THE CHANGES OF METABOLITE CONTENT AND THE CONTROL OF PHOSPHOFRUCTOKINASE IN RAT LIVER IN DIFFERENT DIETARY AND HORMONAL CONDITIONS

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1. Introduction

It is generally believed that, in most tissues, the control of the glycolytic flux is regulated at the phosphofructokinase (PFK) step and that the main effectors are ATP and citrate (inhibitors) and cAMP, AMP, Pi, F6P and FDP (activators) [1–5, see also 6]. Although little doubt exists that these effectors act on the PFK of all tissues, lipogenic and non-lipogenic alike, it seems probable that some differences exist in their relative effectiveness between tissues of different function. Thus, it is logical that in muscle, where glucose metabolism is largely directed to energy production, the main control is vested in ATP and citrate as inhibitors and AMP as an activator. In a lipogenic tissue, such as liver, other considerations apply, namely the requirement for a high glycolytic flux to provide the acetyl-CoA for lipogenesis at a time when there is no necessity for an appreciable change in the activity of the tricarboxylic acid cycle. In this situation a logical case could be made for a control system which is linked to the blood glucose. Such a system would act to permit a high flux rate in glycolysis when the blood glucose is high and the distribution of the acetyl-CoA so produced between oxidation and lipogenesis would then be regulated by the redox state of the nicotinamide nucleotides and the phosphorylation state of the adenine nucleotides in the mitochondria. The present communication examines changes in the content of effector molecules in intact livers from rats in different dietary and hormonal conditions and, by a comparison of this in vivo data with the known kinetic properties of the enzyme, attempts to relate these to the specific requirements for control of the glycolytic flux in liver at PFK. It would appear that, in liver under these conditions, F6P is the most significant modulator of PFK activity, thus relating the activity of this enzyme to the rate of glucose phosphorylation. The modification of the K_i for citrate by F6P [7] permits a rapid rate of glycolysis and lipogenesis without feed-back inhibition by citrate.

2. Methods

2.1. *Animals and treatments*

The nutritional and hormonal treatments used were such as to produce metabolic patterns where the carbon flux was mainly in the direction of gluconeogenesis (i.e. starvation, starvation followed by refeeding a high fat diet or diabetes) or of glycolysis and tricarboxylic acid cycle oxidation (i.e. starvation followed by refeeding a high carbohydrate diet or diabetic animals treated with insulin). Details of the diets and treatments are as given by Novello, Gumaa and McLean [8] except that the rats were starved for 72 hr instead of 48 hr. Diabetic rats were not used until 3 weeks after the administration of alloxan and insulin treatment was for 3 days (2 I.U./day). The number of rats in these groups was not less than 6.

2.2. *Assay procedures*

Measurements were made of the cell contents of the glycolytic and tricarboxylic acid cycle intermediates, using perchloric acid extracts of freeze-clamped livers prepared as described by Lagunas, McLean and Greenbaum [9], by the procedures given in Gumaa and McLean [10] or Lagunas, McLean and Greenbaum [9]. Carbon fluxes were also measured as described previously [9].

The intracellular distribution of citrate was calculated according to Williamson [11].

3. Results and discussion

Table 1 shows the cell content of some of the metabolites known to affect PFK together with an estimate of the flux of carbon through the glycolytic pathway as measured by the rate of production of $^{14}CO_2$ from 3,4-^{14}C-glucose. Although this latter may not be a complete measurement of glycolytic flux since it omits any formation of lactate, it does provide a reasonable approximation to the total flux.

From the data provided in the table, there is no obvious correlation between the changes of flux rates and the tissue content of cAMP, AMP, Pi, FDP, NH_4^+, total ATP, the ATP/ADP × Pi ratio (either total cell or cytoplasmic) or of total cell citrate. It should be noted that, in their study of the effect of butylmalonate on gluconeogenesis in perfused liver, Williamson, Anderson and Browning [12] also found changes in the activity of PFK which could not be accounted for by the observed changes in the content of effector molecules and ascribed the effect to a redistribution of citrate between mitochondria and cytosol, although this was not actually calculated. In the present case, we have calculated the cytosolic content of citrate (last column) by the procedure described by Williamson [11] and it is apparent that there is no systematic relationship between the cytoplasmic citrate and the activity of PFK, assuming that this enzyme is the rate-limiting step in glycolysis. Similarly, Start and Newsholme [13, 14] also observed that the direction of change of the adenine nucleotides and citrate in starvation did not correspond to that expected if they were effective agents in decreasing glycolysis and increasing gluconeogenesis. Instead, they proposed that F6P may act as an important modulator of hepatic PFK activity, the fall of F6P in starvation acting to reduce the activity of PFK.

In the present experiments, a good correlation (correlation coefficient = 0.85, 5% limit = 0.81) appears to exist between flux rate and fructose-6-phosphate content. Three points established by Passonneau and Lowry [7] concerning the properties of hepatic PFK are germane to an understanding of this correlation. First, that the inhibition of PFK by ATP can be largely reversed by AMP (0.3 mM) and Pi (10 mM) in combination. Second, that, in contrast to brain, the ATP inhibition of hepatic PFK is not reversed by levels of F6P ten times higher than those occurring in vivo. Third, that an increase in F6P concentration raises the K_i for citrate inhibition. This last point seems to be of paramount importance in a tissue such as liver where it is necessary to maintain a high glycolytic flux to provide the acetyl-CoA, reducing equivalents and ATP necessary for lipogenesis, which, as stated above, itself requires a high level of citrate both as a substrate for lipogenesis via the citrate cleavage enzyme and as an activator of acetyl-CoA carboxylase. An increase of F6P in conditions of increased glycolytic flux and higher lipid synthesis will, by raising the K_i for citrate, simultaneously release PFK from inhibition and allow substrate to be present for fatty acid synthesis in the same compartment. Conversely, in conditions of decreased glycolytic flux and lowered activity of the fatty acid synthesising system, F6P falls. This, in turn, will lower the K_i for the citrate inhibition of PFK and thus inhibit the enzyme without the necessity for any change in citrate concentration, or even in the presence of a lower cytoplasmic citrate content. Passonneau and Lowry found that the value of the K_i for citrate inhibition in the presence of 0.08 mM F6P (approximately the same level as found in the liver in the present experiments, which ranges from 0.036 mM to 0.09 mM) was 0.14 mM. The calculated value for the cytoplasmic citrate in the present experiments ranges from 0.18 mM to 0.31 mM, i.e. not far removed from the K_i found by Passonneau and Lowry [7]. Atkinson [15] has emphasised that, to achieve maximum sensitivity and flexibility of control systems, it is essential that the concentrations of the substrates and effectors should approximate closely to the K_m values. The present data fit this requirement in a satisfactory manner.

The question now arises as to the factors regulating the F6P in the liver. It would be expected, by

Table 1
Changes in the tissue content of a number of metabolites modifying the activity of hepatic phosphofructokinase.

Condition	Glycolytic flux[a] (μmoles/g wet wt/hr)	Activators and inhibitors					cAMP[b] (%)	Inhibitors			
		F6P	FDP	Pi	NH_4^+	AMP		Citrate	Citrate[c] "cyt"	ATP	$\frac{ATP}{ADP \times Pi}$ "cyt"
		(nmoles/g wet weight liver)						(nmoles/g wet weight liver)			
Control	13.4 ± 1.9	60.4 ± 6.2	11.5 ± 1.2	3500 ± 167	360 ± 38	234 ± 23	100	296 ± 23	218 ± 20	1920 ± 57	814
Starved, 3 days	0.28 ± 0.07	24.4 ± 1.8	14.3 ± 1.6	4080 ± 269	400 ± 60	315 ± 33	167	224 ± 21	119 ± 14	1657 ± 122	765
Starved, 3 days, refed fat diet 3 days	1.55 ± 0.33	33.0 ± 2.3	16.7 ± 2.4	4145 ± 288	205 ± 37	365 ± 56	139	263 ± 26	118 ± 21	1396 ± 116	471
Starved, 3 days, refed carbohydrate diet 3 days	14.9 ± 2.0	52.9 ± 8.0	24.4 ± 1.0	2760 ± 182	392 ± 96	149 ± 15	95	294 ± 45	135 ± 24	2124 ± 117	1775
Diabetic	1.86 ± 0.92	42.1 ± 6.1	11.2 ± 2.4	4100 ± 78	295 ± 65	314 ± 29	127	297 ± 36	182 ± 22	1581 ± 59	253
Diabetic + insulin	8.55 ± 1.24	60.0 ± 1.3	9.9 ± 0.6	3840 ± 183	315 ± 75	242 ± 27	118	205 ± 22	170 ± 18	1892 ± 86	340

The results are given as means ± S.E.M. and represent values from not less than six animals in each group. Values represent total cell contents except where "cyt" is used where they represent the calculated cytoplasmic values.

[a] The rate of 3,4-^{14}C-glucose conversion to $^{14}CO_2$ by liver slices incubated in Krebs Ringer bicarbonate medium.

[b] B.L. Brown, J.D.M. Albano, R.P. Ekins and K.A. Gumaa (unpublished observations).

[c] Calculated according to Williamson [11].

[d] Calculated according to the method of Veech, Raijman and Krebs [26].

definition of a control enzyme [16], that F6P should decrease in situations where there is an increased flux through PFK, whereas the opposite is true for the liver in the present experiments. In this context, the free permeability of the liver to glucose and the properties of glucokinase are of particular relevance. Liver glucokinase operates only at relatively high glucose concentrations ($K_m = 10^{-2}$ M) and is not subject to feedback inhibition by G6P [17]. Furthermore, both glucose and insulin are required for the maintenance and induction of this enzyme [18]. These properties permit a rapid rate of glucose phosphorylation to occur in liver under conditions where there is a high portal blood glucose and insulin (i.e. in rats starved and refed a high carbohydrate diet or in diabetic rats treated with insulin) the resultant effect of which is to increase the steady state concentration of G6P and, because of the high activity of phosphoglucose isomerase, of F6P. Sillero, Sillero and Sols [19] have shown, from experiments with diabetic rats given diets containing fructose, that glucokinase is unique among the glycolytic and gluconeogenic enzymes in having an absolute requirement for insulin.

The stimulation of PFK by the raised F6P may, in some instances, cause an increase of FDP, itself an activator of PFK. This is evident in the case of the starved rats refed a high carbohydrate diet. In other conditions, other mechanisms may assume a more important role. For instance, it has been reported [9] that, in the livers of rats treated with nicotinamide, the main control of PFK appeared to be vested in ATP and citrate and a typical cross-over was found in the metabolite profile.

It is, perhaps, appropriate at this point to consider some of the properties of the enzymes involved in lipogenesis with respect to citrate. Inspection of the table shows that there is no correlation between cytoplasmic citrate and the rate of lipogenesis. However, there is no reason to believe that the levels of citrate present are not adequate to support the observed rates of fatty acid synthesis. The quoted K_m for citrate cleavage enzyme is 5.8×10^{-4} M [20] while the calculated cytoplasmic content here is approximately 2×10^{-4} M. However, it is probable that the true substrate for the cleavage enzyme is the Mg^{2+}-citrate complex and the K_m for this is 1.62×10^{-6} M [21]. Further, it is probable that the K_a for the activation of acetyl-CoA carboxylase by citrate of 0.05 M quoted by Waite and Wakil [22] is also too high. Fang and Lowenstein [23] have shown that the activation of the carboxylase is both time and concentration dependent and Gregolin et al. [24] have found an extraordinarily tight binding of citrate by the carboxylase (2–3 μM for chicken liver). Thus taking both of these observations into account, it is probable that the native enzyme in vivo is fully activated. It would therefore appear that the regulation of the citrate inhibition of hepatic PFK by F6P is the valve allowing greater glycolytic flux and lipogenesis without postulating changes in citrate distribution or concentration.

Another point worth noting is that a parallel situation holds for pyruvate kinase (PK) as for the relief by F6P of inhibition of PFK by citrate. A high cytoplasmic ATP is required to drive the synthetic reactions of fatty acid synthesis and for glucose phosphorylation, but is incompatible with a high activity of PK, a key enzyme in the supply of acetyl-CoA for fatty acid synthesis. Here again, the control is not by alteration of the ATP concentration, which could not be made to suit both enzyme systems without "compartmentation", but rather by a modification of the kinetic properties of the key enzyme involved, in this case pyruvate kinase. It is also interesting that further coordination is achieved by the stimulation of fatty acid synthesis by fructose diphosphate [25]. Since this stimulation by fructose diphosphate is achieved by decreasing the K_m of fatty acid synthetase for NADPH, it is clear that additional coordination will be brought into play involving, in this instance, the pentose phosphate pathway for which there is clear evidence for rate-limitation by the supply of $NADP^+$.

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