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THE EFFECT OF HEXOSAMINES ON THE ACTIVE TRANSPORT OF SUGARS IN THE HAMSTER

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SUMMARY

D-Glucosamine, D-mannosamine and D-galactosamine are not actively accumulated by hamster intestinal rings. The aminosugars are weak, non-competitive inhibitors of D-galactose accumulation, as are other substances containing an amino group. The inhibition is probably not caused by specific interaction with the carrier protein, osmotic or metabolic effects, and may be due to non-specific alteration of membrane properties.

INTRODUCTION

A recent model[1,2] for active intestinal transport of sugars in the hamster intestine proposes a covalent bond between the carrier protein and C-2 of the sugar. A sugar capable of forming an amide bond at this position might lead to a stable inactive carrier–sugar complex, since an amide bond is more stable than an ester.

It has been reported that D-glucosamine is not actively transported into mammalian intestine[3], but that it is an inhibitor[4,5]. It has also been reported that D-galactosamine is not an inhibitor[5]. The work described in this paper investigates the extent and nature of the inhibition of D-galactose accumulation into hamster intestinal rings in the presence of hexosamines.

METHODS AND MATERIALS

Tissue/medium ratios

Tissue accumulation experiments were performed as previously described[2] using everted hamster intestinal rings. The rings 2–3 mm wide were cut from the everted small intestine of two hamsters and added to the incubation flasks so that each flask contained rings from each part of the intestine. Flasks containing rings (2–300 mg wet wt.) were preincubated in Krebs–Henseleit bicarbonate buffer (pH 7.4)[6] gassed with O_2–CO_2 (95:5, v/v) for 15 min. For the determination of tissue/medium ratios the tissue was incubated with the sugar (1 mM) for 30 min, the incubation stopped by decanting off the incubation medium (final vol. 6 ml) and washing the tissue twice in ice-cold glucose-free buffer. The tissue samples were blotted to remove excess moisture and weighed. After homogenization in water (4 ml) the protein was precipitated[7].

Aminosugars were then assayed in both the tissue extract and medium using the method of ELSON AND MORGAN[8]. Alternatively, radioactive substrates were used and the tissue homogenized in 2 ml of water, centrifuged and a portion counted. The tissue/medium concentration ratio was determined assuming that 80 % of the tissue weight was water, as determined in a separate experiment and that all the radioactivity was in the original sugar.

Inhibition of D-*galactose accumulation*

D-[^{3}H]-Galactose accumulation was measured over the concentration range 1–5 mM. Incubation was terminated after 10 min and the tissue concentration was measured. D-Fructose (1 mM) was added to the incubation medium as an energy source. In its absence variable results were obtained when D-glucosamine was used as an inhibitor at the higher concentrations of D-galactose. The inhibitor (20 mM) adjusted to pH 7.4 with HCl and in buffer was preincubated with the tissue for times between 0 and 60 min, generally 30 min. Addition of the inhibitor increased the osmolarity of the solution and the D-galactose accumulation was therefore compared with its accumulation in the presence of the equivalent amount of D-mannitol (40 mM). The presence of 40 mM D-mannitol did not alter the rate of D-galactose accumulation significantly.

Chromatography of tissue homogenates

Samples of tissue homogenates after incubation with [U-^{14}C]glucosamine or [U-^{14}C]galactosamine were deproteinized by addition of ethanol to give a final concentration of 80 %. After centrifugation the supernatant was applied to Whatman No. 1 chromatography paper using acetone–acetic acid–water (18:1:5, by vol.) as solvent. Radioactivity was detected using a Baird and Tatlock chromatogram scanner. Samples of the medium were treated in the same way.

Materials

[U-^{14}C]Glucosamine, [U-^{14}C]galactosamine and [1-^{3}H]galactose were obtained from the Radiochemical Centre, Amersham, Bucks, Great Britain. D-[1-^{3}H]Mannitol was made by $NaBH_4$ reduction of D-mannose.

RESULTS

Tissue/medium ratios

Tissue/medium ratios and recoveries for D-glucosamine, D-galactosamine and D-mannosamine, measured by the method of ELSON and MORGAN[8] are shown in Table I. None of the sugars was actively accumulated, but the entry of both D-glucosamine and D-galactosamine was greater than that of D-mannitol. D-Mannosamine entry was the same as that of D-mannitol.

Inhibition of D-*galactose accumulation*

The inhibitor (20 mM) and fructose were preincubated with the tissue rings for 30 min. The inhibition given by glucosamine, galactosamine, and mannosamine was almost identical (Fig. 1). Lineweaver–Burk plots showed that the inhibition appeared to be non-competitive. Using 1 mM glucosamine no inhibition could be detected.

Other substances containing the ammonium group NH_4Cl, methylammonium chloride, ethanolamine hydrochloride, hydroxylysine hydrochloride and Tris chloride gave a similar inhibition (Table II), but *N*-acetylglucosamine did not. LiCl did not

TABLE I

TISSUE/MEDIUM RATIOS FOR AMINOSUGARS

Sugar	*Tissue/medium ratio*	*Recovery*(%)
D-Glucosamine	0.42 ± 0.02	77.4
D-Galactosamine	0.35 ± 0.02	80.5
D-Mannosamine	0.19 ± 0.01	91.2
D-Galactose	7.4 ± 0.35	—
D-Mannitol	0.17 ± 0.03	—

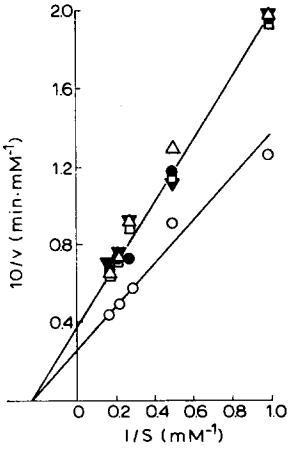

Fig. 1. Inhibition of D-galactose accumulation. Lineweaver–Burk plot of reciprocal of D-galactose accumulation against medium concentration after preincubation with amino sugars for 30 min. ○, D-galactose; ●, D-glucosamine; △, D-galactosamine; ▼, D-mannosamine; □, ammonia.

TABLE II

INHIBITION OF D-GALACTOSE ACCUMULATION INTO HAMSTER INTESTINE BY AMINES

P is the probability that the percent inhibition is the same as that caused by 40 mM D-mannitol. *n* is the number of paired observations. Concn. of amines, 20 mM.

Addition (as HCl salt)	*Inhibition* (%)	*P*	*n*
D-Glucosamine	33 ± 3	<0.001	19
D-Galactosamine	34 ± 2.5	<0.001	9
D-Mannosamine	25 ± 3	<0.001	5
Ammonia	26 ± 4	<0.001	10
Methylamine	34 ± 9	<0.01	5
Ethanolamine	31 ± 2.5	<0.001	3
Tris	25 ± 1	<0.001	10
Hydroxylysine	26 ± 5	<0.01	4
Mannitol(40 mM)	4.5 ± 2.5	–	5
LiCl	3 ± 3	Approx 1	5
N-Acetylglucosamine	2 ± 5	Approx 1	5

inhibit. Since the inhibition was non-competitive, the percent inhibition was independant of D-galactose concentration. Table II shows the percent inhibition, standard error, and probability, for paired experiments, with and without inhibitor (20 mM) for D-galactose concentrations between 1 and 5 mM.

Time course of inhibition by D-glucosamine

Tissue rings were preincubated with D-glucosamine (20 mM). The sugar was then washed off after times between 0 and 60 min, and the D-galactose transport measured compared with tissue preincubated in α-methyl-D-glucoside (Fig. 2). The proportional inhibition remained about the same.

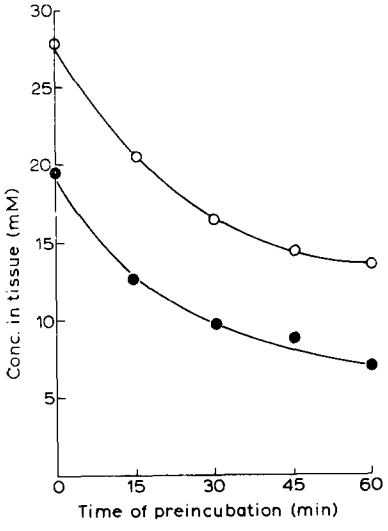

Fig. 2. Effect of preincubation with D-glucosamine sugar (20 mM) for various times on the accumulation of D-galactose (10 mM) after 10 min incubation. Control contained α-methyl-D-glucoside (20 mM). ●, D-glucosamine; ○, α-methyl D-glucoside.

Chromatographic analysis of tissue homogenates

Very little metabolism of either D-glucosamine or D-galactosamine into ethanol-soluble products was detected, but using the former, traces of sugar phosphates and some fructose was detected.

DISCUSSION

Previously it has been noted that D-glucosamine is not a substrate for the carrier for active sugar transport in the hamster intestine[3]. We have confirmed this observation, although the entry into tissue rings of both D-glucosamine and D-galactosamine but not D-mannosamine is greater than that of D-mannitol. We also confirmed, using everted sacs as described previously, that D-glucosamine disappears from the serosal, but not the mucosal compartment[3]. It therefore seems possible that the enhanced entry is *via* the serosal surface of the tissue rings.

In contrast to D-galactosamine, D-glucosamine has been reported to be a good

inhibitor of active sugar transport in hamster jejunum[5], and an inhibitor in the guinea pig[4]. We have found that in the presence of D-fructose as an energy source that both sugars give only a very weak, non-competitive inhibition of D-galactose accumulation. Using 1 mM D-glucosamine the inhibition was not detectable. Using D-glucosamine in the absence of D-fructose, the extent of the inhibition was variable. At the highest concentration of D-galactose (5 mM), and 30 min preincubation with 20 mM D-glucosamine, D-galactose accumulation was stimulated compared with the control. When 1 mM D-fructose was added to the medium, however, 20 mM D-glucosamine was always inhibitory. It seems that D-fructose which enters the cell by a different route to D-galactose can provide the energy required by the cell, and that D-glucosamine can also act in this way, so that the inhibition was cancelled out by the increased energy supply.

We originally had considered that if a transported sugar forms an ester at C-2 (ref. 2), D-glucosamine might form an irreversible complex with the carrier by forming an amide bond at C-2, although the concentration of the free unprotonated amine which would be required for amide formation is less than 1 % at pH 7.4. Such an inhibition might appear kinetically to be non-competitive, but would be progressive and irreversible. Tissue rings were treated with D-glucosamine which was immediately washed off and in subsequent experiments the tissue rings were incubated with glucosamine for times up to 60 min. The inhibition appeared to be irreversible but not progressive (Fig. 2) relative to a control incubated with α-methyl-D-glucoside suggesting that such a bond was not formed. Furthermore, if the inhibition were by such an amide bond, D-galactosamine would have a similar inhibition, but D-mannosamine should not inhibit. All three aminosugars inhibited in the same way and to the same extent (Fig. 1). The weak inhibition must therefore be due to an alternative non-specific interaction with the membrane or transport system.

In an attempt to identify the cause of inhibition NH_4Cl was tested as an inhibitor, since the aminosugars may have given rise to ammonia in the tissue. It was found to have a very similar effect. That this was not the cause of the aminosugar inhibition, however, is indicated by the small amount of metabolism of D-galactosamine and D-mannosamine and also by the similar inhibition by other compounds containing an ammonium group. In contrast *N*-acetylglucosamine does not inhibit. Cl^-(LiCl) is not inhibitory and the pH did not alter during the incubations. It must be concluded that the weak non-competitive inhibition is a property of the ammonium group and may be due to a non-specific interaction with the membrane. The failure of the aminosugars to interact with the sugar binding site of the carrier is probably due to the fact that at the pH used they exist almost entirely in the protonated form.

REFERENCES

1 J. E. G. Barnett, A. Ralph and K. A. Munday, *Biochem. J.*, 116 (1970) 537.
2 J. E. G. Barnett, A. Ralph and K. A. Munday, *Biochem. J.*, 118 (1970) 843.
3 T. H. Wilson and R. K. Crane, *Biochim. Biophys. Acta*, 29 (1958) 30.
4 E. Riklis, B. Haber and J. H. Quastel, *Can. J. Biochem. Physiol.*, 36 (1958) 373.
5 R. G. Faust, M. G. Leadbetter, R. K. Plenge and A. J. McCaslin, *J. Gen. Physiol.*,52 (1968) 482.
6 H. A. Krebs and K. Henseleit, *Z. Physiol. Chem.*, 210 (1932) 33.
7 M. Somogyi, *J. Biol. Chem.*, 160 (1945) 69.
8 L. A. Elson and W. T. J. Morgan, *Biochem. J.*, 27 (1923) 1824.