UTILE AND FUTILE CYCLES IN THE LIVER

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SUMMARY : Glucagon or glucose have been administered to normal fed rats in order to induce high rates of glycogen breakdown or synthesis. The administration of glucagon caused a 3-fold increase in the intrahepatic concentration of glucose 6-phosphate. The effect of the administration of glucose on the level of glucose 6-phosphate varied according to whether glycogen synthetase had or not been activated, being slightly elevated in the absence of activation and being lowered (40 %) when an important activation had occurred. These observations are discussed with regard to the recycling of glucose and glucose 6-phosphate in the liver. It appears that the advantage of this recycling is to allow large changes in the net flux of metabolites in one or the other direction, controlled by substrate concentration only.

The simultaneous operation of glucokinase and of glucose 6-phosphatase in the liver is expected to cause the recycling of glucose in a "futile" manner since ATP is consumed with no net flux of metabolites. A similar situation exists for the interconversion of fructose 6-phosphate and fructose diphosphate by phosphofructokinase and fructose diphosphatase. The major difference between the two systems is that, whereas phosphofructokinase and fructose diphosphatase are low Km enzymes, tightly regulated by various metabolites, there is no known effective regulatory mechanism for the two high Km enzymes, glucokinase and glucose 6-phosphatase, other than by substrate concentration.

There exists now a clear evidence that, in the liver *in vivo* as well as in the isolated liver cells, recycling occurs between glucose and glucose 6-phosphate (1). A major factor for the control of the recycling process is the intrahepatic concentration of glucose 6-phosphate. This concentration is known to be low when glycogen synthetase has been activated by glucose (2) or glucocorticoids (3,4).

MATERIAL AND METHODS

All biochemicals were purchased from Sigma Chemical Company. Purified enzymes were obtained from Boehringer G.m.b.H. Glucagon was

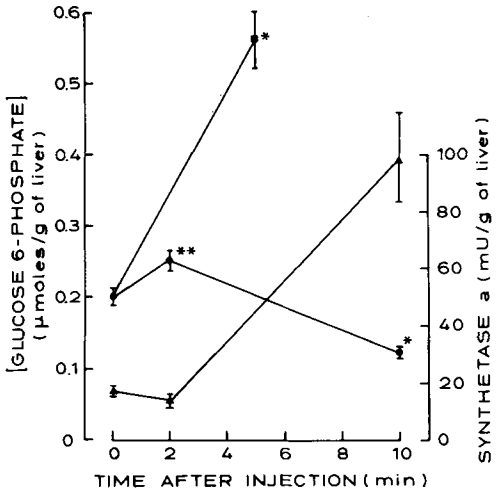

Fig.1. *Concentration of glucose 6-phosphate (●, ■) and activity of glycogen synthetase a (▲) in the liver of normally fed rats, after the administration of glucose (●, ▲) or of glucagon (■).* The activity of synthetase *a* found in the liver of rats treated with glucagon was equal to 20 ± 2 mU per g of liver. Values shown are means ± SEM of at least four animals. *, ** : Values significantly different from control values ($P < 0.001$ and $P < 0.05$ resp.).

obtained from Novo Laboratories. Glucose (1.5 mg/g of body weight) or glucagon (1 μg/g of body weight) were injected into a tail vein of young male Wistar rats weighing 90-120 g. The animals were killed by decapitation and the liver was freeze-clamped (5) within 8 sec. Glucose (6) and glucose 6-phosphate (7) were measured enzymatically in neutralized perchlorate extracts. Activities of glycogen synthetase *a* and phosphorylase *a* were assayed as previously described (8).

RESULTS

Fig. 1 shows that the concentration of glucose 6-phosphate measured in the quick-frozen livers of fed rats was close to 0.2 μmole/g of tissue . Similar values were found by several groups of workers (9-11) and lower ones (below 0.1 μmole/g) by others (2,3). The administration of glucagon caused, within 5 min, a nearly 3-fold increase in the level of glucose 6-phosphate, whereas the administration of glucose caused a moderate but significant elevation at 2 min, followed by a marked fall at 10 min. This fall is confirmatory of previous observation (2). The concentration of glucose in the liver (not shown) was increased by 50 %, 5 min after glucagon, and 3-fold, 2 and 10 min after glucose.

The proportion of phosphorylase which is in the active form *in vivo*, cannot be measured in non-anesthetized animals, because of artefactual conversion of phosphorylase *b* into phosphorylase *a*, upon killing of the animals (8). However, 2 min as well as 10 min after the administration of glucose, the measurable amount of phosphorylase *a* was about 50 % of the initial value (not shown). This indicates that the well-established effect of glucose on the inactivation of phosphorylase *in vivo* (8) was nearly complete 2 min after the injection. The activity of glycogen synthetase *a* was low before glucose administration and was unchanged 2 min later. It was markedly increased at 10 min. This latency in the activation of glycogen synthetase corresponds to the time required for converting phosphorylase *a* into phosphorylase *b* (8).

DISCUSSION : SIGNIFICANCE OF THE GLUCOSE:GLUCOSE 6-PHOSPHATE RECYCLING

The recycling of glucose and glucose 6-phosphate could play an important role in the liver by allowing the net flux of glucose, in one or the other direction, to be adapted to the rates of glycogen synthesis or degradation, glycolysis, gluconeogenesis, and pentose phosphate pathway and this only by substrate concentration. Although we cannot discard the possibility that more elaborate and still undiscovered regulatory mechanisms might control the activity of glucokinase and of glucose 6-phosphatase *in vivo*, the purpose of this discussion is to show that such mechanisms are not necessary.

Properties of the enzymes

It has been emphasized by many authors that, with a Km of 10 mM (12), the rate of phosphorylation of glucose by glucokinase is dependent upon glucose concentration in the physiological range. A Vmax equal to 1 µmole/min per g of liver was observed at 22° (12), and of 1.25 µmoles/min per g at 28° (13). Assuming twice the latter value at 37°, one calculates a rate of phosphorylation of 0.88 µmole/min per g at 5.5 mM glucose and of 1.31 µmoles/min per g at 10 mM glucose (see Fig. 2, points a and b). It is, however, recognized that the change in glucose uptake resulting from a doubling of the normal blood glucose level is far greater than 1.5-fold (2).

The fact that the intrahepatic concentration of glucose 6-phosphate (0.1-0.3 mM) is usually one order of magnitude below the Km of glucose 6-phosphatase (2 to 4 mM) has, to our knowledge, not been emphasized. It is clearly apparent that *in vivo* the hydrolysis of glucose 6-phosphate is a first order reaction. In acute conditions, in which the total amount of enzyme is not expected to change, the level

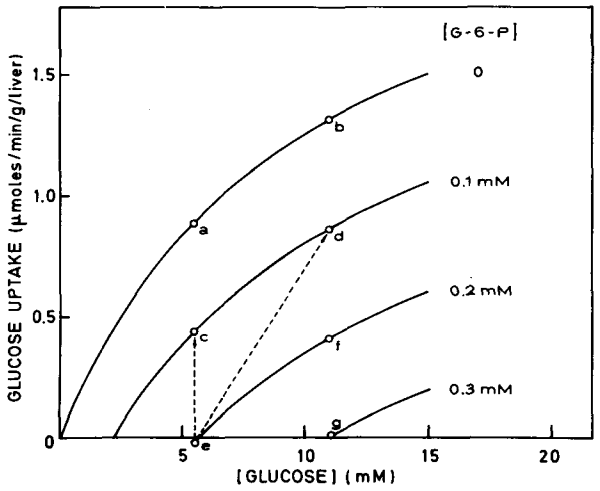

Fig. 2. *Influence of the concentration of glucose and of glucose 6-phosphate on the net flux of glucose to glucose 6-phosphate.* The upper curve represents the activity of glucokinase, assuming a Vmax of 2.5 µmoles/min per g and a Km of 10 mM. The other curves have been obtained by subtracting the activity of glucose 6-phosphatase for which a Vmax of 10 µmoles/min per g and a Km of 2 mM have been adopted. At 0.2 mM glucose 6-phosphate, considered as physiological, the rate of hydrolysis is close to 0.9 µmole/min per g. It appears that at 5.5 mM glucose (100 mg %), although glucose is phosphorylated at a rate of 0.88 µmole /min per g (point a), there is a slight output of glucose (point e). At the same concentration, glucose uptake occurs if glucose 6-phosphate concentration is lowered (point c), as results for instance from the activation of glycogen synthetase by glucocorticoids (3, 4). If glucose concentration is raised to 11 mM without changing glucose 6-phosphate concentration (point f), there is an uptake of 0.41 µmole / min per g, whereas, if glucose 6-phosphate concentration is simultaneously raised to 0.3 mM, the uptake remains close to zero (point g). Similar conditions have been observed in the first minutes after a glucose load, before activation of glycogen synthetase had occurred. Later on, glucose 6-phosphate concentration is lowered by about 50 %; the glucose uptake is now 0.86 µmole /min per g (point d), reaching then 66 % of the rate of glucose phosphorylation by glucokinase (point b). The dotted arrows show the change of glucose flux due to a decrease in glucose 6-phosphate concentration with (point d) and without (point c) increase in glucose concentration.

of glucose 6-phosphate in the tissue is thus indicative of the rate of its hydrolysis. Unfortunately, there exist some uncertainties concerning the Km and Vmax of glucose 6-phosphatase in the liver *in vivo*, since these parameters are affected *in vitro* by a number of treatments (14). The concentration of glucose 6-phosphate in the liver seems somewhat variable since values below 0.1 µmole/g have been reported (2,3), whereas we have observed concentrations close to 0.2 µmole/g. These

values need also to be corrected for the absence of glucose 6-phosphate in the extracellular and mitochondrial spaces. Calculations shown in Fig. 2 have been based on a Km of 2 mM and a Vmax of 10 U/g for glucose 6-phosphatase. At 0.2 mM glucose 6-phosphate, the rate of hydrolysis is then close to 0.9 µmole/min per g of liver.

Both enzymes display Michaëlis-Menten kinetics. No modulator of glucokinase activity is known. Glucose 6-phosphatase is inhibited by inorganic phosphate and by glucose (15), but this inhibition is too small to play a role in the control of the enzymic activity *in vivo*.

Insofar as their Vmax is well above the highest flux that needs to be realized, the irreversible reactions catalysed by glucokinase and glucose 6-phosphatase are rate limiting only during adaptation periods that follow rapid changes in the overall flux of metabolites and do not require control mechanism other than substrate concentration. Indeed, when glucose 6-phosphatase becomes limiting, its substrate concentration increases, adapting automatically the net flux of metabolite to the rate of the other reactions of the sequence. On the other hand, when glucose concentration is elevated, the rate of glucokinase is increased although to a much smaller degree than is usually the rate of glycogen synthesis; the decreased concentration of glucose 6-phosphate (2-4 and Fig. 1) is then an important factor, which, by decreasing the recycling of glucose (see below), allows the net flux of glucose phosphorylation to equal that of glucose polymerisation. Glucokinase presumably becomes limiting when its concentration is low as is the case in the liver of guinea pigs (16), or of new-born (13), diabetic or starved rats (12,17). The parameters used in Fig.2 have been estimated for a normal fed rat.

Properties of the recycling system

The simultaneous operation of two high Km enzymes, a kinase and a phosphatase, catalyzing irreversible antagonistic reactions constitutes an interesting ATP consuming system of which several properties deserve consideration.

a) When the rates of the 2 antagonistic reactions are equal, there is no net flux of metabolites through the system, other than the hydrolysis of ATP to ADP and P_i. As far as the values of the various parameters have been correctly estimated, this situation is reached at a level of glycemia equal to 5.7 mM; the rate of recycling is then 0.91 µmole/min per g of liver. As a first approximation, it is assumed that, under these conditions, formation and utilization of glucose 6-phosphate by other pathways either are negligible or compensate each other.

b) Increasing the concentration of the substrate of one of the two reactions will increase the rate of its conversion to product, of which a higher level will favor the reverse reaction. Therefore, little or no change in the net flux of metabolite is expected, and it is clear that the large increase in glucose uptake by the liver which follows a relatively small elevation of blood glucose level cannot be simply explained by such a push mechanism (see also legend of Fig.2). In contrast, when glycogenolysis is intense, the concentration of glucose 6-phosphate and the rate of its hydrolysis is increased several fold, causing, however, a relatively small change in product concentration, since glucose diffuses out of the liver and is dispersed in the whole body. In this case, the push mechanism is efficient, allowing a marked increase in the net rate of glucose 6-phosphate hydrolysis.

c) As shown in Fig.1, the increase in glucose 6-phosphate concentration after a load of glucose, is only temporary, being followed by a marked decrease. This is due to the fact that glucose, independently of its action on glucokinase, causes an activation of glycogen synthetase (see below) which pulls out UDPG and glucose 6-phosphate (2). In such a system, a relatively small change in glucose concentration allows a great change of glucose uptake. There is indeed a threshold of efficiency of glucokinase, which is determined by the activity of the antagonistic enzyme (see paragraph a). As illustrated in Fig.2, a doubling of the physiological (5.5 mM) concentration of glucose may change the glucose flux from a slightly negative value (point e) to 0.86 μmole/min per g (point d), which is enough to sustain a very high rate of glycogen synthesis. About one half of this large change is due to the increased activity of glucokinase and the other half to the decreased activity of glucose 6-phosphatase, secondary to a decreased concentration of glucose 6-phosphate. Glycogen synthetase can also be activated by glucocorticoids (3,4) with no change in blood glucose concentration. The subsequent decrease in glucose 6-phosphate level allows an important flux of metabolites (point c).

Glycogen metabolism

The control of glycogen metabolism in the liver occurs by interconversion of the active (*a*) and inactive (*b*) forms of glycogen synthetase and glycogen phosphorylase through specific kinases and phosphatases. The system is of the on-off type and allows as large as 50-fold changes in the concentration of the active forms of these rate-limiting enzymes, and consequently in the actual rates of glucose polymerization or of glycogen breakdown. The problem under discussion is to know

how the other enzymes that catalyze irreversible steps in the sequence of reactions leading from glucose to glycogen and from glycogen to glucose adapt their rate of reaction to these very large changes in the activity of synthetase and phosphorylase. As we have seen above, this is made possible due to the recycling of glucose and glucose 6-phosphate. As regards synthesis, it is remarkable that the stimulation of the conversion of glucose to glycogen by a high glucose level is not a push mechanism on the glucokinase, but a pull mechanism by activation of glycogen synthetase, which leads to a depletion of UDPG and of glucose 6-phosphate. As discussed in more detail elsewhere (8, 18, 19), this activation of glycogen synthetase occurs by a complex mechanism which involves binding of glucose to phosphorylase, conversion of phosphorylase *a* to phosphorylase *b*, and the release of the inhibition of synthetase phosphatase by phosphorylase *a*. The stimulation of glycogen synthesis by glucocorticoids is also due to an activation of glycogen synthetase, although by a different mechanism. With regards to glycogen breakdown, as pointed out above, the main point is the remarkable increase in concentration of glucose 6-phosphate in the liver after the activation of phosphorylase, associated with only a slight elevation of glucose concentration.

Other pathways involving glucose 6-phosphate

The above reasoning also applies to gluconeogenesis and to the oxidation of glucose 6-phosphate by its specific dehydrogenase. In the latter case, a greater availability of appropriate electron acceptors for NADPH could increase the flux of glucose 6-phosphate converted to pentose phosphate, of which a certain amount will be resynthetized by the transketolase-transaldolase conversion. Here also, the expected decrease in the concentration of glucose 6-phosphate will allow oxidation to occur at the expense of hydrolysis.

Glycolysis seems to occur only when the concentration of fructose 6-phosphate is high (10) and will therefore not cause depletion of substrate for the other pathways.

The expense of energy

It appears from Fig.2 and from experimental data (1) that the order of magnitude of the recycling of glucose in the liver is close to 1 µmole/min per g of liver. Assuming 10 KCal per mole of ATP, the expense of energy is approximately 15 KCal/day per kg of liver, a value which may be considered as negligible and which can certainly be adequately used for maintaining the body temperature at 37°.

Useful mechanisms are not futile

From the above discussion it appears that the main advantage of the glucose:glucose 6-phosphate continuous recycling in the liver is

to allow large changes in the net metabolic flow in both directions, controlled by substrate concentration only. If glucokinase and glucose 6-phosphatase were not operating simultaneously, they would have to be tightly regulated by some kind of complex and entirely hypothetical on-off mechanism, which would need to be adapted, not only to the rates of glycogen synthesis and breakdown, but also of gluconeogenesis, glycolysis and pentose phosphate oxidation. The expense of energy involved in the recycling is therefore largely justified by the economy in control mechanisms. In contrast,the benefit of the fructose 6-phosphate:fructose diphosphate recycling is less apparent; it may be to increase the sensitivity of the system to various stimuli (20). This recycling seems not to be operative in the liver but, possibly, to operate at a slow rate in normal mammalian muscle (1). A fast recycling has been observed in the flight muscle of *Bombus affinis* (21),where it serves the useful purpose of maintaining the tissue at a minimum temperature of 30°. It also operates in the muscle of some susceptible animals intoxicated with halothane (22) and there, it is not only futile but also highly toxic and causes the death of the animals in a few hours.

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