Effects of Insulin on the Permeability of D- and L-Xylose and D- and L-Arabinose in Rat Diaphragm Muscle

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ABSTRACT The permeability characteristics of D- and L-xylose and D- and L-arabinose have been compared in isolated intact rat diaphragm muscle preparations, in the absence and presence of exogenous insulin. In the absence of added insulin, these pentoses distribute in less than a third of the total cell water. In the presence of added insulin, intracellular distribution of all these pentoses is increased. L-Xylose and D-arabinose distribute in 50 per cent of the intracellular water, whereas D-xylose and L-arabinose distribute in 80 per cent of the cell water. A significant lag period was observed before the insulin effect upon the penetration of L-xylose and D-arabinose was evident whereas the effect upon D-xylose and L-arabinose was rapid. The lag period with L-xylose could be abolished by pretreating the tissues with insulin for 1 hour, but such pretreatment had little effect on D-xylose. These results indicate that insulin has a biphasic effect upon the monosaccharide exclusion system in diaphragm muscle. In dinitrophenol-treated tissues, in which all permeability processes are irreversibly damaged and in which sucrose and pentoses penetrate into most of the cell water, the entry rate of pentoses and sucrose is initially similar but subsequently D-xylose and L-arabinose penetrate more rapidly than their corresponding optical isomers.

It is now well established that insulin facilitates the entry of certain monosaccharides in muscle by modifying a barrier which excludes sugars (1, 2). The entry route made available by insulin is reported to be selective in that it differentiates between closely related sugar structures. Thus it has been reported that insulin facilitates the penetration of D-xylose or L-arabinose in dog or cat skeletal muscle (3, 4) without effect upon the corresponding optical isomer. The permeability differences in muscle between these pairs of optical isomers have been an important consideration in discussion of the mechanistic

basis of cellular selectivity of structurally related monosaccharides. For example, it has been suggested that differences in conformation between D- and L-isomers may be the chemical basis for selection by a cell membrane "carrier" which differentiates between optical isomers (5).

In contrast to the reports cited concerning the selectivity of the sugar entry route made available by insulin, we found in the isolated rat diaphragm muscle that insulin facilitates the entry of L-xylose as well as of D-xylose, although the effects were less marked with the L-isomer (1). Thus (*a*) the calculated intracellular distribution of L-xylose in insulin-treated preparations was less than for D-xylose, and (*b*) there was an appreciable lag period before the effect of insulin upon L-xylose penetration was evident, whereas the effect that facilitated penetration of D-xylose was rapid. In the present work, we have extended our comparison of the effect of insulin upon the permeability characteristics of D- and L-xylose in intact diaphragm muscle *in vitro*, and have also studied L- and D-arabinose in this system as well.

METHODS

The intact diaphragm muscle preparations studied were obtained from exsanguinated male rats weighing 100 to 200 gm (fasted for 18 to 24 hrs.) using procedures previously described (1). Unless otherwise stated, the diaphragm preparations were incubated in 50 ml of Krebs-Ringer phosphate containing 4 mg of pentose per ml and 10 mg of sucrose per ml. In certain experiments, in which the distribution of pentose, sucrose, and inulin was measured simultaneously, C^{14}-labeled inulin (S.A. 3.0 μc/mg) was added to the basal medium at concentration 1 μg/ml. The tissues were incubated at 37.5° with shaking (100 per cent O_2–gas phase) in the absence of added glucose. When insulin was added, the concentration was 0.5 I.U. per ml of a glucagon-free preparation (Lilly). In certain experiments in which 2,4-dinitrophenol (DNP) was employed, the preparations were first incubated with 0.2 mM of DNP for 1 hr. in the absence of added sugars, and were then transferred to fresh Ringer media containing one of the four pentoses, plus sucrose and inulin. The methods used for estimating pentose, sucrose, and cations have been previously described (1, 6); C^{14}-inulin was measured in a flow gas counter.

The sugars employed, all of reagent grade, were D(+)-xylose (Fisher Scientific), L(−)-xylose, D(−)-arabinose, and L(+)-arabinose (all from Nutritional Biochemicals), and sucrose (Mallinckrodt). C^{14}-inulin (carboxyl-labeled) was obtained from the New England Nuclear Corporation. The optical rotation of the D- and L-pairs of both pentoses was measured in a polarimeter and found to correspond to the values reported for pure isomers.

It had been previously found that the distribution of D-xylose and D-galactose in diaphragm muscle *in vitro* is independent of the external sugar concentration over a wide range of concentrations (1, 2); it has therefore been assumed that L-xylose, D-, and L-arabinose likewise distribute in diaphragm muscle *in vitro*, independent of external concentration. The sugar penetration results are expressed either as an apparent volume of distribution in the total tissue or in intracellular water.

The tissue distribution of a sugar is obtained by the ratio:

$$\frac{\text{Sugar in wet tissue (mg/gm)}}{\text{Sugar in medium (mg/ml)}} \times 100.$$

The tissue distribution of pentose and sucrose was determined in each diaphragm preparation, and the sucrose space was assumed to represent a measure of the volume

TABLE I

INTRACELLULAR DISTRIBUTION OF VARIOUS SUGARS IN INTACT RAT DIAPHRAGM PREPARATIONS

Sugar	Insulin	Distribution at varying incubation times, *per cent*			
		15 min.	30 min.	60 min.	90 min.
D-Xylose	+	42±5 (5)*	56±4 (7)	78±3 (16)	82±2 (4)
	−	13±2 (5)	26±5 (4)	30±2 (17)	26±5 (4)
L-Xylose	+	5±1 (5)	21±3 (8)	42±4 (8)	51±5 (8)
	−	3±1 (5)	11±1 (8)	20±3 (8)	26±2 (8)
D-Arabinose	+	7±2 (4)	15±3 (3)	48±3 (7)	52±1 (5)
	−	6±1 (5)	4±2 (4)	17±3 (10)	27±3 (5)
L-Arabinose	+	35±4 (5)	47±5 (4)	78±3 (7)	82±3 (5)
	−	13±3 (5)	18±2 (4)	18±4 (8)	31±3 (5)

* Mean, standard error, and number of observations.

of extracellular water in each tissue. The intracellular distribution (I.D.) of pentose is obtained by the following calculation:

$$\text{I.D.} = \frac{\text{P.D.} - \text{S.D.}}{0.78 - \text{S.D.}} \times 100$$

where P.D. is the individual tissue distribution of pentose and S.D. is the individual tissue distribution of sucrose, with 0.78 ml per gm as the average value for the water content of tissues incubated under our conditions (1) (as determined by drying overnight at 105°).

RESULTS

Table I shows the penetration of the four pentoses into intact diaphragm muscle in the presence and absence of added insulin. In the absence of added insulin after 90 min. of incubation, all the pentoses appear to distribute in

about 30 per cent of the total intracellular water, no significant differences being observed between the corresponding optical isomers. However, there is a difference in the rate of penetration between D- and L-optical isomers, D-xylose and L-arabinose entering more rapidly. The differences between D-xylose and L-xylose at 15 and 30 min. are statistically significant; with the small number of animals employed the differences between L-arabinose and D-arabinose are statistically significant at 30 min., but not at 15 min.

TABLE II
EFFECT OF PREINCUBATION UPON D- AND L-XYLOSE ENTRY

		L-Xylose		D-Xylose	
	Insulin added to media	Intracellular distribution		Intracellular distribution	
		15 min.	30 min.	15 min.	30 min.
Preincubation 1 hr. in KRP and insulin	+	16±2 (9)*	30±1 (9)	50±5 (7)	68±2 (7)
Preincubation 1 hr. in KRP only	+	6±1 (8)	16±2 (9)	31±5 (5)	62±3 (6)
	−	7±1 (9)	11±2 (9)	2±1 (6)	11±4 (6)
No preincubation	+	5±1 (5)	21±3 (8)	42±5 (5)	56±4 (7)
	−	3±1 (5)	11±1 (8)	13±2 (5)	26±6 (3)

* Mean, standard error, and number of observations.

The intracellular distribution of all the four pentoses is increased by insulin. However, there are characteristic differences between the two pairs of optical isomers in insulin-treated muscle. After 90 min. of incubation D-xylose and L-arabinose distribute in about 80 per cent of the total cell water, whereas L-xylose and D-arabinose have distributed in only about 50 per cent of the cell water at this time. The intracellular distribution values at 60 and 90 min. for each sugar are not significantly different; thus, the differences between D- and L-xylose and L- and D-arabinose appear to represent the equilibration of these sugars in different volumes of intracellular water. As previously noted with L-xylose (1), Table I shows that there is a significant lag period before an insulin effect upon the penetration of L-xylose and D-arabinose is evident, whereas the effect of hormone upon D-xylose and L-arabinose entry is observed more rapidly. Thus, after 15 min. incubation the effect of insulin in increasing L-arabinose and D-xylose penetration is significant, whereas with D-arabinose and L-xylose, the effect of insulin is detectable at 30 min. but not at 15 min.

The lag period observed before insulin influences L-xylose or D-arabinose penetration can be abolished if the tissues are preincubated with insulin for 1 hr. Table II shows the results of experiments in which diaphragm prepara-

tions were preincubated for 1 hr. in 25 ml Ringer's phosphate at 37.5°, without added pentose or sucrose, both in the presence and absence of insulin; D- or L-xylose and sucrose in 25 ml Ringer's was then added to the incubation flask, and the tissues were incubated for an additional 15 or 30 min. In some experiments, tissue was preincubated for 1 hr. in Krebs-Ringer, without added insulin, to check the effect of preincubation alone. It will be seen that in tissues preincubated for 1 hr. with insulin, L-xylose entry is significantly greater than in tissues preincubated for 1 hr. in the absence of insulin, whether or not insulin is subsequently added in the second incubation period. Thus, preincubation in the absence of insulin, did not affect the lag period with L-xylose, which, however, was abolished by pretreating with insulin. Table II also shows that the D-xylose tends to enter more rapidly in tissues pre-

TABLE III

THE ENTRY OF VARIOUS SUGARS AND INULIN IN TISSUES PREINCUBATED WITH DNP (0.2 mM) FOR 1 HOUR

	Distribution in whole tissue					
	Incubation time, *min.*					
Sugar	7.5	15	30	45	60	90
D-Xylose	31±3 (4)*	51±2 (7)	60±2 (11)	65±2 (6)	70±1 (9)	70±3 (5)
L-Xylose	33±4 (4)	47±4 (7)	53±2 (10)	58±3 (6)	65±1 (9)	69±2 (5)
D-Arabinose			54±3 (9)		65±2 (10)	73±4 (5)
L-Arabinose			59±2 (9)		71±1 (10)	72±2 (5)
Sucrose	28±3 (6)	39±2 (15)	41±2 (15)	49±2 (11)	61±2 (10)	64±2 (19)
Inulin	11±2 (6)	19±2 (6)	23±1 (17)	25±1 (12)	31±1 (12)	38±1 (20)

* Mean, standard error, and number of observations.

incubated with insulin; however, the difference between tissues preincubated with and without insulin (but receiving insulin in the second period) is not statistically significant ($p = 0.1$). While preincubation in Krebs-Ringer did not markedly influence entry of L-xylose relative to non-preincubated tissues, there is a suggestion that such preincubation did influence D-xylose entry. Thus, in tissues preincubated in Ringer's for 1 hr. and then for an additional 30 min. with sugar, the rate of D-xylose entry tends to be less at 15 and 30 min. than that observed in tissues not preincubated; likewise the effect of insulin at 15 min. in tissues preincubated in Ringer's without insulin, is less than that observed in fresh tissues, although the difference is not statistically significant.

It had previously been shown that when diaphragm muscle is incubated with a metabolic inhibitor such as DNP (6, 7), D-xylose is no longer excluded but penetrates to equilibrate in most of the cell water. It was therefore of interest to determine whether the difference observed between optical isomers

was retained in tissues in which the monosaccharide exclusion system is no longer operative. Table III shows the results obtained in tissues preincubated for 1 hr. with DNP (0.2 mM) prior to the addition of pentose, sucrose, and inulin. The results are presented in terms of tissue distribution, rather than distribution in cell water (as in the previous tables) because the very high tissue distribution of sucrose in DNP-treated tissues (60 to 65 per cent) relative to normal tissues (19 to 25 per cent), indicates that sucrose is not excluded from the fibers in this preparation. Inulin, which has been employed to measure extracellular space in hemidiaphragm preparations (1), likewise seems unsuitable as a measure of extracellular space in DNP-treated tissues, since it does not give an equilibrated value in 90 min. DNP also profoundly disturbs cation distribution in the diaphragm preparation. The K content of DNP-pretreated diaphragms, after an additional hour of incubation is about 20 μM per gm, in contrast to 100 μM per gm in tissues incubated in normal Ringer's; the corresponding values for tissue Na are about 150 and 47 μM per gm, respectively.

Despite the marked alterations in permeability processes observed in DNP-treated tissues, Table III shows that differences between optical isomers are retained to a certain degree in such tissues. Although all four pentoses distribute in about 70 per cent of the tissue wet weight, D-xylose enters more rapidly in such preparations than L-xylose, the differences at 30 and 60 min. being statistically significant. While the data with D- and L-arabinose are limited, the evidence with this pair likewise indicates that L-arabinose tends to penetrate more rapidly than D-arabinose. During the first 7.5 min. period of incubation, however, D-xylose, L-xylose, and sucrose enter at about the same rate; therefore, the differences among these sugars relate to differential rates of diffusion during the subsequent period of incubation.

DISCUSSION

The present studies with D- and L-xylose, and L- and D-arabinose help to define some of the characteristics of the effect of insulin upon sugar permeability in the isolated rat diaphragm muscle. As a result of these studies, it now appears that insulin has a biphasic action upon the monosaccharide exclusion system in isolated rat diaphragm muscle. In the first phase which occurs rapidly, insulin appears to alter the barrier, so that sugars like D-xylose and L-arabinose, but not L-xylose or D-arabinose, are permitted to enter. After an appreciable lag period, of the order of 15 min. or so, the barrier system appears to be further modified by insulin, so that L-xylose and D-arabinose are now permitted to penetrate. Narahara *et al.* (9) have recently presented evidence that there is an appreciable lag period in insulin action to facilitate glucose entry in isolated frog sartorius muscle. The nature and locus of the

monosaccharide exclusion barrier in muscle fibers are unknown, hence it does not seem profitable to speculate whether the biphasic action of insulin involves a single route for entry, available first for D-xylose and then later for L-xylose as well, or whether two separate routes are involved.

The present studies also show that the intracellular water made available for pentoses by insulin does not appear to be a single aqueous region or compartment, since in insulin-treated diaphragms L-xylose and D-arabinose distribute in only about 50 per cent of the total cell water, whereas D-xylose and L-arabinose distribute in 80 per cent of the cell water. This difference between optical isomers in insulin-treated muscle can be interpreted in various ways, depending upon the fundamental assumptions made concerning the role of the cell membrane, the state of water in the cell, and the locus of insulin action. Although it is widely believed that the effect of insulin upon permeability processes is a cell membrane phenomenon (3, 4, 8, 9), actually there is no definitive evidence for this view. Evidence is accumulating which indicates that cellular selection and exclusion may not be an exclusive property of the cell surface, but a bulk property of the cell as a whole (1, 6, 11, 13). Indeed, in the case of insulin action, substantial evidence exists in diaphragm muscle, both with regard to sugar penetration and with respect to the biosynthesis of protein and glycogen, that the hormone modifies the cellular interior, in addition to a possible effect at the cell surface (6, 10–12). The present studies with pentoses could be explained by either of these alternatives as well as by other possibilities which could be advanced.

However, the finding that diaphragm preparation preincubated with DNP retains differences in the penetration of D- and L-optical isomers of xylose and arabinose is particularly difficult to explain in terms of a hypothesis that the cell membrane is the exclusive determinant of selectivity. Such preparations, presumably as a result of interference with cellular bioenergetics, suffer irreversible damage to processes involved in cation transport and sugar exclusion so that sucrose as well as pentoses penetrates. If it were to be postulated that the cell membrane retains monosaccharide selectivity despite the unavailability of metabolic energy, one would expect these differences to be most marked during the initial phase of entry. However, the results demonstrate that during the first 7.5 min. the rates of entry of D-xylose, L-xylose, and sucrose are approximately the same; the differences among these sugars are the consequence of a secondary diffusion process. Accordingly, the data appear to be more explicable on the basis that in these damaged cells water is so "sequestered" or "compartmentalized" that the intracellular diffusion of D-xylose > L-xylose > sucrose.

Finally, our *in vitro* finding that insulin facilitates the penetration of L-xylose and D-arabinose in rat diaphragm muscle is an apparent contradiction of reported results that the sugar transfer mechanism in skeletal muscle of

dogs and cats, and heart muscle in rats, when activated by insulin, operates with D-xylose and L-arabinose, but not with their optical isomers (3, 4). However, our *in vitro* findings are in accord with the results of Helmreich and Cori (14); they reported that insulin increased the penetration of D-arabinose in rat skeletal muscle *in vivo*, although the effects were less marked than with L-arabinose. While it is possible that the differences discussed may be due to a difference between species, it also seems possible that the insulin effects observed with L-xylose and D-arabinose, since they are less pronounced and have a longer latent period, may not have been detected in the other studies cited. In any case, it is clear that at least in rat muscle (whether diaphragm or skeletal), any theory which purports to describe the mechanism of insulin action upon sugar permeability as well as the basis of monosaccharide selectivity must take into account the finding that insulin appears to have a biphasic action to facilitate the penetration of D- and L-xylose and L- and D-arabinose.

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