Biochimica et Biophysica Acta, 772 (1984) 259–263
Elsevier

BBA 72083

EFFECTS OF KETOHEXOSEMIA ON THE KETOHEXOSE TRANSPORT IN THE SMALL INTESTINE OF RATS

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(Received December 5th, 1983)

Key words: Hyperglycemia; Hyperketosemia; Ketohexose; Carbohydrate transport; Phlorizin; Phloretin; Na^+ dependence; (Rat intestine)

It was observed previously (Csáky, T.Z. and Fischer, E. (1981) Diabetes 30, 568–574), that sustained hyperglycemia enhances the intestinal transport of aldohexoses; on the other hand, hyperfructosemia affects primarily the transport of fructose. The present study examines in detail the hyperketosemia-induced intestinal ketose transport. Intravenously infused 3-*O*-methylfructose produces marked 3-*O*-methylfructosemia without concomitant hyperglycemia; in such animals the intestinal transport of both fructose and 3-*O*-methylfructose increased. The hyperketosemia-induced increased ketose transport was inhibited by phloretin but only if placed on the serosal compartment. Phlorizin affects neither the basal nor the induced intestinal ketohexose transport. The enhancement of the intestinal ketohexose transport is not sodium-dependent and is not inhibited by ouabain.

Introduction

It has been repeatedly observed that the rate of glucose transport increases in the isolated small intestine of rats rendered diabetic by the administration of alloxan or streptozocin (for references see Ref. 2). It was also shown that one symptom of diabetes, hyperglycemia, alone is sufficient to produce the enhanced intestinal glucose transport [1,2]. In connection with the latter observation, it was apparent that: (a) hyperglycemia has to be sustained for at least 4 h to achieve the increased sugar transport in the gut; (b) the sugar transport enhancement was not due to an increased mucosal metabolism of sugar as the transport of 3-*O*-methylglucose, a non-metabolized analog, was also enhenced; (c) the enhanced glucose transport was inhibited by phloretin but not by phlorizin; (d) there was a degree in specificity in the induction, namely the aldohexoses (glucose, galactose, 3-*O*-methylglucose) induce each others transport, while the transport of fructose was not influenced by high levels of glucose, galactose, or 3-*O*-methylglucose in the blood but was increased by hyperfructosemia sustained for several hours; (e) the transport enhancing effect of high blood sugar was eliminated in animals treated with a protein-synthesis inhibitor, cycloheximide. Based on these observations, it was hypothesized that sustained high blood sugar induces the synthesis of new sugar carriers which are probably localized in the basolateral membrane [2].

The intestinal glucose transport system is not an ideal model for the examination of the question whether a given treatment, such as sustained high concentration of sugar in the circulating blood, effects the carriers. Glucose and some other aldoses, are transported across the intestinal epithelium by carriers, one of which is definitely

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energized and involved in sodium-dependent energy-required uphill transport. This renders the exploration of the effect of high blood sugar upon the carrier proper complicated. On the other hand, the intestinal transport of fructose appears to be mediated by an uncomplicated equilibrating carrier [3–5] and as such would lend itself more favorably to the study of the effect of high blood sugar upon the hypothetical induction of new intestinal sugar-carriers sites. Because of this, it was worth examining whether the simple equilibrating intestinal ketose carrier could serve as a model for the quantitative study of the induction of high blood sugar upon the carrier synthesis.

The experiments to be reported here were carried out with two main objectives in mind: (a) to solve the difficulty caused by the rapid bodily conversion of fructose to glucose which makes it impossible to provide pure hyperfructosemia by intravenous infusion of the ketohexose, and (b) to examine whether the basic and induced ketose carrier is sodium-dependent, viz. mediated by active transport or facilitated diffusion.

Materials and Methods

Preparation of 3-O-methylfructose. This preparation was carried out according to the procedure previously described [6,7] with modifications.

α-(1 : 2-4 : 5)-Diisopropylidene fructose. Fructose (1000 g) was stirred at room temperature in 7 liters of dry acetone and 1400 g anhydrous zinc chloride until a filtered sample did not reduce Fehling's solution (about 50 h). To the cooled mixture sodium hydroxide (650 g in 650 ml water) was added drop-wise. The inorganic precipitate was filtered and was washed with acetone. The filtrate and washings were combined and evaporated in vacuum. One liter water was added to the residue which was then extracted four times with each 400 ml of chloroform. The chloroform solution was washed with water, dried with Na_2SO_4 and concentrated. The crude crystals were recrystallized from hexane, m.p. 120°C.

3-Methyl-α-diisopropylidene fructose. 52 g of α-diisopropylidene fructose were dissolved in 100 ml dry acetone, 21.4 g powderized NaOH was added. 28.4 ml methylsulfate was then added drop-wise with stirring at 40–50°C in 75 min. The mixture was stirred at 50°C for 90 min, then at 55–60°C for 3 h. It was diluted with water and extracted with chloroform (3 × 100 ml). The chloroform extracts were washed with water, dried with Na_2SO_4 and concentrated in vacuum. The residue was recrystallized from hexane, m.p. 112°C.

3-O-Methylfructose. 27.4 g 3-methyl-diisopropylidene fructose were stirred for 6 h in 100 ml water with 5 g Amberlite IR 120 (washed with 10% HCl and rinsed with distilled water). The mixture was filtered, treated with 4 g of charcoal and concentrated. The residue crystallized when rubbed with acetone. Recrystallized from methanol, m.p. 125°C.

On paper chromatography, using *n*-butanol/acetic acid/water (4 : 1 : 5, v/v) one reducing spot was observed, the location of which was identical with that of reference 3-*O*-methylfructose.

D-Fructose, phlorizin, phloretin and ouabain were commercial preparations.

Analytical procedures. Fructose and 3-*O*-methylfructose were determined colorimetrically with phloroglucinol-HCl at 80°C [8]. Serum glucose was determined by the glucose oxidase method [9].

Animal experiments. Male Sprague-Dawley rats weighing 200–250 g were used. The animals were routinely starved before the experiment for 18 h with free access to drinking water. The intravascular sugar infusions were carried out for the minimum of 5 h essentially with the methods of Terkel [10] with slight modifications as described previously [2]. A 30% solution of the sugar was routinely infused at the rate of 2 ml/h. The in vitro flux studies were preformed in everted loops of the small intestine as described previously [11]. The fluxes were expressed as μmol of substrate appearing in the trans-compartment/g dry gut per h. *n* in the tables denotes the number of intestinal loops used in the particular experiment.

Results

1. Effect of intravascular 3-O-methylfructose infusion upon the plasma concentration of glucose and 3-O-methylfructose and intestinal transport of fructose and 3-O-methylfructose

30%(w/v) 3-*O*-methylfructose solution was infused intravascularly. After 6 h arterial blood was withdrawn into a heparinized syringe and centri-

TABLE I

EFFECT OF 3-*O*-METHYLFRUCTOSE INFUSION (30%, 2.0 ml/h) FOR 6 h UPON THE PLASMA SUGAR LEVEL AND THE MUCOSAL-TO-SEROSAL FLUX OF FRUCTOSE AND 3-*O*-METHYLFRUCTOSE (μmol/g dry gut per h)

Plasma glucose (mg%)	98.8 ± 2.9	(n = 6)
Plasma 3-*O*-methylfructose (mg%)	410.0 ±70.8	(n = 6)
Fructose flux		
Control	8.39± 0.35	(n = 47)
Infused	20.98± 1.10	(n = 4)
3-*O*-Methylfructose flux		
Control	9.45± 0.59	(n = 12)
Infused	27.27± 5.12	(n = 4)

fuged. Glucose and 3-*O*-methylfructose were determined in the plasma. Table I shows that the infustion of 3-methyl ether considerably increased the level of the ketohexose without changing the level of the glucose in the blood plasma. The data presented in Table I also indicate that the isolated everted small intestine of rats in which hyper-3-*O*-methylfructosemia was maintained the mucosal-to-serosal flux of both fructose and 3-*O*-methylfructose significantly increased.

2. Is the enhanced ketose transport sodium-dependent?

Animals were infused with fructose for 5 h. The mucosal-to-serosal flux of fructose was measured in Na_2SO_4-Ringer and in Li_2SO_4-Ringer, placed either on the mucosal or on the serosal side. In the Li_2SO_4-Ringer, all sodium was substituted (for the composition of Li_2SO_4-Ringer see Ref. 12). The data presented in Table II indicate that replacement of sodium in the Ringer bathing either side of gut did not alter either the basal or enhanced fructose transport. This indicates that an active process is not likely to be involved in either transport.

TABLE II

FRUCTOSE INFUSION, EFFECT OF LACK OF SODIUM ON THE MUCOSAL-TO-SEROSAL FLUX OF FRUCTOSE (μmol/g dry gut)

Na_2SO_4-Ringer		
Control	8.39±0.35	n = 47
Infused	20.24±1.02	n = 26
Li_2SO_4-Ringer, mucosal side		
Control	9.03±0.78	n = 10
Infused	18.27±2.08	n = 6
Li_2SO_4-Ringer, serosal side		
Control	6.84±0.67	n = 8
Infused	16.91±1.38	n = 11

TABLE III

FRUCTOSE INFUSION, EFFECT OF 10^{-4} M OUABAIN (SEROSAL SIDE) ON THE MUCOSAL-TO-SEROSAL FLUX OF FRUCTOSE (μmol/g dry gut)

Control	8.39±0.35	n = 47
Infused	20.24±1.02	n = 26
Control + ouabain	7.28±0.40	n = 18
Infused + ouabain	16.54±1.20	n = 13

This assumption was further corroborated by the finding (presented in Table III), that ouabain in high concentration did not drastically reduce either the base or induced fructose transport (for the effect of ouabain see also Discussion).

3. Effect of phlorizin and phloretin upon the intestinal fluxes of fructose and 3-O-methylfructose

The animals were infused with fructose. Data on Table IV indicate that the mucosal-to-serosal flux of 3-*O*-methylfructose was enhanced in the gut of the infused animal. Phlorizin, placed in both mucosal and serosal compartments, did not inhibit either the basal or enhanced transport. Phloretin, placed on both sides of the gut, did not influence the basal transport of the fructose methyl ether but depressed the enhanced transport in the gut of the fructose-infused animal.

TABLE IV

FRUCTOSE INFUSION, EFFECT OF PHLORIZIN AND PHLORETIN ON THE MUCOSAL-TO-SEROSAL FLUX OF 3-*O*-METHYLFRUCTOSE (μmol/g dry gut)

Control	9.45±0.59	n = 10
Infused	17.40±0.99	n = 8
Control + 10^{-3} M phlorizin	10.93±0.71	n = 10
Infused + 10^{-3} M phlorizin	19.47±1.1	n = 6
Control ± (10^{-4} M) phloretin	10.69±0.70	n = 14
Infused + (10^{-4} M) phloretin	12.68±0.78	n = 14

TABLE V

FRUCTOSE INFUSION, EFFECT OF PHLORETIN PLACED EITHER ON THE MUCOSAL OR SEROSAL SIDE ON THE MUCOSAL-TO-SEROSAL FLUX OF FRUCTOSE (μmol/g dry gut)

Control	8.39 ± 0.35	$n = 47$
Infused	20.24 ± 1.02	$n = 26$
Phloretin on the mucosal side (10^{-4} M)		
Control + phloretin	6.56 ± 0.70	$n = 3$
Infused + phloretin	19.77 ± 2.17	$n = 6$
Phloretin on the serosal side (10^{-4} M)		
Control + phloretin	6.79 ± 0.73	$n = 4$
Infused + phloretin	9.43 ± 0.89	$n = 7$

Phloretin affected the transport of fructose similarly as shown in Table V. However, the aglucone inhibited the enhanced ketose transport only if placed in the serosal but not if in the mucosal compartment.

Discussion

One of the objectives of the present study was to explore the problem of producing hyperketosemia without concomitant hyperglycemia. This was achieved by substituting 3-*O*-methylfructose for fructose.

The stable methyl ethers of glucose were first employed in the study of biological transport some 40 years ago [13], and served since then as rather valuable research tools. 3-*O*-Methylglucose was found to behave like glucose in various transport systems; moreover, it is not metabolized in the animal body [14,15]. By analogy it was assumed that the 3-methyl ether of fructose will also be metabolically inert. The experiments presented in this paper seem to justify this assumption. Fructose is rapidly converted in the body to glucose, consequently when infused intravascularly it produces both hyperfructosemia and hyperglycemia. With 3-*O*-methylfructose the situation was quite different; when this ketose ether was infused intravascularly, high level of hyperketosemia was achieved without any change in the concentration of glucose in the blood indicating that 3-*O*-methylfructose was not converted into glucose in the body. On the other hand, sustained hyperfructosemia enhanced the intestinal transport of both fructose and 3-*O*-methylfructose; sustained 3-*O*-methylfructosemia produced the same effects, thus, as far as the effect of high blood sugar levels upon intestinal sugar transport is concerned, fructose and its 3-methyl ether behaved the same way. Consequently in the present study fructose and its 3-methyl ether could be used interchangeable with confidence.

The exact mechanism by which experimental diabetes or sustained hyperglycemia enhances the intestinal sugar transport is awaiting definite clarification. Based on our finding that the protein synthesis inhibitor, cyclohexamide eliminated the hyperglycemia-induced enhanced intestinal hexose transport it was hypothesised that high blood sugar induces the sugar carrier synthesis in the intestinal mucosa [1]. However, other theories were also offered [19]; one of them assumes an increased lumen-to-epithelium sodium gradient [20]. This theory would require that the enhanced intestinal sugar transport be sodium dependent. Indeed we could not demonstrate the hyperglycemia-induced enhanced hexose transport in isolated intestine bathed in a sodium-free medium [2]. On the other hand, the data presented in this paper clearly indicate that, in the case of ketohexoses, sustained high sugar concentration in the blood may cause a sodium-independent enhanced sugar transport in the isolated intestine. The difference between aldoses and ketoses in this respect may suggest an enhanced uphill transport of the aldoses or may imply that the aldose carrier per se is sodium-dependent while the ketose carrier is not. At this time no clear-cut answer is available to these questions. The present study indicates that, in the case of the ketoses, one deals with a simple equilibrating transport system unlike the one responsible for the transport of aldoses.

Regarding the use of ouabain in these experiments a word of caution is justified. It is known that the $(Na^+ + K^+)$-ATPase in the intestine of the rat is relatively insensitive to the action of ouabain [15]. Nonetheless, the lack of sensitivity is relative and very large concentrations of the cardioglycoside, such as used in this experiment, would be expected to produce some inhibition.

The present observation corroborates a previous one that phlorizin has no effect on the intestinal transport of ketoses [17]. This is another indi-

cation that the intestinal ketose carrier is different from the aldose carrier which is known to be inhibited by phlorizin. On the other hand, there is some similarity between the two sugar carriers witnessed by the previous finding in this laboratory, that the intestinal absorption of D-xylose, which utilizes the aldose carrier, is inhibited in the presence of an excess amount of fructose [18].

The aglucone of phlorizin, phloretin does not seem to inhibit the base transport of ketoxoses, however, it markedly diminishes the hyperfructosemia-induced enhanced ketose transport. The inhibition occurs only if the aglucone is present on the serosal but not on the mucosal side. This strongly suggests that the inhibition occurs on the basolateral membrane. The lack of inhibition of the basal, but not the induced, fructose transport by phloretin is a puzzling observation. No clear explanation can be offered at this time for the phenomenon. The assumption that the hyperfructosemia-induced transport is different from the one involved in the noninduced basal transport is an attractive one but should be considered, at this time, mere speculation.

The observations presented in this paper indicate that the system transferring ketoses in the intestine presents an adequate model for quantitative study of the induction of sugar transport system by sustained high sugar levels in the blood.

Acknowledgments

This work was supported by grant No. AM 27041 from the U.S. Public Health Service and by a grant from the Kroc Foundation.

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