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Influence of Luminal Na^+ on the Intestinal Absorption of Sugars *in vivo*

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The effect of substituting Na^+ with Tris, Li^+, K^+ or mannitol on the intestinal absorption of sugars, in successive periods of 1 minute duration, has been studied in rat and hamster *in vivo*. The absorption of 2 mM D-glucose, D-galactose, 3-0-methyl-D-glucose and D-fructose is clearly inhibited in the absence of Na^+, up to 70-80 %, and returns to its normal value on restoring Na^+. The degree of inhibition varies with the sugar, increases on lowering Na^+ concentration, reaches maximum values with mannitol as substituent, and minimum with Tris. D-arabinose absorption is not affected by Na^+.

These results prove once more how important Na^+ is in sugar intestinal transport *in vivo*, while they reveal additional influences of the different substituents on the transport system.

The role of Na^+ in intestinal transport of sugars has often been the object of revisions (4, 5, 17, 25, 29). It is generally agreed that the presence of Na^+ in intestinal lumen is essential for sugar transport to occur against gradient when working *in vitro*. No other ion nor any other assayed substance can substitute Na^+ in allowing active transport.

The level of sugar accumulation and the penetration rate in the epithelial cells are a function of the mucosal concentration of Na^+, which seems to influence, depending on the species, the transport constant (K_T) or the V_{max} (29). This Na^+ dependence has been confirmed on various types of preparations *in vitro:* intestinal sacs or rings, isolated enterocytes (18), or membrane vesicles (7).

The importance of Na^+, however, in the intestinal absorption of sugars *in vivo* has been the subjected of discussion. Inhibitions have been observed under very low levels of Na^+, in rat (8, 19), in dog (1) and in man (24, 32). LLUCH and PONZ (20, 27) showed that absorption in rat depended on Na^+ concentration but that it was also affected by the nature of the

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Na^+ substituent employed, reaching even a 58 % with total substitution by mannitol. Other results *in vivo*, however, have been negative or have only showed a very weak influence of Na^+ (2, 9, 12, 13, 28).

These conflicting results with *in vivo* techniques, made advisable the revision of Na^+ influence on the absorption rate of different sugars by using a recently described method (26) with perfusion of the intestinal lumen during very short successive periods of absorption. Results are very expressive and show that absorption in rat and hamster depends, as *in vitro*, on Na^+ concentration.

Materials and Methods

Wistar rats weighing 110-230 g and hamsters 80-120 g of either sex were used after a 24 hour fast. Preparation of the animal for intestinal perfusion was done following the technique of PONZ *et al.* (26). The intestinal segment between cannulae was about 20 cm in length. The sugars were added to the perfusion medium Krebs-Ringer-Tris (22, 34), in which NaCl was substituted in various proportions by LiCl, KCl, Tris-HCl or mannitol, just in the amount required to preserve osmolarity.

Perfusion was carried out at the rate of 5.6 ml/min, without recycling the solution. The successive periods of absorption were 1 minute long. The perfusion system was washed after each period with a solution chemically identical to that used in the subsequent period, minus sugar.

In experiments with arabinose the perfusion medium was recycled and the successive periods lasted five minutes, due to the slow absorption quality of this sugar.

The sugars D-glucose, D-galactose, D-fructose, 3-0-methyl-D-glucose, and D-arabinose were at 2 mM concentration. Sugars were determined after NELSON-SOMOGYI (23, 33). Absorption values are expressed in nmoles of sugar absorbed per cm of intestine per minute (26).

Results

Rat. Six successive absorption periods of one minute were performed on each animal. Na^+ was totally absent from the perfusion medium in the second period, whereas 127 mEq Na^+/l was present in all the others. The washing between the first and second period was more thorough to remove residual Na^+. Only the absorption of one sugar was measured in any given animal.

Figure 1 shows the results from total substitution of Na^+ on the absorption of the four hexoses. In all cases absorption remains clearly inhibited in a Na^+ free solution, and its normal value is restored as soon as perfusion is carried out again with Na^+. The degree of inhibition caused by the absence of Na^+ varies somewhat depending on the sugar used. It also varies for a given sugar according to the

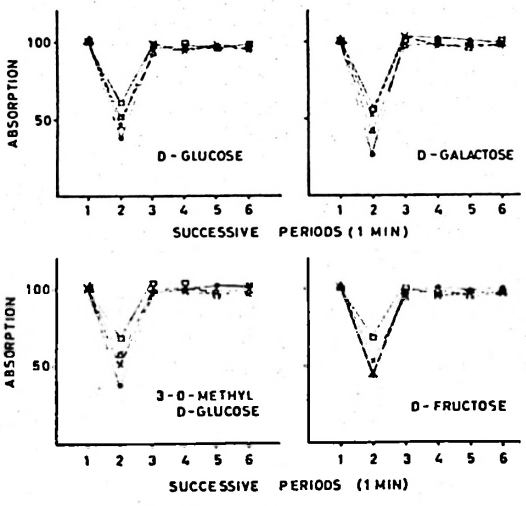

Fig. 1. *Influence of total substitution of Na^+ in the perfusion medium by Li^+ (×), K^+ (△), Tris (□) or mannitol (●) on the intestinal absorption of distinct sugars (2 mM) in rat.* Perfusion without recycling (5.6 ml/min). Successive periods of 1 minute duration.

Table I. *Influence of partial substitution of Na^+ in the perfusion medium (Krebs-Ringer-Tris) on the intestinal absorption of sugars (2 mM) in rat.*
Perfusion without recycling (5.6 ml/min). Successive periods of 1 minute duration. Mean values with their standard error. Number of experiments in parentheses.

Sugar (2 mM)	Substitute	[Na^+] (mEq/l):	nmoles absorbed/cm/min 127	64	32	16	0
D-glucose	Li^+	(6)	124±8	108±6	97±7	86±7	66±2
	K^+	(6)	152±10	117±9	103±6	89±7	75±5
	Tris	(6)	137±7	115±7	104±5	86±7	68±6
	Mannitol	(6)	135±4	122±2	105±2	58±2	33±4
D-galactose	Li^+	(6)	160±13	146±13	134±10	107±4	82±5
	K^+	(10)	149±5	125±5	96±2	78±3	56±2
	Tris	(6)	150±7	128±6	115±6	89±5	70±3
	Mannitol	(6)	138±7	124±5	101±4	48±2	29±2
3-0-methyl-D-glucose	Li^+	(10)	113±6	95±4	81±6	46±2	34±2
	K^+	(6)	142±13	125±10	94±8	71±6	49±5
	Tris	(6)	121±5	107±3	93±3	80±2	67±2
	Mannitol	(6)	134±4	116±2	93±3	71±3	53±6
D-fructose	Li^+	(6)	77±4	71±3	62±3	47±2	43±3
	K^+	(6)	94±8	83±6	70±5	54±3	43±2
	Tris	(6)	115±7	102±6	90±6	77±5	72±4
	Mannitol	(6)	107±8	93±6	67±2	53±3	43±3

Na^+ substituent employed. The greatest inhibitions for all sugars were observed with mannitol as substituent, and the smallest with Tris.

Analogous experiments were performed with D-arabinose, but with 5 minute long absorption periods and recycling the solution. Total substitution of Na^+ by Li^+ did not affect arabinose absorption at all, which remained identical throughout the six successive periods.

In other groups of rats the absorption of distinct sugars was studied under Na^+ concentrations of 127, 64, 32, 16, and 0 mEq/l in the medium. Ten 1 minute successive periods of absorption were verified in each animal with the same sugar and the same substituent under the above mentioned Na^+ concentrations (two periods per each concentration). The concentration in the first and the last period was always 127 mEq Na^+/l, but the order of succession of the remaining concentrations was changed at random. Other groups of animals were formed by combining different sugars and substituents.

In all cases (table I and figure 2) the lower the Na^+ concentration, the lower the values of the absorption rate were. Decreases in absorption, usually statistically significant, had already been noted when passing from 127 to 64 mEq Na^+/l. Total absence of Na^+ in the medium produced the greatest inhibitions, as much as 70-80 % in the case of absorption with glucose or galactose with mannitol as substituent. It was not possible to establish a uniform serial arrangement for the inhibitions produced by the different substituents, since differences are conditional both on the sugar absorbed and on the proportion of substituted Na^+.

For glucose and galactose, when Na^+ substitution reaches 87.5 % and 100 %, the serial degree of inhibition according to the substituents is: mannitol > K^+ >

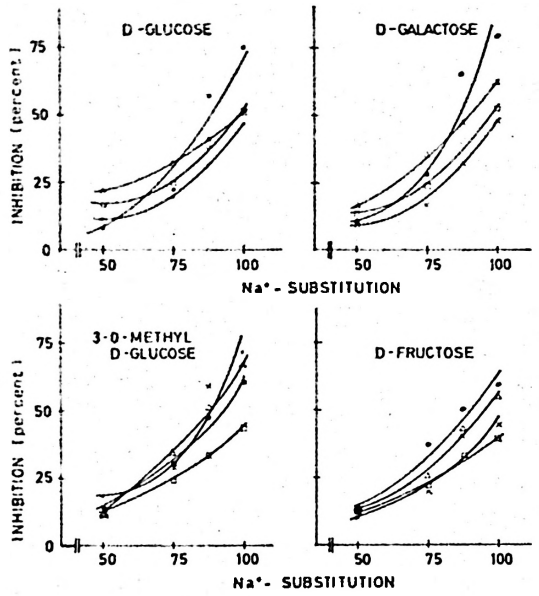

Fig. 2. *Inhibition of intestinal absorption of sugars (2 mM) in rat, as a function of the percentage of Na^+ substitution by Li^+ (×), K^+ (△), Tris (□) or mannitol (●).* Perfusion without recycling (5.6 ml/min). Successive periods of 1 minute duration.

$Li^+ >$ Tris. With substitutions, however, of 75 % and 50 % the greatest inhibitions correspond to K^+.

With fructose the inhibition levels with the distinct substituents of Na^+ are somewhat inferior to those corresponding to the other sugars.

Hamster. In hamster, experiments with total substitution of Na^+ were performed following the procedure described for rat. The results for the four sugars and the distinct substituents are showed in table II. Lack of Na^+ in the second period produces a clear inhibition in absorption, as in rat, and it disappears as soon as perfusion resumes with 127 mEq Na^+/l in the solution. The inhibition for all sugars decreases according to the series: mannitol $> K^+ > Li^+ >$ Tris. Inhibition values from total absence of Na^+ are quite similar in hamster and rat. In hamster, inhibition differences due to the sugars employed are also observed, being somewhat higher with glucose and galactose.

Discussion

Intestinal absorption of sugars *in vivo*, both in rat and hamster, is markedly inhibited by the total suppression of Na^+ from the perfusion medium. The highest inhibitions reach 70-80 % and correspond to the absorption of glucose and galactose with mannitol as substituent of NaCl for osmotic purposes. When Na^+ is partially substituted, inhibition increases to the degree of the substitution, i.e. according to the decrease of Na^+ concentration.

The effect from lack of Na^+ appears in glucose, galactose, and 3-methyl-glucose, as well as in fructose. The absorption of D-arabinose, instead, does not seem to be sensitive to the lack of Na^+. The three aldohexoses are well known as actively transportable sugars. The digestive epithelium metabolizes glucose with ease, galactose to a scant extent, but none of 3-methyl-glucose. For this reason the role played by Na^+ must be related to the transport process and not to the metabolizing of sugar. The fact that the absorption of fructose is inhibited by the absence of Na^+ confirms indirectly the results *in vitro* that uphold its inflow into the epithelial cells by means of a Na^+ dependent transport system (15, 21).

The observed inhibitions cannot be assigned exclusively to the absence of Na^+, since they vary considerably according to the nature of the substituent employed to keep the osmolarity of the medium constant.

These results confirm those previously obtained *in vivo* in rat, where the solution was kept static and the absorption periods were twenty times longer (27). With 1 minute long periods inhibitions are much higher. This might be due to the fact that

Table II. *Influence of total substitution of Na+ in the perfusion medium (Krebs-Ringer-Tris) on the intestinal absorption of distinct sugars (2 mM) in hamster.*
Perfusion without recycling (5.6 ml/min). Successive periods of 1 minute duration. Mean values with their standard error. Number of experiments in parentheses.

Sugar (2 mM)	Substitute		Absorption in sucessive periods (nmoles/cm/min) 1st Na+	2nd Na+-free	3rd Na+	4th Na+	5th Na+	6th Na+
D-glucose	Li^+	(4)	132±3	64±2	132±3	130±4	131±4	131±3
	K^+	(4)	123±1	64±2	121±1	121±1	122±1	123±1
	$Tris^+$	(4)	125±3	73±3	123±1	123±1	123±3	123±1
	Mannitol	(4)	120±5	35±3	121±1	121±1	122±1	121±1
D-galactose	Li^+	(4)	130±1	64±2	132±3	130±4	131±4	131±3
	K^+	(4)	141±4	64±1	139±4	139±5	138±4	139±5
	$Tris^+$	(4)	141±0.8	79±3	141±3	141±1	142±1	141±1
	Mannitol	(4)	137±7	38±4	136±5	136±6	137±4	136±6
3-0-methyl-D-glucose	Li^+	(4)	124±2	64±1	125±4	124±4	123±4	124±4
	K^+	(4)	128±2	55±1	124±2	126±3	125±3	124±3
	$Tris^+$	(4)	126±4	84±4	126±3	124±2	126±5	124±1
	Mannitol	(4)	124±4	46±3	123±5	124±3	122±5	122±1
D-fructose	Li^+	(4)	82±4	44±1	82±4	79±4	80±5	79±4
	K^+	(4)	86±1	50±1	86±1	86±1	86±2	87±1
	$Tris^+$	(4)	87±2	60±2	86±5	85±1	85±1	87±3
	Mannitol	(4)	79±1	33±1	78±3	78±1	77±1	78±1

sugar concentration decreases very little during such short periods. Furthermore the outflux of endogenous Na^+ toward lumen (28, 30, 31) for 1 minute has to exert very little influence on the concentration of the cation at the outer part of the membrane.

Another difference observed in 1 minute periods is the complete reversibility of the inhibition on returning to the control concentration of Na^+, whatever sugar or substituent is employed. Reversibility became increasingly difficult with longer periods, especially when Na^+ was substituted by mannitol (27). This pointed to perturbations in the absorbing capacity of the enterocytes, probably related to intracellular ionic and metabolic changes, which would not be possible in 1 minute periods.

The present data contradict some negative results on the effect of Na^+ *in vivo* (2, 9-14, 28), due perhaps to differences in experimental conditions: rats with very high hyperglucaemia, and use of glucose concentrations far above the K_T values recently obtained *in vivo* (16). Other negative results in rat (28), with solution recycling, might be due to the fact that only solutions with 75 and 0 mEq Na^+/l were compared, which reduces the possible differences, and by the sharp decline of glucose concentration in the medium during the 50 minute long periods.

Lack of Na^+ in the perfusion medium does not entirely block absorption since it persits up to 20-40 % according to the sugars and the substituents used.

A possible explanation for this residual absorption is that lack of Na^+ reduces sharply the transport system affinity for sugar, but does not make it void (6). With *in vivo* techniques, besides, it becomes extremely difficult to elimitate

completely through intestinal washings the Na^+ trapped in the unstirred liquid layers close to the brush border membrane. Furthermore it is well known (28, 30, 31) that appreciable amounts of endogenous Na^+ find their way to the intestinal lumen in favour of the strong gradient, reaching concentrations, not at all insignificant, in those layers and at the sites of the transport system.

An explanation for the inhibition differences that appear with equal concentrations of Na^+ depending on the substituent used cannot be oferred. It is difficult to think that with periods as short as 1 minute, the intracellular ionic changes will be significant enough to account for those differences. Perhaps Li^+, K^+, Tris and mannitol may have somewhat different effects on the interaction of sugar and Na^+ with the carrier.

These results, as others previously found on the K_T values for sugar transport *in vivo*, lead to the conclusion that the transport systems of sugar working *in vivo* are the same as those that have been characterized from *in vitro* experiments and that they possess the same properties. Due, however, to the upkeeping of blood circulation, the component of passage of sugar by diffusion, and the greater thickness of the unstirred layers, deviations occur leading some people to appreciate incompatibility between the properties of the systems operating *in vivo* and *in vitro*.

The Na^+ influence on sugar absorption *in vivo*, aside its interest in relation to the transport mechanism, ought to be considered also in itself, since Na^+ concentration in the intestinal lumen under normal feeding conditions is somewhat lower than 127 mEq/l and becomes still lower along the small intestine (3).

Resumen

Se ha estudiado en rata y hamster *in vivo* el efecto de la sustitución del Na^+ por Tris, Li^+, K^+ o manitol sobre la absorción intestinal de azúcares (2 mM), en períodos sucesivos de 1 minuto. La absorción de D-glucosa, D-galactosa, 3-0-metil-D-glucosa y D-fructosa se inhibe claramente (hasta 70-80 %) en ausencia de Na^+ y vuelve a su valor normal al restaurar el Na^+. La intensidad de la inhibición varía con el azúcar, aumenta al disminuir la concentración de Na^+ y es máxima al sustituir por manitol y mínima con el Tris. La absorción de D-arabinosa no se afecta por el Na^+.

Estos resultados confirman la importancia del Na^+ para el transporte intestinal de azúcares *in vivo* y revelan influencias adicionales de los distintos sustituyentes sobre el proceso de transporte.

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