]. It has been reported recently that the tissue content of Fru-2,6-P_2 is decreased in liver and adrenal glands in diabetes [12,30]. Fru-2,6-P_2 is present in micromolar concentrations in heart and skeletal muscle,

Table 3. Effect of alloxan-diabetes on the level of fructose 2,6-bisphosphate in rat hearts.

	CONTROL	DIABETIC	Diabetic x 100 / Control %	P
FRUCTOSE 2,6-BISPHOSPHATE	(nmol/g)			
No of observations	10	8		
Fru-2,6-P_2	1.45 ± 0.07	1.16 ± 0.10	80	*

Fru-2,6-P_2 was measured in extracts of freeze clamped heart as described by Van Shaftingen et al [24]. Values are means ± SEM, Fisher's P value, * P< 0.05

and in the perfused hind limb muscle insulin and adrenaline respectively caused a 2- and 4-fold increase in Fru-2,6-P_2 and a stimulation of glycolysis [9]. As shown in Table 3, there is a decline of 20% in Fru-2,6-P_2 concentration in the diabetic rat heart, a change which could contribute to the decline in glycolytic flux in this tissue in diabetes.

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