Regulation of expression of the human fructose transporter (GLUT5) by cyclic AMP

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The effect of cyclic AMP on the expression of the fructose transporter, GLUT5, was studied in Caco-2 cells, a human colon cancer cell line that differentiates spontaneously in culture into cells with the properties of small intestine enterocytes. Treatment of differentiated Caco-2 cells with 50 μM forskolin, which stimulates adenylate cyclase and raises intracellular cyclic AMP levels, increased fructose uptake 2-fold and raised GLUT5 protein and mRNA levels 5- and 7-fold respectively. The increased GLUT5 mRNA levels in forskolin-treated cells are a result of stabilization of GLUT5 mRNA in these cells and increased transcription. The effect of cyclic AMP on GLUT5 transcription was assessed by measuring the activity of human GLUT5 promoter–reporter gene constructs in forskolin-treated differentiated Caco-2 cells. The results showed that forskolin stimulated the activity of the GLUT5–reporter gene constructs and this stimulatory effect was mediated by *cis*-acting regulatory sequences.

INTRODUCTION

Glucose, galactose and fructose are the major hexose sugars in the human diet. Their absorption from the lumen of the small intestine is carried out by members of two families of membrane transport proteins [1]. Glucose and galactose are taken up by the Na^+/glucose co-transporter SGLT1 which utilizes the electrochemical Na^+ gradient to transport these sugars against their concentration gradient. Fructose is transported across the apical surface of the enterocyte by a member of the facilitative sugar transporter family GLUT5 which transports fructose down its concentration gradient in an energy-independent manner. The release of fructose from the enterocyte into the interstitium is probably carried out by another member of the facilitative glucose-transporter family, GLUT2, which is located in the basolateral membrane of these cells and also has the capacity to transport fructose although not as efficiently as GLUT5 [2].

GLUT5 is expressed in a number of tissues besides small intestine including kidney, muscle, adipose tissue, brain and testis, at least in humans [2–7]. It is also expressed by Caco-2 cells [8,9], a human colon cancer cell line that differentiates spontaneously in culture into cells with the properties of enterocytes having apical- and basolateral-membrane domains and expressing specific proteins involved in the uptake of sugars including sucrase–isomaltase [10] and SGLT1 [11], in addition to GLUT5 [9]. These cells represent a useful *in vitro* cell model for studying regulation of hexose-transporter expression.

Previous studies using the same frozen stock of cells at the same passages have shown that treatment of confluent differentiated cultures of parental Caco-2 cells with agents that increase cellular cyclic AMP (cAMP) levels such as forskolin (FK) decreases the expression of sucrase–isomaltase [12,13], the enzyme that hydrolyses sucrose into fructose and glucose. As fructose is generated by the action of sucrase–isomaltase, this result would suggest that GLUT5 expression may also be regulated by cAMP. We therefore examined the effects of FK treatment of differentiated Caco-2 cells on fructose-transport activity and GLUT5 mRNA and protein levels. These studies show that increased cAMP levels are associated with an increase in fructose transport and GLUT5 mRNA and protein levels. This increased expression results from stabilization of GLUT5 mRNA as well as increased transcription of the GLUT5 gene.

MATERIAL AND METHODS

Cell culture

Caco-2 cells were obtained from Dr. J. Fogh (Sloan Kettering Institute for Cancer Research, Rye, NY, U.S.A.). The cells were passaged and cultured as described [9]. Experiments were carried out using the same parental population of Caco-2 cells taken from a frozen stock of cells at the same passages (70–80) as those characterized for their response to FK and their cAMP levels by Rousset et al. [12]. FK (50 μM) and 1,9-dideoxyforskolin (DDFK, 50 μM) (France-Biochem, Calbiochem, Meudon, France) were added as ethanol stock solutions (final ethanol concentration 0.1 %) as described [12]. Dibutyryl-cAMP (But_2-cAMP, 1 mM) and cycloheximide (CHX, 10 μM) (Sigma, St. Louis, MO, U.S.A.) were added as a stock solution in sterile water, and 5,6-dichloro-1-β-D-ribofuranosylbenzimidazol (DRB, 20 μM) (France-Biochem, Calbiochem) was prepared as a 10 mM (100 ×) stock solution in dimethyl sulphoxide. Cell viability was unchanged. Cell content of glycogen was measured as described previously [14]. The glucose content of the media was determined using a glucose analyser (Beckman, Gagny, France). Protein concentration was assayed using the BCA Protein Assay Reagent (Pierce, Rockford, IL, U.S.A.). CHO, HepG2 and COS-1 cells

Abbreviations used: FK, forskolin; DDFK, 1,9-dideoxyforskolin; CHX, cycloheximide; But_2-cAMP, dibutyryl cyclic AMP; DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazol; CRE, cyclic AMP-regulatory element.

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The nucleotide sequence(s) reported in this paper will appear in the GenBankTM/EMBL Data Bank under accession number U05344.

were kindly given by J. L. Danan (CEREMOD, CNRS, Meudon-Bellevue, France).

Fructose-transport assay

Fructose-transport assays were performed in 24-well plates (Corning) essentially as described by Blais et al. [11]. The cells were seeded at a concentration of 4×10^4 cells per cm^2 and cultured in the presence or absence of 50 μM FK. After 15 days, at which time the cells were fully differentiated into small-intestine-like cells, the cell monolayer was washed four times with transport medium which contained 137 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$ and 10 mM Hepes, pH 7.2, before incubation at 37 °C in the presence of either 1 mM D-[U-^{14}C]fructose (248 mCi/mmol, Amersham) or L-[1-^{3}H]glucose (16.2 Ci/mmol, New England Nuclear/Dupont de Nemours). Uptake of the labelled sugar was stopped by the addition of ice-cold transport medium containing 50 mM D-fructose. The monolayers were rinsed four times with this solution and the cells were solubilized in 0.1 M NaOH and counted for radioactivity. Triplicate determinations were made and radioactivity values of the samples were transformed into mol/mg of protein (means ± S.E.M.). L-glucose uptake was used to monitor cell integrity and non-specific transport of glucose.

Northern-blot analysis

The human GLUT5 cDNA probe was phJHT5 [7] and the human sucrase–isomaltase probe was SI2 (obtained from D. Swallow, MRC Human Biochemical Genetics Unit, London, U.K.) [15]. Cell monolayers (day 15 of culture) were rinsed twice with ice-cold sterile saline and stored at −70 °C. Total RNA was extracted using guanidinium thiocyanate and centrifugation through a CsCl gradient [16]. Glyoxal-denatured samples (25 μg) of total RNA were fractionated by electrophoresis on 1% agarose gels and subsequently transferred to Hybond-N membranes (Amersham). Hybridization of the membranes with ^{32}P-labelled probes (Multiprime labelling system, Amersham) was carried out as described [17]. The membranes were washed using high-stringency conditions with a final 15 min wash in 0.1 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) and 0.1% SDS at 65 °C before exposure to X-ray film. The X-ray films were scanned with a densitometer to quantify the hybridization signal.

Western-blot analysis

The protein content of the brush-border membrane-enriched fraction (P2 fraction) was analysed by Western blotting. Cells were rinsed twice with cold PBS and then frozen at −70 °C. Extracts were prepared as described [10]. Briefly, cell homogenates in 2 mM Tris/HCl, pH 7.1, and 50 mM mannitol were prepared using conical grinding tubes and sonicated. The brush-border membrane-enriched fraction was prepared using the calcium (20 mM) precipitation method [18]. The protein samples were solubilized in Laemmli buffer as modified by Haspel et al. [19] (250 mM Tris/HCl, pH 6.8, 8 M urea, 10% SDS, 20% glycerol, 0.05% Bromophenol Blue and 0.1 M dithiothreitol) and subjected to SDS/PAGE (10% gels). Molecular-mass markers (Rainbow Markers, Pharmacia) were run in parallel. After electrophoretic separation, the proteins were transferred to Hybond-ECL membranes (Amersham) and proteins were visualized by staining the membrane with a solution of 0.25% Ponceau Red in 5% trichloroacetic acid. The membranes were then allowed to react with an anti-(human GLUT5) antibody (1:800 dilution) [9] that had been affinity-purified on a peptide-bound column (Affi-Gel 15, Bio-Rad, Richmond, CA, U.S.A.). The primary antibody was detected using a rabbit immunoglobulin horseradish peroxidase-linked antibody from donkey and the Luminol ECL detection system (Amersham).

Isolation of the human GLUT5 gene

Overlapping fragments of the human GLUT5 gene were isolated from the partial *Hae*III–*Alu*I fetal human liver genomic library in λCh4A of Lawn et al. [20] by hybridization with ^{32}P-labelled human GLUT5 cDNA clone as described [21]. The sequences of the exons, adjacent intron segments and promoter region were determined by using the dideoxy-chain-termination method after subcloning appropriate DNA fragments into M13mp18 and M13mp19.

Mapping of transcriptional start site by primer extension

Primer extension was conducted as described previously [21] using the primer, 5′-ATGCTTGCTCTGGAAGGGCAGAGT-3′ (complementary to nucleotides 75–98; see Figure 8a) and adult human kidney RNA. Briefly, 100 μg of adult human kidney RNA was mixed with 75 fmol of ^{32}P-labelled primer in a solution of 50 mM Tris/HCl, pH 8.3, 50 mM KCl and 8 mM $MgCl_2$. After denaturation at 94 °C for 4 min, the primer was allowed to anneal with the RNA at 56 °C for 1 h. Primer extension was initiated by the addition of deoxynucleotide triphosphates and Moloney murine leukaemia virus reverse transcriptase, and the reaction mixture was incubated at 42 °C for 1 h. The primer-extended products were separated on a 5% acrylamide sequencing gel. A sequencing ladder obtained with the same primer on the appropriate gene template was run in adjacent lanes to determine the size of the extended product and to predict its sequence.

Expression of human GLUT5–reporter gene constructs in Caco-2 cells

Plasmid DNAs were prepared using standard methods and banded twice on CsCl/ethidium bromide density gradients before being used for transfection studies [22]. Caco-2 cells were seeded and cultured in 60 mm-diameter culture dishes. On day 4 of culture, a few hours before the cells had reached confluence and begun to differentiate, they were co-transfected with the human GLUT5 promoter–luciferase constructs described in Figure 1 and the control plasmid, pCH110, using the calcium phosphate precipitation method [22]. The plasmid pCH110 (Pharmacia, St. Quentin-en-Yvelines, France) contains a functional *Escherichia coli lacZ* gene which is expressed from the SV40 early promoter and was included to provide an internal marker for monitoring and normalizing expression levels obtained between different experiments. On day 6 (i.e. after the onset of the differentiation process and at a time when significant levels of GLUT5 mRNA are present in FK-treated cells; see Figure 5), half of the cells were subcultured in the presence of 50 μM FK and maintained in this medium until the end of the experiment at which time the cells were rinsed twice with cold PBS, frozen at −70 °C and then resuspended in a solution of 25 mM Tris/HCl, pH 7.8, 8 mM $MgCl_2$, 1 mM EDTA, 1% Triton X-100, 15% glycerol, 1 mM dithiothreitol and 0.4 mM phenylmethanesulphonyl fluoride. The homogenates were frozen at −70 °C and centrifuged at 13000 *g* for 15 min at 4 °C.

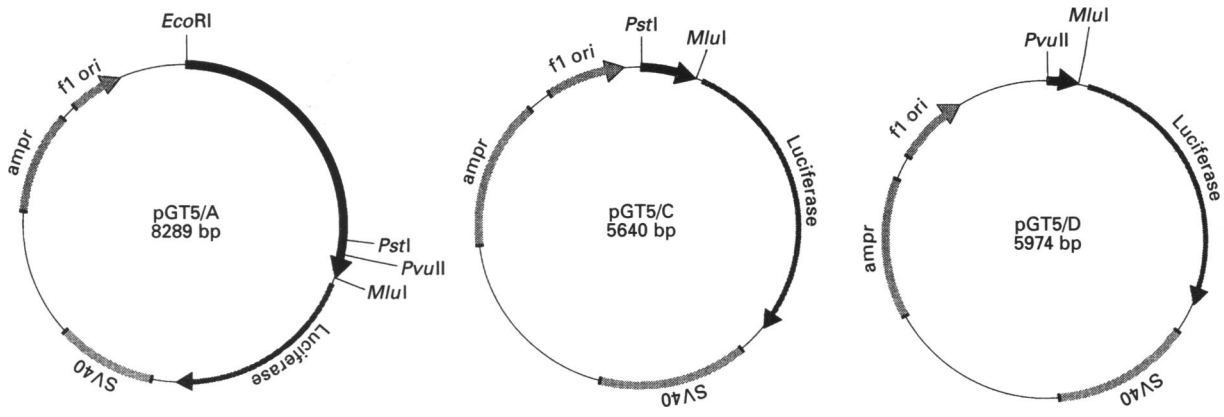

Figure 1 Human GLUT5 promoter–luciferase constructs

A 2.5 kb *Eco*RI–*Mlu*I fragment containing the promoter region of the human GLUT5 gene was cloned between the *Sac*I–*Mlu*I sites of the GeneLight Reporter Vector, pGL2-Basic (Promega, Lyon, France), to generate the construct pGT5/A (−2500 to +21) (the 5′ *Eco*RI site in this fragment is an artificial site caused by the ligation of an *Eco*RI linker to a *Hae*III–*Alu*I site). Promoter-deletion constructs were prepared by digesting pGT5/A with *Kpn*I and *Pst*I, and *Kpn*I and *Pvu*II to generate pGT5/C (−290 to +21) and pGT5/D (−186 to +21) respectively.

The activity of the luciferase reporter gene was assayed using the Luciferase Assay System (Promega) and Luminometer (Lumat LB 9501; Berthold, St Quentin-en-Yvelines, France). The linearity of the light response was determined using purified luciferase (Sigma) and all measurements were made in the linear portion of the curve. Addition of extracts prepared from control and FK-treated cells showed that the cell extracts had no effect on activity of purified luciferase. β-Galactosidase activity was measured in cell extracts by monitoring the hydrolysis of 4-methylumbelliferyl β-D-galactopyranoside and measuring the fluorescence at 445 nm [23,24]. The β-galactosidase activity was not significantly affected by treatment with FK as assessed by the ratio of β-galactosidase activity measured in cells transfected with the same amount of pCH110 (5 μg) DNA, in the presence or absence of FK [mean ratio: 0.93±0.16 ($n = 41$)]. Initial studies showed that there was a linear relationship between the amount of pGT5/A DNA added (5, 10, 15 or 20 μg) and luciferase activity. The transfection studies were carried out using 10 μg of each GLUT5 promoter–luciferase reporter construct and 5 μg of pCH110 DNA. Results are expressed as light units generated by luciferase per fluorescence units of β-galactosidase activity (means±S.E.M.).

Table 1 Increased expression of a functional protein GLUT5 in the brush-border membrane of FK-treated Caco-2 cells

Transport of 5 mM D-[U-^{14}C]fructose or L-[1-^{3}H]glucose by cell monolayers cultured for 15 days in the absence (control) or presence of either 50 μM FK or DDFK for the last 3 days of culture is reported. The values are 30 min uptakes at 37 °C and are expressed as nmol/mg of protein (means±S.E.M. of three experiments). Similar results (not shown) were obtained for 10 min uptake determinations.

	Uptake (nmol/mg)		
	Control	+FK	+DDFK
D-Fructose	58.3±7.3	95.1±7.4	52.9±3.8
L-Glucose	16.9±1.2	15.2±2.9	16.9±1.9

RESULTS

cAMP increases fructose transport and GLUT5 protein levels in confluent differentiated Caco-2 cells

Treatment of differentiated Caco-2 cells with 50 μM FK results in a permanent ~40-fold increase in cAMP above the basal levels [12,13]. With such treatment, differentiated Caco-2 cells exhibited a 2-fold increase in the net uptake of fructose (Table 1). This effect was specific to FK as DDFK, an analogue of FK that is unable to stimulate adenylate cyclase, had no effect on the capacity of cells to transport fructose. The uptake of the non-transported sugar L-glucose, which is a marker for non-specific glucose transport, was identical in control and treated cells.

Western-blot analysis of the GLUT5 content of brush-border-enriched fractions prepared from cells cultured in the presence of FK for up to 13 days showed a 5-fold increase in GLUT5 protein levels (Figure 2). GLUT5 migrated as a single band of 45–50 kDa in the control cells (no FK) and the amount of immunoreactive material increased with the duration of the exposure to FK. In cells expressing high levels of GLUT5, immunoreactive proteins of other sizes were also evident including a component of ~30 kDa. These proteins were not detected when the anti-GLUT5 serum was preincubated with the peptide against which it was raised (not shown).

cAMP increases GLUT5 mRNA levels

Treatment of differentiated cultures of Caco-2 cells with FK also caused an increase in GLUT5 mRNA levels that was evident as early as 8 h after addition of FK to the culture medium (Figure 3a). The multiple GLUT5 transcripts seen in the RNA blot of Caco-2 cells shown in Figure 3(a) are also seen in normal human tissues. The levels of GLUT5 mRNA were maximal after about 3 days of treatment at which time the levels were about 7-fold greater in treated than control cells. Transmission scanning of the corresponding Northern blots (not shown) indicate that in cells treated with FK for 24 h, the amount of the 5.1 kb transcript is not increased whereas the levels of the 2.8 and 2.0 kb transcripts exhibited a 2-fold increase. This effect was transient as the three transcripts were increased to the same extent after 3 days of

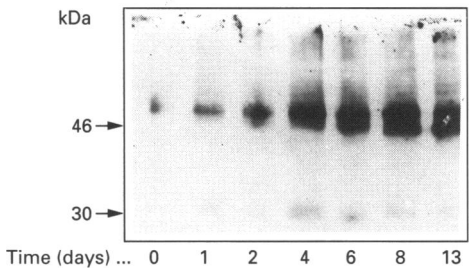

Figure 2 Western-blot analysis of GLUT5 in the brush-border membrane of FK-treated Caco-2 cells

Western-blot analysis of 10 μg of a brush-border-membrane-enriched fraction prepared from Caco-2 cells grown in the absence (0) or presence of 50 μM FK for 1, 2, 4, 6, 8 or 13 days was carried out. Proteins were electrophoresed on an SDS/10% polyacrylamide gel as described in the Materials and methods section. Note the appearance of a second band at 30 kDa in long-term treated cells.

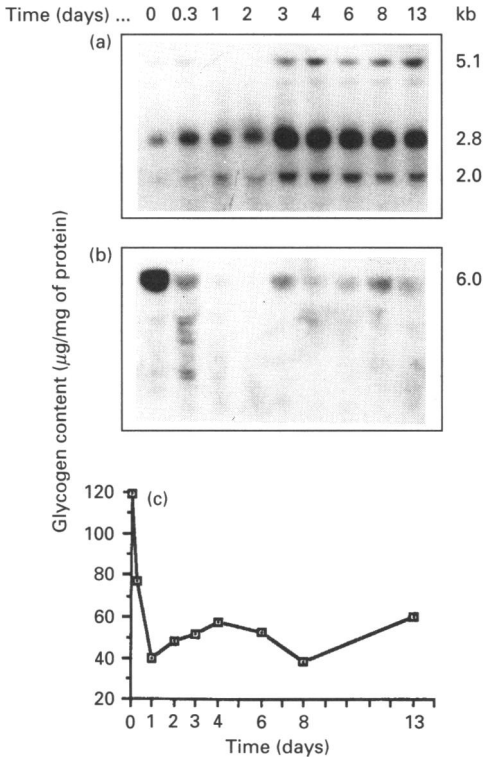

Figure 3 Effect of FK treatment on GLUT5 and sucrase–isomaltase mRNA levels and glycogen content in Caco-2 cells

Cells were cultured for 15 days in the absence or presence of 50 μM FK. Northern-blot analysis was carried out using 25 μg of total RNA extracted from control cells (0) or cells treated with FK for 8 h (0.3) or 1, 2, 3, 4, 6, 8 or 13 days. The blot was hybridized with the cDNA probes for GLUT5 (**a**) and sucrase–isomaltase (**b**). Glycogen content (**c**) was assayed in cells from the same culture. Note the difference in response to FK of the mRNA of sucrase–isomaltase and the glycogen content of the cells.

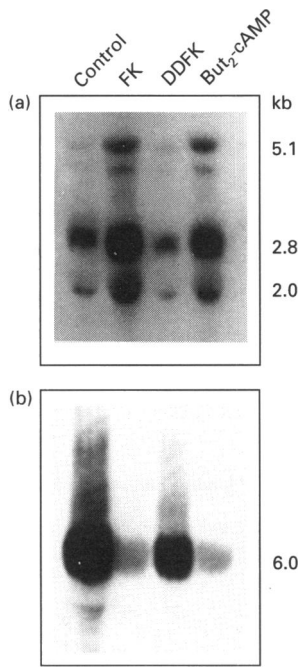

Figure 4 Effect of FK, DDFK and But_2-cAMP on GLUT5 and sucrase–isomaltase mRNA levels

Total RNA extracts (25 μg) from cells grown for 15 days were analysed. Cells were either cultured for the last 4 days in the absence of any drug (control) or in the presence of 50 μM FK, 50 μM DDFK or 1 mM But_2-cAMP. The Northern blot was hybridized with the GLUT5 (**a**) or sucrase–isomaltase (**b**) cDNA probe.

treatment with FK. In contrast with the increase in GLUT5 mRNA levels, the levels of sucrase–isomaltase mRNA decreased in the same period and after 8 h were about 10% of the levels seen in control cells (Figure 3b). Forskolin treatment also resulted in an approx. 65% reduction in glycogen content of Caco-2 cells (Figure 3c). To determine if the increased GLUT5 mRNA levels were indeed a consequence of increased cAMP levels, we compared the effects of FK with those of the non-metabolizable analogue of cAMP, But_2-cAMP, and of DDFK. As shown in Figure 4, But_2-cAMP treatment increased GLUT5 mRNA levels and decreased sucrase–isomaltase mRNA levels, a result consistent with a direct effect of cAMP on the regulation of GLUT5 expression. DDFK treatment did not induce an increase in GLUT5 mRNA. The effect of FK on the expression of GLUT5 mRNA was reversible: removal of FK, which results in a decrease in cAMP to the basal value within 48 h [13], resulted in a slow return (more than 3 days) of GLUT5 mRNA levels to basal values (not shown).

We also examined the effect of FK treatment on growing preconfluent Caco-2 cells, i.e. before the onset of their differentiation, and in postconfluent differentiating cells. The results presented in Figure 5 show that FK treatment can induce an increase in GLUT5 mRNA levels only in postconfluent cells. In preconfluent cells the level of GLUT5 mRNA is undetectable by RNA blotting using either total or polyadenylated mRNA (8 μg, not shown). At day 7, i.e. in early postconfluent cells, GLUT5 mRNA is detectable in the FK-treated cells, indicating that the gene is indeed responsive to cAMP.

Protein synthesis is necessary for increased GLUT5 mRNA levels

In order to determine if protein synthesis was required for the induction of GLUT5 mRNA by FK, cells were treated with 10 μM CHX $\pm$ 50 μM FK for 3 days. GLUT5 mRNA was undetectable in cells grown in the presence of CHX alone (Figure 6) and the stimulation of GLUT5 mRNA levels in cells treated

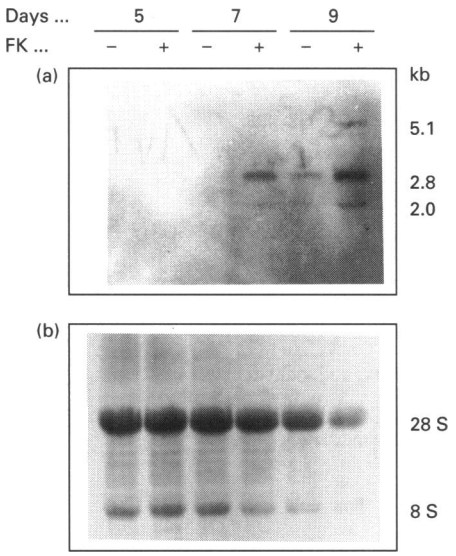

Figure 5 FK stimulation of GLUT5 mRNA levels in early postconfluent cells

Northern-blot analysis of 30 μg of total RNA (**a**) extracted from cells cultured in the absence (−) or presence of 50 μM FK (+) during the last 3 days of each culture: day 2–5 (5), day 4–7 (7), and day 6–9 (9). Note the difference between the amount of RNA loaded per lane, as estimated by staining of the blot with Methylene Blue (**b**), and the increase in the specific signal for GLUT5 mRNA, indicating an underestimation of the increase on day 9 (9+).

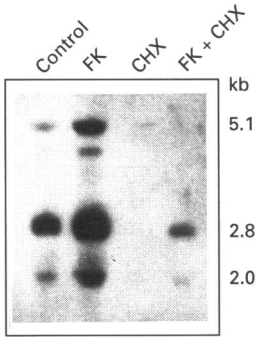

Figure 6 Necessity for protein synthesis for FK stimulation of GLUT5 mRNA levels

Total RNA was extracted on day 15 of culture. Cells were cultured on day 12 in the control medium or in 50 μM FK, 10 μM CHX or both drugs together (FK + CHX). The Northern blot of 25 μg of total RNA was hybridized with a human GLUT5 cDNA probe.

with both FK and CHX was significantly less than that noted in cells treated with FK alone (Figure 6), implying that protein synthesis is necessary to observe the full stimulatory effect of FK on GLUT5 mRNA levels.

Forskolin alters GLUT5 mRNA stability

The increase in GLUT5 mRNA levels in FK-treated cells could be due to increased stability of GLUT5 mRNA, increased transcription of the GLUT5 gene or a combination of both mechanisms. The effects of FK treatment on GLUT5 mRNA stability was assessed by culturing control and FK-treated cells in the presence of the transcription blocker DRB for 4 days. The $t_{1/2}$ of GLUT5 mRNA was 60 h in FK-treated cells compared

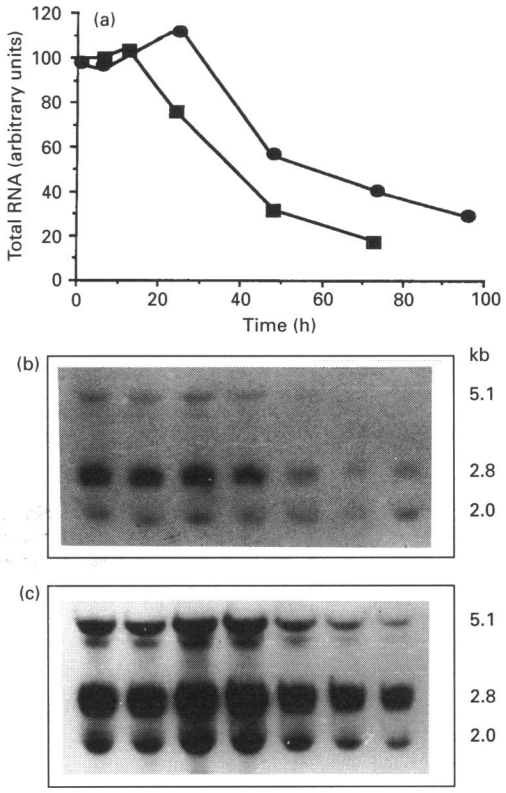

Figure 7 Effect of FK on GLUT5 mRNA stability

(**a**) Total RNA was isolated from control (■) and FK-treated (●) Caco-2 cells at the indicated times after the addition of 0.1 mM DRB. Total RNA (25 μg) was blotted and hybridized with the GLUT5 cDNA probe. The results were quantified using transmission scanning of the X-Ray film (two separate experiments) and are presented as percentage of control GLUT5 density surface area in control (**b**) or FK-treated (**c**) cells for 4 days.

with 40 h in untreated cells (Figure 7a); the levels of the different GLUT5 transcripts (5.1, 2.8, 2.0 kb) appeared to decrease at similar rates. Thus stabilization may account, at least in part, for the difference in GLUT5 mRNA levels noted between control and FK-treated cells.

Isolation and sequence of the promoter region of the human GLUT5 gene

In order to determine if cAMP increases transcription of the human GLUT5 gene, we first isolated the gene and partially sequenced the promoter region (Figure 8a). Primer extension studies localized the 5′ end of the mRNA to a site 96 nucleotides upstream from the start of translation (Figure 8b). Analysis of the sequence of the promoter region of the human GLUT5 gene indicated that there was an *Alu*-dispersed middle repetitive sequence element beginning at nucleotide −441. The functional significance of a repetitive sequence in this region of the gene is uncertain but it may indicate that the sequence elements that are required for appropriate tissue-specific and regulated expression of this gene are located in the region between nucleotide −440 and the transcriptional start site at nucleotide 1. The promoter of the GLUT5 gene contains a classical TATA motif as well as a CAAT box and two potential cAMP-response elements (CRE), a motif found in many genes, the expression of which is regulated by cAMP [25].

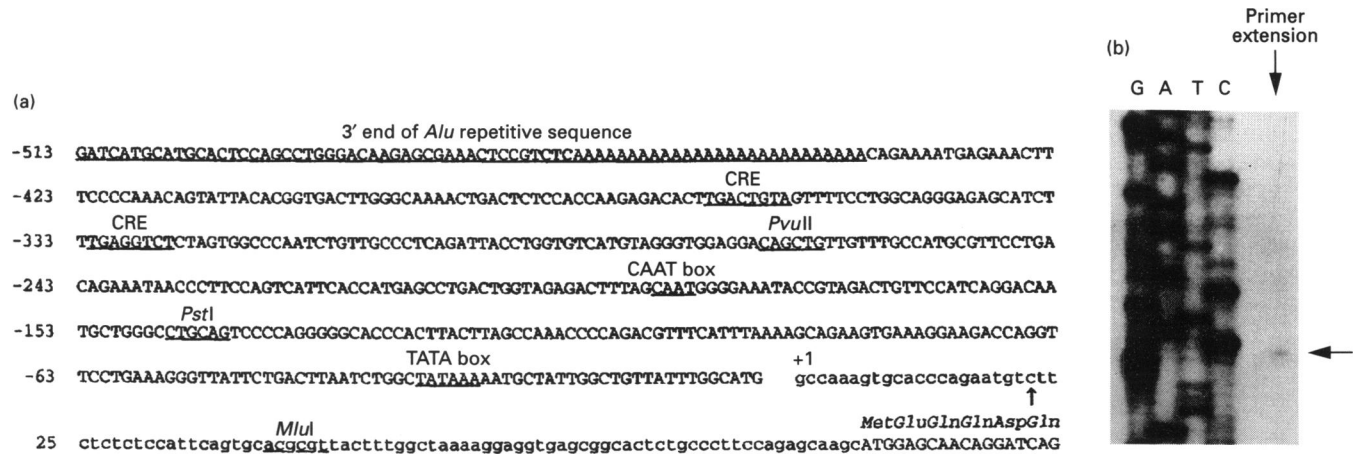

Figure 8 Sequence of the promoter region of the human GLUT5 gene

(**a**) The sequence of the promoter is indicated. Putative regulatory elements, restriction sites used to prepare the constructs described in this report, and the starts of transcription and translation are noted. The arrow shows the 5′ end of the human GLUT5 cDNA sequence reported in Kayano et al. [7]. (**b**) Mapping the transcriptional start site of the human GLUT5 gene. The results of the primer extension carried out as described in the Materials and methods section using human kidney mRNA are presented. A sequence ladder obtained using the same primer and the cloned fragment of the gene as a template are shown.

Table 2 Expression of the human GLUT5 and SV40 promoter–luciferase reporter gene constructs in differentiated Caco-2, HepG2, CHO and COS cells: effect of FK

Transfected cells were cultured in the presence or absence of FK for 24 h as described in the legend of Figure 2. The cells were transfected with the pGL2-basic, pGL2-SV40 promoter and pGL2-pGT5/A constructs. Results are expressed as the ratio of light units generated by luciferase per unit of β-galactosidase activity (means ± S.D. for the number of experiments given in parentheses). Results obtained with deletion constructs pGL2-pGT5/D and pGL2-pGT5/C were identical with that obtained with the pGL2-pGT5/2 construct (not shown). The relative increases in luciferase activity in FK-treated compared with control cells are shown in the column headed FK/C.

Construct	Cell line	Control	FK	FK/C
Basic	Caco-2	5 ± 1 (9)	12 ± 3 (9)	2.3
	HepG2	14 (1)	22 (1)	1.6
	COS	81 ± 23 (2)	61 ± 14 (3)	0.7
	CHO	17 ± 3 (4)	15 ± 3 (4)	0.9
GT5/A	Caco-2	34 ± 2 (6)	397 ± 25 (4)	11.7
	HepG2	123 ± 34 (3)	107 ± 37 (3)	0.9
	COS	22 ± 4 (7)	18 ± 3 (8)	0.8
	CHO	44 ± 9 (11)	52 ± 9 (11)	1.2
SV40	Caco-2	176 ± 17 (6)	776 ± 198 (6)	4.4
	HepG2	2509 (1)	2934 (1)	1.2
	COS	16201 ± 3197 (2)	16481 ± 240 (2)	1.0
	CHO	632 ± 183 (6)	551 ± 167 (6)	0.9

Table 3 Expression of human GLUT5 promoter–luciferase reporter gene construct in Caco-2 cells and stimulation by FK

Transfection of the cells by the calcium phosphate precipitation method was carried out on day 4 of culture. Cells were transfected with 5 μg of pCH110 and 10 μg of the pGL2 promoter–luciferase gene constructs (see the Materials and methods section) and cultured from day 5 to day 6 in the absence (control) or presence of 50 μM FK or DDFK. Results are expressed as the ratio of light units generated by luciferase per unit of β-galactosidase activity (means ± S.E.M. for the number of experiments shown in parentheses). The constructs are: pGL2-basic (Basic), pGT5/A (5/A, *Eco*RI–*Mlu*I, −2500 to +21), pGT5/C (5/C, *Pst*I–*Mlu*I, −290 to +21), pGT5/D (5/D, *Pvu*II–*Mlu*I, −186 to +21). This transfection assay was repeated at least four times with similar results.

	Basic	5/A	5/C	5/D
Control	5 ± 1 (9)	34 ± 2 (6)	74 ± 20 (2)	36 ± 10 (2)
+ FK	12 ± 3 (9)	397 ± 25 (4)	238 ± 75 (2)	208 ± 54 (2)
+ DDFK	5 ± 0 (4)	38 ± 5 (4)	73 ± 6 (2)	40 ± 13 (2)

Activity of human GLUT5 promoter–reporter gene constructs in Caco-2 or HepG2, COS and CHO cells

Preconfluent undifferentiated Caco-2 cells were transfected with GLUT5 promoter–reporter gene constructs. Promoter activity was measured in postconfluent cells, after the onset of differentiation. The GLUT5 promoter construct pGT5/A which includes 2.5 kb of 5′-flanking region showed a 7-fold increase in luciferase activity compared with the promoterless vector pGL2-basic; the SV40 promoter-based construct, pGL2-pro, gave a 35-fold increase in activity (Table 2). The GLUT5 promoter constructs pGT5/C and pGT5/D which have 270 and 145 bp of 5′-flanking region respectively showed similar relative promoter activities in this assay implying that the *cis*-acting elements necessary for the expression of this gene in Caco-2 cells are located in a 145 bp region upstream of the transcriptional start site. This region includes the TATA motif but neither of the putative CREs are located in this region. The promoter activity of the pGT5/A construct was also tested in HepG2, COS and CHO cells, cell lines not known to express GLUT5. This construct showed a 9, 0.3 and 2.6 increase in luciferase activity in transfected HepG2, COS and CHO cells respectively; the pGL2-pro SV40 promoter construct gave a 19 to 200-fold increase in luciferase activity (Table 2). The reason for the relatively efficient expression of the GLUT5 promoter in HepG2 cells is unknown.

FK treatment of postconfluent differentiating Caco-2 cells transfected with pGT5/A showed a 7.7 ± 2.4-fold ($n = 5$) increase in luciferase activity compared with control cells (Table 3). This increase in luciferase activity was not seen in DDFK-treated cells. The FK stimulation of luciferase activity was also seen, although to a smaller extent, in cells transfected with pGT5/C [4.2 ± 1.4 ($n = 6$)] or pGT5/D [4.1 ± 0.6 ($n = 6$)]. These results suggest that *cis*-acting sequences involved in cAMP regulation of

the transcription of GLUT5 are located between nucleotides −513 and −145 although additional sequences may also be required for the full response because there was a greater response noted with pGT5/A than with either pGT5/C or pGT5/D. By contrast, FK had no effect on promoter activity in HepG2, COS or CHO cells transfected with pGT5/A, suggesting that tissue-specific elements are required to obtain the full activating effect observed in Caco-2 cells.

DISCUSSION

Fructose is the predominant monosaccharide in many fruits, and high-fructose corn sweeteners are used in the preparation of a large number of food products. Because of the introduction of high-fructose corn sweeteners, the consumption of fructose has been increasing and now accounts for about 8 % of total caloric intake per day [26]. The long-term consequences of the recent increase in fructose consumption are uncertain but there is evidence suggesting that this increased consumption may be associated with an increase in plasma triacylglycerol levels as well as contributing to insulin resistance [27,28]. Thus an understanding of the regulation of fructose transport across the plasma membrane may provide a basis for studying the effects of fructose on other metabolic processes. The transport of dietary fructose from the lumen of the small intestine is carried out by the facilitative sugar-transporter GLUT5 [2]. This protein is also responsible for fructose transport in the kidney, brain and insulin-responsive tissues such as muscle and adipose tissue [29]. However, the uptake of fructose by the liver, the main site of fructose metabolism, is probably carried out by GLUT2, a low-affinity high-capacity glucose transporter that is also able to transport fructose.

Differentiated Caco-2 cells express GLUT5 [8,9] and can be used to study the regulation of GLUT5 expression. The studies described in this report demonstrate that cAMP increases GLUT5 expression in these cells. This was an unexpected result as cAMP decreases expression of sucrase–isomaltase [12,13], the enzyme responsible for the hydrolysis of sucrose to fructose and glucose. Thus cAMP regulates the activities of these two key proteins of fructose uptake in different directions. *In vivo*, fructose feeding leads to an increase in intestinal fructose-transport activity, the molecular basis of which is unknown [30]. However, if fructose stimulates intestinal GLUT5 expression *in vivo*, it would regulate its own expression. It may be possible to test this hypothesis directly by studying the effects on GLUT5 expression of culturing Caco-2 in increasing concentrations of fructose.

Our studies suggest that the *cis*-acting sequences necessary for expression of GLUT5 in Caco-2 cells, and presumably enterocytes as well, are located in close proximity to the start of transcription. GLUT5 is also responsible for fructose transport in kidney, brain, muscle and adipose tissue. It is important to determine if the sequences responsible for tissue-specific expression of GLUT5 in these tissues are also located in close proximity to the transcriptional start site or elsewhere, and if GLUT5 expression in these tissues is regulated by cAMP.

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