Regulation of Carbohydrate Metabolism in Muscle and Liver

Introduction

In the heart, like in many other tissues, glucose uptake and metabolism is regulated by the availability of substrates including oxygen, by the energy demand and by the hormonal and dietary status of the organism. It is reasonable to assume that heart glycolysis is controlled at several steps, including glucose transport and phosphorylation, and the reaction catalysed by 6-phosphofructo-1-kinase (PFK-1), the so-called first committed step of glycolysis. PFK-1 is a multimodulated enzyme, which is inhibited by ATP, citrate and protons, and which is stimulated by fructose 6-phosphate and AMP. All PFK-1 isoenzymes studied so far are sensitive to fructose 2,6-bisphosphate (Fru-2,6-P₂) [1]. At physiological concentrations of substrates and effectors, heart PFK-1 [2], like liver PFK-1 [3], is almost completely inactive unless physiological (micromolar) concentrations of Fru-2,6-P₂ relieve the inhibition by ATP. Thus Fru-2,6-P₂ can be regarded as an intracellular signal that controls glycolysis in various tissues [4]. We were particularly interested to know whether this also applies to the heart.

The synthesis and degradation of Fru-2,6-P₂ are catalysed by the bifunctional enzyme, 6-phosphofructo-2-kinase (PFK-2)/fructose-2,6-bisphosphatase (FBPase-2) [1,4,5]. In the heart, two isoforms of this bifunctional enzyme (a 58000 Mᵋ-form and 54 000 Mᵋ-form) have been described, which originate from the same primary transcript by alternative splicing [6]. Other tissue-specific isoenzymes exist [7] but are not relevant to this discussion.

The purpose of this short review is to briefly describe the mechanisms involved in the regulation of heart glycolysis by substrate availability, energy demand and hormones. We will mainly consider the regulation under physiological conditions, which is of interest for the biochemist and the physiologist. The dysfunction of this regulation in pathological conditions such as ischaemia is briefly alluded to in the last section, where some recent work is described.

Substrate availability

Under normal conditions, the heart takes its energy from substrates in the bloodstream so that the endogenous energy stores, triacylglycerols and glycogen, are preserved. Moreover, there is a hierarchy among the oxidizable substrates: glucose is not a preferred substrate and heart glycolysis is inhibited by fatty acids or ketone bodies in what is known as the glucose/fatty acid cycle [8,9]. The glucose-sparing effect of these alternative oxidizable fuels results from a citrate-mediated inhibition of PFK-1 [10], which is re-inforced by a fall in Fru-2,6-P₂ concentration [11]. This fall is also explained by citrate, a potent inhibitor of heart PFK-2 activity. Thus, citrate signals the on-going oxidation of fatty acids or ketone bodies and inhibits glycolysis by a dual lock imposed on PFK-1 and PFK-2.

The glucose/fatty acid cycle concept can be extended to include lactate [12]. The latter, which is continuously produced by glycolysing erythrocytes, and which accumulates during exercise, is a pre-
ferred substrate for the heart. Lactate inhibits both glycolysis and fatty oxidation in the heart in vivo [13]. We found that the inhibition of glycolysis by lactate was mainly exerted on PFK-1 and PFK-2 and was mediated by citrate [12]. In this case, like in the presence of fatty acids or ketone bodies, Fru-2,6-P_2 content was decreased. Such a fall in Fru-2,6-P_2 was also found in the muscles of flying locusts [14]. Therefore Fru-2,6-P_2 behaves as a glycolytic signal that is turned on by glucose availability and switched off by alternative fuels.

**Glucose re-routing versus glucose sparing effect**

Despite the inhibition of glycolysis, glucose phosphorylation was barely affected by lactate, suggesting a re-orientation of glucose metabolism. Lactate induced a dose-dependent increase in glycogen content [12], which was explained by the accumulation of UDP-glucose, the substrate, and glucose 6-phosphate, a stimulator of glycogen synthase.

Thus it appears that physiological concentrations of lactate inhibit glycolysis and re-route glucose towards glycogen synthesis. The other oxidizable fuels share with lactate the ability to inhibit glycolysis and to favour the conversion of glucose into glycogen [8,15]. However, in vivo, the overall effect of these fuels on glucose metabolism might differ, since they also depend on other factors such as the dietary and hormonal status. For instance, the concentration of fatty acids in blood increases mainly during fasting, under which condition insulin concentration and, hence, glucose transport and uptake are decreased. Therefore both the decrease in insulinaemia and the increase in fatty acid concentration lead to the 'glucose-sparing' effect of starvation. By contrast, the concentration of circulating lactate is only slightly affected by fasting, but is very much increased during and after muscular exercise. Thus, the prevailing effect of lactate on heart glucose metabolism in vivo is to divert glucose from glycolysis and re-route it towards glycogen rather than truly sparing it. This effect also depends on the dietary and hormonal status, and may explain why the heart rapidly recovers its glycogen stores after exercise [16].

Therefore, the 'glucose/fatty acid, lactate' cycle leads to a glucose sparing or a glucose re-routing effect depending on the fuel available and on the dietary and hormonal status.

**Energy demand**

In the isolated working rat heart, glycolysis is stimulated by increasing the work load [17-19]. Under this condition, the flux through PFK-1 is increased and related to an increase in Fru-2,6-P_2 content [20]. To explain this increase, an activation of PFK-2 was proposed, since the concentrations of fructose 6-phosphate and ATP, the substrates of PFK-2, were not affected [20]. Heart PFK-2/FBPase-2 is a substrate of the cyclic AMP-dependent protein kinase (PKA), which activates PFK-2 by decreasing its K_m for fructose 6-phosphate but has no effect on FBPase-2 activity [6,21,22]. We demonstrated that heart PFK-2/FBPase-2 is also a substrate for the calcium- and calmodulin-dependent protein kinase (Ca/CAMK). Both kinases probably phosphorylate the same sites [20], which is in agreement with the low specificity of Ca/CAMK compared with that of PKA [23]. Phosphorylation in vitro by PKA or Ca/CAMK results in the same change in activity, namely an activation of PFK-2. Similar changes in PFK-2 activity were observed in preparations of PFK-2/FBPase-2 purified from hearts submitted to high workload [20]. We proposed that the change in K_m of PFK-2 in vivo resulted from phosphorylation by Ca/CAMK, because the concentration of cyclic AMP was not affected under this condition.

**Hormonal control**

Adrenaline and insulin stimulate heart glycolysis, although they exert opposing effects on glycogen metabolism [2,24,25]. Adrenaline promotes glycogen breakdown, thereby providing glycolysis with glucosyl units. Thus, the glycolytic substrate comes mainly from glycogen. As a result of the stimulation of glycogenolysis, the concentration of hexose 6-phosphate increases, as does the content of Fru-2,6-P_2. Adrenaline action is mediated by cyclic AMP and PKA which activate the glycogenolytic cascade as well as PFK-2 [6,21,22].

Insulin stimulates both glycolysis and glycogen synthesis. Therefore, and in contrast with the situation in adrenaline-treated hearts, exogenous glucose is the major substrate for the insulin-stimulated glycolysis, and the overall effect of insulin on glucose metabolism is an increased glucose uptake. The stimulation of glycolysis by insulin results from a concerted regulation at different levels. It involves stimulation of glucose transport and phosphorylation, as well as activation of PFK-2 and of pyruvate dehydrogenase [25,26]. Our preliminary results suggest that the insulin-dependent activation of PFK-2 is, like glucose transport, completely inhibited by wortmannin, an inhibitor of phosphatidylinositol 3-kinase, one of the components of the insulin signalling pathway. This observation offers
new possibilities of investigating the molecular mechanism responsible for insulin action on glycolysis.

**Anoxia and Ischaemia**

Both conditions are known to decrease the work developed by the heart, and to stimulate glycolysis and glycogen breakdown [27,28]. No increase in Fru-2,6-P$_2$ can be detected in these conditions [20]. Therefore, Fru-2,6-P$_2$ cannot be regarded as a major regulator under anaerobic conditions. The same conclusion had already been drawn for the stimulation of glycolysis by anoxia in liver and skeletal muscle [4].

In a recent study of the ischaemia-stimulated glycogen breakdown, we found that the concentration of cyclic GMP was increased during ischaemia [29]. Cyclic GMP is known to mediate the effects of nitric oxide (NO) in certain tissues. This biological messenger is synthesized from L-arginine by the enzyme NO synthase. This observation prompted us to investigate the functional and metabolic effects of NO synthase inhibitors in ischaemic hearts, the more so since these inhibitors were found to be proportional to the ischaemic damage [31]. The onset of ischaemia was rapidly followed by a marked increase in glucose uptake and lactate production. However, this enhancement was only transient and started to decline at a time corresponding to the onset of ischaemic contracture. The addition of inhibitors of NO synthase to this model resulted in (i) a decrease in ischaemic contracture, (ii) the maintenance, through the whole ischaemic period, of a stimulation of glycolysis from exogenous glucose, and (iii) a preservation of the glycogen stores. This resulted in a slower decrease in ATP and phosphocreatine concentration. During reperfusion, the functional recovery was more than twice that of the controls. The protective effects of the inhibitors were lost in the presence of L-arginine, but, rather unexpectedly, they were not antagonized by cyclic GMP analogues, indicating some direct involvement of NO in the ischaemic damage. Finally, the functional and metabolic effects of the inhibitors were observed at concentrations that were at least two orders of magnitude lower than those known to have vasoconstrictive effects.

As to the regulation of glycolysis during low-flow ischaemia in the control hearts, the early response (i.e. stimulation of glycolysis) corresponds to the well-known Pasteur effect. A careful analysis of the changes in the concentrations of intermediary metabolites during the second phase indicated that the progressive inhibition of glycolysis could result primarily from a decrease in glucose transport, the inhibition of other steps, such as PFK-1 or glyceraldehyde-3-phosphate dehydrogenase being secondary events. Similarly, the stimulation of glycolysis by NO synthase inhibitors results from an increased glucose transport. Whether the mechanism involved corresponds to a recruitment of transporters, as is the case for insulin, is not known.

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Mechanisms underlying suppression of glucose oxidation in insulin-resistant states

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Introduction

Insulin elicits a range of diverse biological effects on cellular metabolism. In the long term, insulin has a general anabolic effect to increase cell growth, both within the physiological context of tissue renewal and in the more adaptative sense of tissue hyperplasia. In the shorter term, insulin has a primary role in fuel homoeostasis. As well as inhibiting the breakdown of storage fuels (adipose tissue triglyceride, hepatic and muscle glycogen) and inhibiting endogenous glucose production, insulin stimulates glucose uptake into adipose tissue and muscle, both of which contain the insulin-regulatable glucose transporter (GLUT 4). In adipose tissue, the predominant fate of glucose after its phosphorylation to glucose 6-phosphate (Glc-6-P) is its use for glycerol 3-phosphate and fatty acid synthesis. In muscle, Glc-6-P enters one of two major pathways, glycogen synthesis or glycolysis. In turn, glycolysis leads either to lactate formation or pyruvate oxidation.

The conversion of pyruvate into acetyl CoA for oxidation or use as a precursor for fatty acid synthesis is catalysed by the mitochondrial pyruvate dehydrogenase holocomplex (PDHC). PDHC comprises three component enzymes, E1 (pyruvate dehydrogenase, PDH), E2 (dihydrolipoamide acetyltransferase) and E3 (dihydrolipoamide dehydrogenase). PDHC activity is regulated by end-product inhibition and by reversible phosphorylation (inactivation) of E1 [1]. Glucose oxidation is suppressed in states associated with relative insulin deficiency (starvation and type 1 diabetes mellitus) (reviewed in [2]). This occurs through inactivation (phosphorylation) of PDHC, a reaction catalysed by PDH kinase. Increased PDH kinase activity is, at least in part, a consequence of activation by increasing mitochondrial concentration ratios of ATP/ADP, acetyl CoA/CoA and NADH/NAD+ secondary to fatty acid (FA) oxidation [3]. In addition, the specific activity of PDH kinase increases in response to long-term starvation in a number of oxidative tissues, e.g. heart, liver, skeletal muscle and mammary gland [4]. The

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Abbreviations used: DBcAMP, dibutyl cyclic AMP; FA, fatty acids; Glc-6-P, glucose 6-phosphate; MAP, mitogen-activated protein; NEFA, non-esterified fatty acids; PDH, pyruvate dehydrogenase; PDHC, PDH complex; PDHa, active PDHC, PDHPase, PDHP phosphorylase.

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