Differential regulation of the fructose transporters GLUT2 and GLUT5 in the intestinal cell line Caco-2

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Régulation différentielle de l'expression des transporteurs de fructose GLUT2 et GLUT5 dans la lignée intestinale Caco-2

RÉSUMÉ

L'apport accru du fructose dans l'alimentation moderne peut avoir des conséquences à long terme qui restent mal definies, avec semble-t-il des augmentations de taux de triacylglycérols plasmatiques et l'installation d'une résistance périphérique à l'insuline. Au delà de son intérêt fondamental, l'étude de la régulation de l'expression des transporteurs de fructose présente donc un intérêt physiopathologique. Deux transporteurs équilibrateurs, GLUT5 et GLUT2 associés respectivement aux membranes en bordure en brosse et basolatérale, assurent le transport de fructose dans les cellules entérocytaires de l'intestin normal ou dans celles de la lignée cancéreuse colique différenciée Caco-2. Des clones de cellules Caco-2 ont été sélectionnés sur la base de leur taux de consommation de glucose et de leur niveau d'expression des protéines associées à l'absorption du fructose. Le niveau d'expression de GLUT2 et GLUT5 est en rapport inverse des taux de consommation de glucose des clones. La restriction de l'apport en glucose à ces cellules affecte de façon différente GLUT2 et GLUT5. Elle augmente le taux d'ARNm de GLUT2 dans les cellules fortes consommatrices et diminue celui de GLUT5 dans les cellules faibles consommatrices qui l'expriment. L'activation de l'adénylate cyclase (*EC* 4.6.1.1) par la forskoline provoque une forte augmentation de l'AMPc, associée à une glycogénolyse et à l'accélération de la consommation de glucose. Son effet sur l'expression des transporteurs GLUT2 et GLUT5 est inverse: le taux d'ARNm de GLUT2 diminue fortement alors que celui de GLUT5 est augmenté. L'activité du promoteur de GLUT5, étudiée en transfection transitoire avec des constructions plasmidiques contenant le gène rapporteur luciférase (*EC* 1.14.14.3) a permis de montrer que l'AMPc module la transcription de GLUT5 par l'intermédiaire de deux régions distinctes du promoteur. Une région contenant deux séquences de réponse à l'AMPc (CRE) assure 50% de la stimulation de la transcription suggérant que le reste de l'activation résulte des effets de l'AMPc sur le métabolisme du glucose. Ces résultats indiquent donc qu'il existe une régulation fine de l'expression des transporteurs de fructose qui dépend de la capacité propre d'utilisation du glucose par les cellules, de la quantité de glucose qui leur est fournie et de l'activation de la voie de transduction de l'AMPc.

Fructose is the predominant sugar in many fruits, and is contained in maize sweeteners that are widely used in the preparation of food products. These sweeteners are

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responsible for the large increase in the consumption of fructose which now accounts for more than 8% of the daily energy intake in developed countries (Glinsmann *et al.* 1986). The long-term consequences of this change in nutritional habits is not known, but there is evidence that high fructose ingestion may provoke increased plasma triacylglycerol levels and contribute to insulin resistance (Hallfrisch, 1990; Bantle *et al.* 1992). Therefore, studies on the cellular mechanisms that control the expression of proteins involved in fructose transport have a physiopathological interest.

Fructose transporters GLUT2 and GLUT5 are members of the facilitative hexose-transporter family (Bell *et al.* 1990). Their function as fructose transporters has been defined mainly in the *Xenopus laevis* oocyte expression system where human GLUT2 (Burant *et al.* 1992) and rat GLUT5 (Rand *et al.* 1993), unlike human GLUT5, were shown to transport glucose (Burant *et al.* 1992; Colville *et al.* 1993). The membrane localization of GLUT2 in the basolateral membrane and GLUT5 in the apical brush-border membrane of small intestinal absorbing cells was identified with specific antibodies directed against the C-terminal peptide of the protein (Davidson *et al.* 1992; Mahraoui *et al.* 1992; Thorens, 1993). The transfer of fructose molecules from the lumen side of the intestine to the blood involves, therefore, two different transporters GLUT5 and GLUT2 (Cheeseman, 1993), which function in series to promote fructose absorption.

Caco-2 cells were characterized in the 1980s and since then have been found to exhibit morphological and functional characteristics of human differentiated intestinal absorbing cells (Pinto *et al.* 1983; Zweibaum *et al.* 1991). They grow as monolayers of polarized cells with an apical brush-border and a basolateral membrane domain separated by tight junctions. The cells differentiate spontaneously after confluence. They express the Na–glucose co-transporter encoded by the SGLT1 gene (Blais *et al.* 1987; Mahraoui *et al.* 1994*a*), and the fructose transporters GLUT5 and GLUT2 which are normally associated with intestinal absorbing cells. The membrane localization of these transporters is identical with that observed in normal intestinal absorbing cells. However, Caco-2 cells also express GLUT1 and GLUT3, which are associated with the fetal and/or transformed phenotype (Mahraoui *et al.* 1992, 1994*a*). GLUT1 was found to be present in the basolateral domain of the cells (Mahraoui *et al.* 1992; Mesonero *et al.* 1994).

Although Caco-2 cells are uniformly differentiated into the phenotype of intestinal absorbing cells, the cell line exhibits some metabolic variability. Indeed, a passage relationship exists between the hexose-transporter and sucrase–isomaltase (*EC* 3.2.1.10, 3.2.1.48) mRNA abundances on one hand and the glucose consumption rate of the cells on the other hand (Zweibaum, 1986; Mahraoui *et al.* 1992). Early passage-cells (P29) exhibit high glucose consumption rates associated with a low abundance of fructose transporter and sucrase–isomaltase mRNA. Conversely late passages (P198) exhibit high fructose transporter and sucrase–isomaltase mRNA abundances associated with low glucose consumption rates. Caco-2 cell clones have been obtained using a limit dilution technique taking cells from the two extreme passages.

In the present paper we present two different sets of results which show that glucose metabolism and/or its alterations control the expression of the fructose transporters GLUT2 and GLUT5 in the human cell line Caco-2. The first series of observations were made with clones of Caco-2 cells which have the morphological phenotypes of differentiated enterocytes but differ dramatically in their glucose consumption rates (Chantret *et al.* 1994). The second set of results focuses on the effect of forskolin which induces a

strong increase in cAMP levels in the cells, together with an accelerated metabolism of glucose.

FRUCTOSE-TRANSPORTER EXPRESSION IN GLUCOSE-RESTRICTED CACO-2 CLONES

Cellular glucose consumption rates vary as a function of growth and from one clone to another. High glucose consumption rates are observed in the exponential phase of growth and decrease to a plateau level of consumption in the stationary phase of growth. This plateau rate of glucose consumption for low-glucose-consuming clones is 40–50-fold lower than that for high-glucose consuming clones.

An inverse relationship was observed between the expression of GLUT2, GLUT5 and SGLT1 mRNA and the glucose consumption rate of the cells. Indeed, these mRNA are abundant in low-glucose-consuming cells and are barely detectable in clones that maintain a high level of glucose consumption after the onset of differentiation. These results indicated that the expression of fructose transporters in Caco-2 cells is related to their level of glucose utilization.

To assess whether modification of the glucose supply to the different clones would alter the expression of GLUT2 and GLUT5, cell cultures were made in either 25 mM- or 1 mM-glucose medium, the latter concentration being the minimum possible input of glucose due to the addition of 200 ml heat-inactivated fetal bovine serum/l to the glucose-free Dulbecco's modified Eagle's medium. Under these conditions of culture, and in the post-stationary phase of growth (day 20), cell number, glucose consumption rates and dipeptidylpeptidase IV (*EC* 3.4.14.5) activity for each clone were similar. In low-glucose-consuming clones, that exhibit spontaneously high GLUT2 and GLUT5 mRNA abundances, glucose deprivation decreased GLUT5 mRNA abundance and did not affect GLUT2 significantly. On the contrary, in high-glucose-consuming cells, the expression of GLUT5 is undetectable with both low- and high-glucose culture conditions, but GLUT2 mRNA abundance is strongly increased in glucose-deprived cells. Similar observations were made in the same clones and under the same culture conditions (Chantret *et al.* 1994) with sucrase–isomaltase, a disaccharidase that hydrolyses sucrose into glucose and fructose, i.e. high glucose consumption rates were associated with low sucrase–isomaltase mRNA abundance. There is an apparent contradiction in the regulation of the expression of GLUT2 in low- and high-glucose-consuming clones which suggests that the expression of GLUT2 involves refined mechanisms that combine their specific metabolic rates and the quantity of glucose supplied to the cells. Alternatively, these experiments may have shown the existence of different mechanisms of controls for GLUT2 depending on the cell clones. Despite these differences, glucose metabolism is involved in the expression of GLUT2 and GLUT5 in Caco-2 cells, with an apparent coordinate regulation of sucrase–isomaltase and GLUT2, but an inverse regulation for the two fructose transporters.

cAMP REGULATION OF THE EXPRESSION OF GLUT2 AND GLUT5

The metabolism of glucose by the cells can be modulated by pharmacological means. Forskolin is a potent stimulator of adenylate cyclase (*EC* 4.6.1.1) activity in cells and raises intracellular cAMP levels (Seamon *et al.* 1981). Forskolin-treated Caco-2 cells

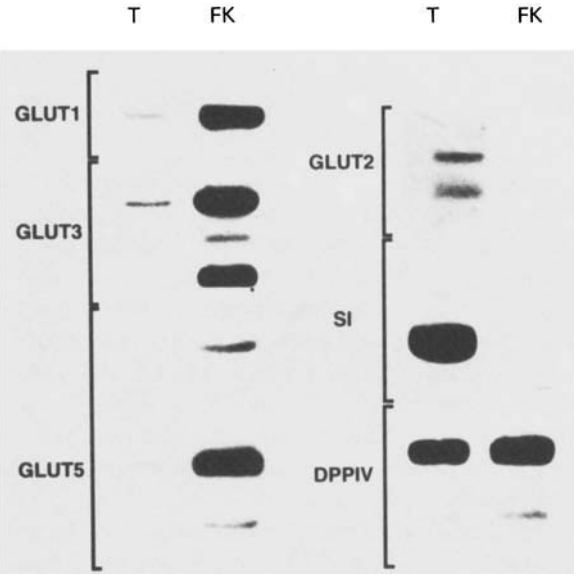

Fig. 1. Hexose-transporter mRNA abundance in forskolin-treated Caco-2 cells. Northern blot of 20 μg total RNA extracts from control (T) and 5×10^{-5} M-forskolin (FK)-treated cells made and hybridized with the ^{32}P-labelled cDNA probes GLUT1, pGEM4Z-HepG2GT, 1·75 kb insert; GLUT2, pBS-HTL210/hGLUT2, 2·4 kb insert; GLUT3, phMGT-31GLUT3, 2·2 kb insert and GLUT5, phJHT5/hGLUT5, 1·9 kb insert; sucrase–isomaltase (SI) with cDNA probe SI2 and dipeptidylpeptidase-IV (DPPIV) with DPI-101. GLUT2 and sucrase–isomaltase mRNA abundances are decreased in (FK)-treated cells whereas GLUT1, GLUT3 and GLUT5 are strongly increased. Caco-2 cells were cultured for 20 d in Dulbecco's modified Eagle's medium supplemented with 25 mM-glucose, 10 ml non-essential amino acids/l and 200 ml heat-inactivated fetal bovine serum/l.

have increased glucose consumption rates and accelerated glycogenolysis (Rousset *et al.* 1985). We examined, therefore, whether forskolin would affect the expression of the fructose transporters GLUT2 and GLUT5 and, if so, by what mechanism(s).

The mRNA abundance of GLUT2 was strongly and transiently reduced in forskolin-treated cells (Fig. 1). Such a decrease is reminiscent of that observed for sucrase–isomaltase in forskolin-treated cells (Rousset *et al.* 1989). This down-regulation of sucrase–isomaltase is now understood as a catabolic repression of the transcription of the gene (Chantret *et al.* 1993), opening the possibility that in Caco-2 cells the gene GLUT2 is repressed by glucose.

On the contrary, forskolin increased markedly the mRNA abundance of GLUT5 in Caco-2 cells. This increase was effective within the first hours after the treatment and could be obtained as soon as the cells express GLUT5. Indeed, it was not possible to obtain any anticipated expression of this transporter by treating undifferentiated cells before the confluence of the monolayer. With dideoxyforskolin, a forskolin analogue that does not stimulate adenylate cyclase activity, GLUT5 mRNA abundances were similar to those of controls. Furthermore, use of the non-metabolizable cAMP, dibutyryl-cAMP, yielded an essentially identical increase in GLUT5 mRNA. The effect of forskolin was reversible within 4–5 d. Although the mRNA half-life of GLUT5 was

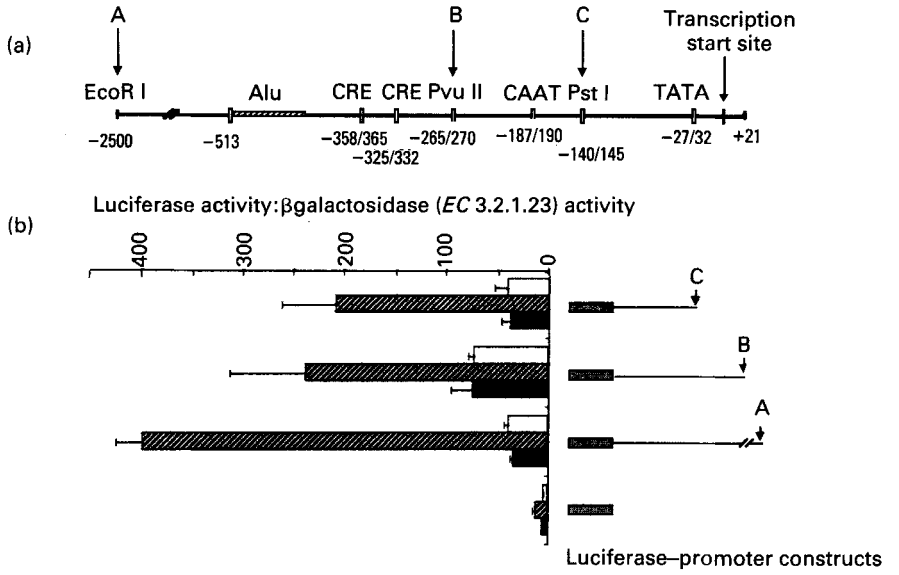

Fig. 2. (a) Scheme of the promoter region of the fructose-transporter GLUT5. The coding sequence of the gene luciferase (*EC* 1.14.14.3) was placed under the control of the three genomic DNA sequences of the 5′ end of the gene GLUT5 containing the promoter region in the transient expression vector pGL2 (Promega). (b) Caco-2 cells were cultured for 20 d in Dulbecco's modified Eagle's medium supplemented with 25 mM-glucose, 10 ml non-essential amino acids/l and 200 ml heat-inactivated fetal bovine serum/l and were transfected with the pGL2-luciferase promoter constructs. Constructs B and C derived from digests of construct A by PvuII and Pst I restriction enzymes respectively that do not contain the cAMP responsive element (CRE), still yield a 47 and 45% luciferase activity increase as compared with that obtained with construct A in forskolin-treated cells. (■), Control; (▨), forskolin; (▒), dideoxyforskolin-treated cells. Values are means with their standard errors represented by vertical bars.

increased, this stabilization could not explain the increase in GLUT5 mRNA abundance, suggesting that transcription was also affected by forskolin treatment (Mahraoui *et al.* 1994*b*).

Transcriptional activation of genes by cAMP involves specific interactions of consensus cAMP-responsive element (CRE) DNA motifs with nuclear transcription factors (CRE-binding protein; Borrelli *et al.* 1992). CRE have been found in a number of genes which are regulated by cAMP, including facilitative glucose transporters (Murakami *et al.* 1992; Takeda *et al.* 1993).

To study the effect of cAMP on the transcription of the gene GLUT5, a genomic DNA clone containing its promoter region was isolated (Mahraoui *et al.* 1994*b*; G. I. Bell and J. Takeda, unpublished results). The analysis of its sequence shows an Alu-dispersed middle repetitive sequence beginning at nucleotide −440, upstream of the transcription start site, suggesting that the regulatory elements of the gene are located between nucleotides −440 and +1. This region contains a classical TATA motif and two potential CRE. In a first attempt to assess the functional significance of these CRE, Caco-2 cells were transfected with two promoter–reporter gene constructs that contained either 2500 base pairs (bp) or the last 270 bp upstream of the transcription start site (Fig. 2). The activity of the promoter sequences on the transcription of the reporter-gene luciferase

(*EC* 1.14.14.3) was measured in control, forskolin- and dideoxyforskolin-treated cells for 2 d. Normalized reporter-gene activities were eight to ten times higher in forskolin-treated cells than in control or dideoxyforskolin-treated cells (Fig. 2), indicating that the regulatory elements responsible for the increase in transcription of the gene GLUT5 by cAMP are present. Transcriptional activation by forskolin was reduced by half, with the constructs that did not contain the CRE consensus sequences indicating that not only CRE are required for the full activation of the transcription of the gene by cAMP but also that nucleotides between −300 and +1 are involved in 50% of its effect, suggesting some cooperativity between these two DNA domains. These activations are tissue specific, as indicated by the absence of stimulation of the transcription of the reporter gene in Cos, HepG2 and CHO cells (Mahraoui *et al.* 1994*b*).

CONCLUDING REMARKS

The aim of our studies was to compare and contrast the regulation of two fructose transporters GLUT2 and GLUT5 in human intestinal absorbing cells. The catabolism of glucose appears to repress GLUT2 in Caco-2 cells. The physiological significance of the effect of altered glucose consumption rates on the expression of the gene GLUT2 is unclear and requires more investigation to decide whether the observed effects result from the pathology of cancer cells or are due to specific aspects of regulation that differ from one tissue to another. Indeed, GLUT2 is up-regulated by glucose in a dose-dependent manner in pancreas and liver cell lines and glucose-dependent nuclear-factor-binding DNA regions have been identified in the close proximity of the gene's transcription start site (Waeber *et al.* 1994; Leibiger & Leibiger, 1995).

The fructose transporter GLUT5 is up-regulated by glucose, thus differing from GLUT2. In addition, the transcription of the gene GLUT5 is strongly stimulated by cAMP. To date it seems that the full activation of the gene by cAMP involves both the 'glucose' effect and CRE. Work is in progress in our laboratory to study the role of each regulation process and their possible relationships.

Fructose-transporter expression clearly involves several mechanisms that probably cooperate to provide a refined control of fructose absorption in the intestine.

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