Transcriptional Glucose Signaling through The Glucose Response Element Is Mediated by the Pentose Phosphate Pathway*

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Bruno Doiron, Marie-Hélène Cuif, Ruihuan Chen, and Axel Kahn‡
From the Institut Cochin de Génétique Moléculaire, INSERM, Unité 129, 24 rue du Faubourg Saint Jacques, 75014 Paris, France

Glucose catabolism induces the expression of the L-type pyruvate kinase (L-PK) gene through the glucose response element (GIRE). The metabolic pathway used by glucose after its phosphorylation to glucose 6-phosphate by glucokinase to induce L-PK gene expression in hepatocytes remains unknown. The sugar alcohol xylitol is metabolized to xylulose 5-phosphate, an intermediate of the nonoxidative branch of the pentose phosphate pathway. In this study, we demonstrated that xylitol at low concentration (0.5 mM) induced the expression of the L-PK/CAT construct similarly to 20 mM glucose alone. In hepatocytes in primary culture, 5 mM xylitol induced accumulation of the L-PK mRNA even in the absence of insulin. Furthermore, the response to xylitol as well as glucose required the presence of a functional GIRE. It can be assumed from these results that glucose induces the expression of the L-PK gene through the nonoxidative branch of the pentose phosphate pathway. The effect of xylitol at low concentration suggests that the glucose signal to the transcriptional machinery is mediated by xylulose 5-phosphate.

Glucose, a major fuel of mammalian tissues, induces the transcription of several glycolytic and lipogenic genes in hepatocytes and adipocytes (1–4). In particular, it induces the expression of the L-type pyruvate kinase (L-PK) gene in the liver through the glucose response element (GIRE) located at position −168 to −144 bp with respect to the cap site (5–7). This GIRE consists of two palindromic binding sites for upstream stimulating factor (USF) proteins separated by 5 base pairs (5, 8, 9). Similar elements, also termed carbohydrate response element, have been identified in the regulatory regions of several glucose-responsive genes, i.e. the spot 14 gene (10) and the fatty acid synthase gene (11, 12). We have shown that activation of the L-PK promoter through its GIRE requires phosphorylation of glucose to glucose 6-phosphate, mediated by insulin-dependent glucokinase induction in hepatocytes (13). However, insulin can be replaced in hepatocytes by transfection of a glucokinase expression vector and by low concentrations of fructose acting through fructose 1-phosphate-dependent activation of residual glucokinase (13). Furthermore, insulin is not necessary in the glucose-responsive hepatoma cell lines in which glucokinase is replaced by other isoforms of insulin-independent hexokinases (14). However, the pathway by which glucose 6-phosphate activates transcription of the L-PK gene and other glucose-responsive genes remains unknown. Glucose 6-phosphate is an important compound at the junction of several metabolic pathways (glycolysis, gluconeogenesis, pentose phosphate pathway, glycosgenesis, and glycogenolysis). In adipocytes, the glucose analogue 2-deoxyglucose (transported in the cell, phosphorylated into 2-deoxyglucose 6-phosphate but was not further metabolized in the Embden Meyerhoff pathway) has been shown to stimulate expression of the fatty acid synthase and acetyl-CoA carboxylase genes (15). Similarly, 2-deoxyglucose can activate the L-PK promoter in the insulinoma cell line INS-1 (16), but not in hepatocyte or hepatoma cells (14). However, the efficiency of 2-deoxyglucose in mimicking the glucose effect in some cells does not signify that the observed induction was mediated by 2-deoxyglucose 6-phosphate itself. Indeed, although its isomerization into fructose 6-phosphate is impossible, 2-deoxyglucose 6-phosphate is partly further metabolized into various compounds (17, 18). Therefore, if the 2-deoxyglucose-dependent induction of glucose-responsive genes in adipocytes and INS-1 cells rules out the involvement of the Embden Meyerhof pathway, it does not rule out the involvement of intermediates rising from 2-deoxyglucose 6-phosphate, especially through the pentose phosphate pathway.

In this study, we show that xylitol is active at a lower concentration than glucose for stimulating the L-PK promoter in both mHAT3F hepatoma cells and hepatocytes. In mHAT3F cells, the activating xylitol concentration is too low to modify intracellular glucose 6-phosphate concentration. The xylitol acts as glucose through the GIRE. Since xylitol is transformed into xylulose 5-phosphate in the cells, we suggest that glucose acts on glucose-responsive genes in the liver, and probably adipocytes, through the nonoxidative branch of the pentose phosphate pathway. Consequently, xylulose 5-phosphate is the major metabolite candidate of the nonoxidative branch of the pentose phosphate pathway responsible for mediating transcriptional machinery induction by glucose catabolism.

MATERIALS AND METHODS

Plasmids—All plasmids were constructed by using standard DNA cloning procedures (19). The constructs were verified by nucleotide sequencing.

The different L-PK/CAT constructs (termed −183 PK/CAT, −150 PK/CAT, −96 PK/CAT, L4mi-L3 −119 PK/CAT) have been previously described (5, 20). The KS2V2 CAT plasmid, used as a transfection control, contains the CAT gene directed by the early promoter and enhancer of simian virus 40 (SV40) (14).
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Cell Culture Conditions—Hepatocytes were isolated by collagenase perfusion method from male Sprague-Dawley rats (180–200 g) fasted since 3 days (5, 21, 22). Hepatocyte suspensions were plated on 10-cm dishes in a final volume of 10 ml of a medium 199 (Life Technologies, Inc.) supplemented with penicillin, streptomycin, and 10% (v/v) dialyzed fetal calf serum. After 12 h of attachment, the medium was removed and replaced by a hormone-supplemented fresh medium 199 with different glucose and/or xylitol concentrations in the presence or absence of insulin (20 nm). The medium was replaced every 24 h.

The mhAT3F hepatocyte-like cell lines were derived from tumoral liver of transgenic mice expressing the SV40 large T and small T antigens under the direction of the liver-specific antithrombin III promoter (14, 23, 24). Cells were cultured in Ham’s F-12-Dulbecco’s modified Eagle’s medium (v/v) (Life Technologies, Inc.) medium supplemented with penicillin, streptomycin, 20 nm insulin, 1 μM triiodothyronine, 1 μM dexamethasone, and 5% (v/v) fetal calf serum. Twenty-four h before the experiment, cells were cultured in a serum-free, glucose-free medium containing 10 mM lactate and supplemented with the same mixture of hormones as described above. Induction of the different L-PK/CAT constructs was measured in the presence of the various concentrations of glucose and xylitol.

Transfections and CAT Assays—Transfection of the mhAT3F was performed by lipofection using N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium methylsulfate (DOTAP, Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s instructions, in cells cultured under lactate conditions. Five micrograms of plasmids was transfected when cells were 60–80% confluent in 60-mm plastic dishes (Falcon, Oxnard, CA). The medium containing the liposome-DNA complex was removed 12 h later and replaced with medium as described above for glucose and xylitol regulation studies. Cells were harvested 36 h later. CAT activity assay was performed as described (5, 14). The CAT activity was normalized with respect to the KSV2 CAT activity as a transfection standard.

Northern Blot Analysis—Total RNAs were isolated from adult rat hepatocytes in primary culture by lysis in guanidine, followed by phenol extraction (25). The RNA concentration was determined spectrophotometrically. RNA was denatured with methylmercury hydroxide, electrophoretically separated on formaldehyde agarose gels, and finally transferred and UV-cross-linked to nylon filters (Hybond-N, Amersham). Prefhybridization (2 h with 100 μg/ml sperm DNA) and hybridization were carried out as described previously (6).

Determination of Glucose 6-Phosphate Concentration—Glucose 6-phosphate concentration in cultured mhAT3F cells was assayed enzymatically according to Steiml (26) as described previously (13).

Statistics—Results are given as means ± S.D. Statistical significance of differences between treatment groups in these studies was determined by Student’s t-test. The minimal level of significance chosen was p < 0.05.

RESULTS AND DISCUSSION

Xylitol Stimulates the L-PK Promoter at Low Concentration in mhAT3F Cells—Glucose 6-phosphate synthesis is the first step of glucose-dependent activation of glucose-responsive genes in the liver and hepatocytes (13). It is consumed through the Embden Meyerhoff pathway and pentose phosphate pathway. It can also be used for the synthesis of glycogen, that occurs mainly in muscle and liver. In the liver, glucose 6-phosphate can be produced by gluconeogenesis and glycogenolysis. The Embden Meyerhoff pathway or pentose phosphate pathway, more or less active in all cells, are therefore the most likely source of active intermediates mediating transcriptional stimulation of glucose responsive genes through their GIRE. However, the efficiency of 2-deoxyglucose for stimulating the L-PK promoter through its GIRE in insulinoma INS-1 cells seems to rule out the involvement of the Embden Meyerhoff pathway (16). To test the role of intermediates of the nonoxidative branch of the pentose phosphate pathway in activation of the L-PK GIRE, we compared the efficiency of various concentrations of either xylitol or glucose in stimulating CAT activity of L-PK/CAT constructs transiently transfected in mhAT3F cells. Fig. 1 shows that 0.5 mM xylitol was as efficient as 20 mM glucose, and a detectable stimulation was observed for xylitol concentrations as low as 0.1 mM. In contrast, we have previously demonstrated that stimulation of the −183 PK/CAT construct was undetectable in mhAT3F cells for a glucose concentration lower than 5 mM (14). Xylitol and glucose-dependent activation appeared to be noncumulative since similar CAT activity was observed in the presence of 20 mM glucose regardless of the presence of 5 mM xylitol.

Both Xylitol and Glucose Effects on the Activity of the L-PK Promoter Are Mediated by the L-PK GIRE—The positive response of the L-PK gene to glucose is mediated by the GIRE, corresponding to box L4 spanning from −168 to −144 bp with respect to the cap site. Any mutation impairing binding of USF proteins to the GIRE suppresses the glucose responsiveness of the L-PK promoter in hepatocytes in primary cultures (5, 8) as well as in mhAT3F cells (14). Fig. 2 shows that all PK/CAT constructs lacking the box L4 (−96 PK/CAT and L3 −119 PK/CAT constructs) or with a mutant box L4 unable to bind USF (L4−L3 −119 PK/CAT construct) were unresponsive to both glucose and xylitol in mhAT3F cells. In the L4−L3 −119 PK/CAT construct, the downstream USF binding site of the GIRE was inactivated by transforming the CCCGTG E box into CCCC TTG (5). These results suggest that xylitol uses the same way as glucose to induce expression of the L-PK gene through the GIRE. Consequently, we suggest that glucose induces L-PK gene expression through the nonoxidative branch of the pentose phosphate pathway. Indeed, xylitol is first oxidized to D-xylulose, which is then phosphorylated to D-xylulose 5-phosphate and further metabolized by the nonoxidative branch of the pentose phosphate pathway (27). The activation of the L-PK promoter by xylitol concentrations as low as 0.1 and 0.5

![Fig. 1. Effect of xylitol on the activation of the L-PK gene promoter in mhAT3F cells.](image-url)
mM practically rules out the hypothesis that this pentose could act through conversion of xylulose 5-phosphate to hexose phosphate by the nonoxidative branch and then re-enter the Embden Meyerhoff pathway. Indeed, 0.5 mM xylitol was unable to increase glucose 6-phosphate in mHAT3F cell. A xylitol-dependent glucose 6-phosphate accumulation through the pentose phosphate pathway and gluconeogenesis was detectable only for much higher xylitol concentrations (Table I). In addition, we have previously shown that in hepatocytes in primary culture, fructose alone at 0.2 mM was inactive on the L-PK promoter, while it was able to stimulate the glucose effect regardless of the presence of insulin (13). This signifies that fructose 1-phosphate, a product of the fructokinase reaction, was able to stimulate glucokinase-dependent phosphorylation of glucose, but was inactive by itself or by its metabolism through the Embden Meyerhoff pathway.

Xylitol Activates the Endogenous L-PK Gene without Insulin in Hepatocytes in Primary Culture—Insulin dependence of the glucose effect in hepatocytes is due to the transcriptional induction of the glucokinase gene by insulin allowing for glucose phosphorylation to glucose 6-phosphate (13). Xylitol is oxidized to D-xylulose by L-iditol dehydrogenase and then phosphorylated to xylulose 5-phosphate by a specific xylulokinase (27). Therefore, the xylitol effect in hepatocytes is not expected to depend on the insulin-dependent glucokinase gene activation. Indeed, detection by a Northern blot of the endogenous L-PK mRNA shows that 5 mM xylitol alone, without insulin, leads to L-PK mRNA accumulation while 25 mM glucose was practically inefficient in an insulin-free medium (Fig. 3). As expected, 20 mM glucose 6-phosphate concentration

| Glucose 6-phosphate concentration | % | nM/mg protein |
|----------------------------------|---|---------------|
| 10 mM glucose                    | 0.63 ± 0.03 | 100 ± 4.8 |
| 0.5 mM xylitol                   | ND | ND |
| 2.5 mM xylitol                   | 0.15 ± 0.04 | 24 ± 6.3 |
| 5 mM xylitol                     | 0.15 ± 0.07 | 24 ± 11.1 |
| 10 mM xylitol                    | 0.49 ± 0.07 | 77 ± 11.1 |

nm insulin strongly stimulated the glucose responsiveness, but did not significantly change the xylitol responsiveness of the L-PK gene. The extent of xylitol-dependent induction of the L-PK gene in hepatocytes, with or without insulin, was 3-fold lower than with glucose plus insulin. As a matter of fact, the use of xylitol as the only fuel can provoke cellular ATP depletion due to inorganic phosphate trapping in the phosphate esters of the pentose phosphate pathway (28, 29), which could explain this apparent lower efficiency of xylitol as compared to glucose plus insulin. Moreover, xylitol was active in hepatocytes at a lower concentration than glucose (5 mM versus more than 10 mM), as already commented upon with mAT3F hepatoma cells. The need for a higher carbohydrate concentration to induce the L-PK gene in hepatocytes compared with mAT3F cells seems to be a general phenomenon which could be explained by several features, such as differences in transporters, membrane permeability, enzyme activities, very low metabolism, and transcriptional activities of hepatocytes in primary culture, etc.

Role of the Pentose Phosphate Pathway in Glucose Signaling Toward the Transcriptional Machinery—The pentose phosphate pathway was described in its classical form as a cycle by Horecker and Mehler (30). This cycle occurs in two separate stages: the oxidative step, which involves oxidations by NADP⁺, occurs in the reaction from glucose or glucose 6-phosphate to ribulose 5-phosphate, and the nonoxidative step, which involves sugar interconversion reactions, occurs in the pathway from ribulose 5-phosphate back to glucose 6-phosphate. The main biological function of the oxidative step exists in the generation of NADPH as a source of reducing equivalents for biosynthetic reactions. Therefore, this oxidative phase is especially important in adipocytes, since NADPH is essential for the biosynthesis of fatty acids. The main function of the nonoxidative step is the maintenance of the monosaccharide pool in the cell cytosol, especially the ribose 5-phosphate for nucleotide and nucleic acid synthesis. Our results with xylitol suggests that the nonoxidative branch of the pentose phosphate pathway is also a crucial step for triggering the induction signal of the L-PK gene expression. Recently, Nishimura et al. (31, 32) demonstrated the stimulation by xylulose 5-phosphate of a protein phosphatase 2A active on phosphophofructokinase-2. The activation of this protein phosphatase 2A requires at least 10 μM xylulose 5-phosphate, and its activation curve was highly sigmoidal. Xylulose 5-phosphate appeared to be a specific activator of the protein phosphatase because none of the other sugar phosphates tested, including glucose 6-phosphate or fructose 6-phosphate, was effective. Consistently with the involvement of protein phosphatase(s) in the response of the L-PK gene to glucose, we found that okadaic acid, that specifically inhibits protein phosphatases 2A and 1 (33), blocks...
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Fold stimulation

Fig. 3. Effects of xylitol on the level of L-type pyruvate kinase mRNA in cultured hepatocytes. Hepatocytes were isolated from 3-day-starved male rats and plated on 10-cm dishes in a medium supplemented with penicillin, streptomycin, and 10% (v/v) dialyzed fetal calf serum. After culture in the presence of 1 μM triiodothyronine, 1 μM dexamethasone, xylitol (5 and 10 mM) or glucose (25 mM), with or without insulin (20 nM), hepatocytes were harvested and total RNA was purified. Total RNA was purified from the hepatic cells. The L-type pyruvate kinase mRNA was quantitated by scanning the autoradiograms of Northern blot analysis. The values are the means of three distinct experiments and are expressed relative to the value obtained under the lactate condition.

In conclusion, we have demonstrated that a pentose entering the nonoxidative branch of the pentose phosphate pathway is active at very low concentrations for stimulating the L-PK gene through its glucose response elements. We suggest that xylulose-5-phosphate, capable of activating a protein phosphatase activity (31, 32), could trigger a phosphorylation/depshorylation cascade modulating the activity of the glucose response complex assembled on the glucose-responsive elements in hepatocytes and adipocytes.

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