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AN EFFECT OF DIET ON THE ACTIVITY OF PHOSPHOFRUCTOKINASE IN RAT HEART

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Summary : High-carbohydrate diet and high-fat diet lead to an increase and a decrease, respectively in the activity of heart extract phosphofructokinase when determined at 10 μM-hexose 6-phosphate and 1 mM-ATP. Neither the maximal catalytic activity nor the total immunoprecipitable phosphofructokinase was significantly altered by these diets. Dialysis of the heart extracts from rats on either high-carbohydrate or high-fat diet decreased the activity of phosphofructokinase in each case but the proportional difference between the two activities remained essentially the same. It appears unlikely that the changes in activity result from changes in intracellular effector concentration that may be introduced by the diets.

Introduction

Short-term control of heart muscle phosphofructokinase has recently been shown in this laboratory to be mediated by catecholamines via an α-adrenergic receptor mechanism (1). α-Agonists activate phosphofructokinase independently of cyclic AMP (1), protein phosphorylation (2,3) and the β-adrenergic receptor-mediated activation of glycogen phosphorylase (1). Although the molecular mechanism for the activation of phosphofructokinase is unknown, the activation is stable to gel-filtration and thus appears to occur independently of changes in the concentration of intracellular effectors (4). In the present study it is reported that long-term dietary background influences the activity of heart phosphofructokinase without any apparent effect on the total catalytic activity, or concentration, of the enzyme.

Methods

Male hooded-Wistar rats maintained in this Division were used for the experiments. Rats were separated into groups at weaning and allowed unrestricted access to the following:- 1) Rat and mouse diet as manufactured

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and supplied by William Charlick Limited, South Australia. In terms of percentage of total calories protein contributed 17, fat 10 and carbohydrate 73. The major carbohydrate in this diet was starch. The diet is referred to as 'Colony' high-carbohydrate. 2) High-carbohydrate diet, consisting of the following (% W/W) sucrose 34.1, cornflour 35.8, casein 19.0, maize oil 5.0, salt mix (5) 5.0, vitamin mix (6) 0.5, choline chloride 0.1 and DL-methionine 0.5. As a percentage of total calories protein contributed 20, carbohydrate 68, and fat 12. 3) High-fat diet, consisting of the following (% W/W) lard 48.8, casein 32.3, maize oil 8.5, salt mix 8.5, vitamin mix 0.85, choline chloride 1.07, DL-methionine 0.85. As a percentage of total calories protein contributed 20 and fat 80. Both diets 2 and 3 were formulated to provide the same amount of protein, vitamins and salt per calorie. 4) High carbohydrate diet (diet 2 above) with the option to drink 62% (W/V) sucrose as well as water (7). Methods for heart perfusion (4,8), extract preparation (8), and the determination of phosphofructokinase activity (8) were as described previously. Assays for phosphofructokinase at 1 mM hexose 6-phosphate and 1 mM ATP included the coupling enzymes aldolase (10 µg), phosphoglucose isomerase (5 µg) and triose phosphate isomerase (5 µg) in 200 µl of the assay mixture (8). Each enzyme preparation was gel-filtered. Extract protein was determined by the method of Lowry et al. (9) using bovine serum albumin as the standard.

Results

Table 1 shows the effects of dietary composition on the activity of rat heart extract phosphofructokinase at saturating and sub-saturating concentrations of hexose 6-phosphate. For newly weaned rats from dams maintained on the 'colony' high-carbohydrate diet, the activity at sub-saturating hexose 6-phosphate (H6-P) (10 µM) and 1 mM ATP was 0.18 nmol/min per mg protein. When the activity of phosphofructokinase was re-examined in similar rats after approximately 200 days on each of the four diets there were marked changes. On the one hand, the high-carbohydrate diets led to an increase in the activity of phosphofructokinase (to approx. 0.4 nmol/min per mg protein). On the other hand, the high fat diet led to a decrease in activity to 0.081.

The differences in activity at 10 µM H6-P resulting from the dietary composition did not appear to be attributable to changes in the concentration of coupling enzymes; exogenously added phosphoglucose isomerase, aldolase and triose phosphate isomerase were without effect on phosphofructokinase activity. In addition there was no significant effect of the dietary composition on the intracellular concentration of H6-P. The concentrations of glucose 6-phosphate + fructose 6-phosphate (H6-P) were 0.026±0.015 (n=3),

TABLE 1

Effect of high-carbohydrate and high-fat diets on the activity of rat heart extract phosphofructokinase at saturating and sub-saturating concentrations of hexose 6-phosphate

Newly weaned male Hooded-Wistar rats were allowed unrestricted access to the diets shown. At approximately 200 days of age or after 175 days on the diets the rats were anaesthetized and the hearts removed for perfusion. Fifteen minutes of non-recirculating perfusion was conducted and the hearts were then freeze-clamped using tongs precooled in liquid N_2. Heart extracts were prepared and the activity of phosphofructokinase at 1 mM and 0.01 mM hexose 6-phosphate determined (both at 1 mM ATP). Calculation of phosphofructokinase activity at 10 μM H6-P required measurement of tissue content of glucose 6-phosphate and fructose 6-phosphate (H6-P) and assumed equilibration between added glucose 6-phosphate and endogenous material. The hexose 6-phosphates were determined on other neutralized perchlorate extracts of the hearts. Details were as given in the Materials and Methods section. Immunoprecipitable phosphofructokinase was determined as described previously (2). Values shown are means ± SEM.

Diet	No. of animals	Age (days)	Wt. (g)	Heart extract			
				Phosphofructokinase			Protein (mg cytosol protein/g fresh heart)
				Activity (nmol/min per mg protein) at 1 mM H6-P	at 10 μM H6-P	Immunoprecipitatate (μg/mg cytosol protein)	
Weaners	4	21	30	123±5.8	0.18±0.04	32.8±2.3	28.0
'Colony' high carbohydrate	3	210±10	450±20	118.6±5.3	0.47±0.13	36.5±2.4	37.3±0.2
High carbohydrate	6	214±14	390±22	107.6±3.4	0.31±0.047	40.8±2.6	40.4±0.1
High carbohydrate + 62% (W/V) Sucrose	2	200	456	99.8	0.42	42.3	37.2
High fat	4	226±43	471±35	95.4±3.7	0.081±0.009	34.1±3.7	44.1±0.3

0.035±0.020 (n=4) and 0.021 (n=2) for high-fat diet, high carbohydrate diet and weaner rats, respectively.

Table 1 also shows that the total activity of phosphofructokinase, the total concentration of immunoprecipitable phosphofructokinase, and the protein content of the heart were largely unaffected by diet.

To test whether the diet-induced changes in activity of phosphofructokinase that occurred at sub-saturating concentration of hexose 6-phosphate resulted from alterations in the concentration of the enzyme's effectors other than H6-P, extracts of the hearts were dialysed. As shown in Table 2 dialysis of heart extracts from rats on either diet lead to a loss of activity particularly when assayed at 10 μM hexose 6-phosphate (and 1 mM ATP). This change possibly reflected the loss of activators from the extracts. However the proportional difference between the activities for the two extracts (high-fat and high-carbohydrate diets) remained essentially the same.

As an assessment of the effectiveness of the dialysis procedure 1 mM AMP and 1 mM citrate were included with separate portions of the extracts. Analysis of phosphofructokinase activity before and after dialysis indicated that removal of AMP was complete for both extracts. Whereas removal of citrate from the high-carbohydrate diet extract was complete, the removal of citrate from the high-fat diet extract was not (Table 2).

Discussion

The present study indicates that the activity of phosphofructokinase in heart is modified by long-term dietary background. As noted by others, for rabbit heart (10), there was no effect of diet on the concentration or maximal catalytic activity of the enzyme.

The assay conditions used to determine the activity at sub-saturating hexose 6-phosphate concentration approach those occurring in the cell where free ATP concentration could be expected to be millimolar and free fructose 6-phosphate might be 10 μM or less. Thus the diet-induced changes that occur in the activity of phosphofructokinase imply that glycolytic flux

TABLE 2

Effect of dialysis on diet-modified phosphofructokinase activity in heart extracts

Details for the rats and diets are given in the Methods and Table 1. Hearts were perfused for 15 min and freeze-clamped. Frozen powdered heart tissue (1 part by weight) was homogenized in 10 parts (by volume) of 100 mM Tris/HCl, pH 7.4, containing 15 mM 2-mercaptoethanol, 30 mM NaF, 0.1 mM EDTA and 3 mM NaN_3 at 0°C using an Ultra-Turrax homogenizer (20,000 rpm for 30s). This buffer was chosen to minimize loss of total catalytic activity. Where indicated, the homogenizing buffer for the hearts contained 1 mM AMP or 1 mM citrate. After centrifugation (8000 g x 5 min) the supernatants (0.5 ml) were dialyzed against homogenizing buffer (two 1 litre volumes) for 5 h at 5°C and then assayed for phosphofructokinase. A portion of the undialyzed supernatant was also assayed at this time. Other details were as for Table 1.

Rat	Additions to heart extract	Phosphofructokinase activity (n mol/min per mg protein)				100 x Activity at 10 μM H 6-P / Activity at 1 mM H 6-P	
		At 1 mM H6-P		At 10 μM H6-P			
		Pre dialysis	Post dialysis	Pre dialysis	Post dialysis	Pre dialysis	Post dialysis
High carbohydrate diet for 189 days	None	98.4±5.3	80.3±7.6	0.41±0.03	0.17±0.02	0.42±0.02	0.21±0.01
	1 mM AMP	105.3±1.6	89.0±4.8	0.89±0.03	0.18±0.02	0.84±0.02	0.20±0.02
	1 mM Citrate	99.5±1.6	81.6±6.2	0.16±0.006	0.18±0.03	0.16±0.006	0.22±0.02
High fat diet for 201 days	None	95.4±3.7	65.8±7.9	0.10±0.006	0.043±0.002	0.10±0.006	0.06±0.001
	1 mM AMP	102.1±10.6	73.4±6.5	0.31±0.02	0.048±0.002	0.30±0.01	0.06±0.007
	1 mM Citrate	89.9±4.6	72.4±4.1	0.05±0.002	0.028±0.001	0.05±0.001	0.038±0.002

rates are increased as a result of the high-carbohydrate diet and are decreased as a result of the high-fat diet. In this context, these findings are consistent with the operation of the glucose fatty acid cycle (11) where high rates of lipid metabolism act to conserve carbohydrate catabolism by inhibiting glycolysis. However the mechanism by which this interrelationship is proposed to occur may not account for the present observations. Citrate has been demonstrated as a potent inhibitor of muscle phosphofructokinase (12). Proponents of the glucose fatty acid cycle have suggested that the effects of increased fatty acid oxidation inhibit glucose metabolism and the concomitant increases in citrate concentration are linked. Thus inhibition of glycolysis has been proposed to result from the inhibitory influence of the increased citrate concentration (12,13). If in the present study the high-fat diet decreases the activity of phosphofructokinase by increasing the intracellular concentration of citrate it could be expected that the extract activity would increase following dialysis. In Table 2, and as noted previously (4), the inhibitory influence of citrate on phosphofructokinase activity in extracts from rats on high carbohydrate diets was reversed by dialysis or gel-filtration. Since the inhibitory effect of citrate on phosphofructokinase activity in extracts from rats on the high-fat diet was not reversed by dialysis it appears possible that the affinity of the enzyme for citrate is enhanced by this diet. Alternatively it may be argued that the high-carbohydrate diet leads to a modification of the enzyme that has less affinity for citrate. Such an argument would presuppose that the enzyme from high-fat diet rats has a significant amount of citrate bound that is not removed by dialysis. Whatever the case it appears unlikely that the changes in activity simply result from changes in intracellular effector concentration introduced by the diets. The possibility that the diet-induced change in activity of phosphofructokinase involves catecholamines acting via an α-adrenergic receptor mechanism (1) is being investigated.

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