EFFECT OF INSULIN ON XYLOSE TRANSPORT IN HUMAN LEUKOCYTES

L. Luzzatto

Istituto di Chimica Biologica
Università di Genova
Genova, Italia

Received June 1, 1960

Since the first evidence produced by Levine et al. (1949) numerous reports have appeared concerning the action of insulin on sugar transport in mammalian tissues. As far as human material is concerned, the readily available erythrocytes have been shown to be completely insensitive to the hormone (Guensberg, 1947), whereas a variable increase in glucose consumption by the leukocytes under the effect of in vitro added insulin has been reported by Dumm (1957) in about 70% of experiments performed on blood from normal and diabetic subjects. In order to further elucidate the pattern of action of insulin, the entry of a non-utilizable sugar[1] was studied: the data presented in this paper indicate that insulin actually increases the transport rate of d-xylose in leukocyte suspensions obtained from normal humans.

[1] Experiments performed with very low sugar concentrations (0.001 M) indicated that leukocytes are actually able of slowly metabolizing xylose. Under these circumstances this could be followed by measuring pentose disappearance from the medium, while no accumulation at all took place in the cells. On the other hand, when xylose transport was studied, extracellular concentration was at least 0.067 M, so as to remain practically constant throughout incubation, and to make utilization negligible with respect to the rapid intracellular accumulation.

Blood was obtained by venipuncture, using either heparin or oxalate-sulphate as anticoagulant, no difference being found in the results. Red cell sedimentation rate was occasionally increased by adding dextran to a final concentration of 0.5%. After 1-3 hours standing at 37° the leukocyte rich plasma was centrifuged three times for 7 min. at 200 x g. Each time the sedimented cells were resuspended in normal saline, pooled together and then allowed to sediment again for 45-60 min. at room temperature, according to Lapin et al. (1958). The supernatant, which contained most of the residual red cells, was discarded, and the sedimented cells (which were 60-80% leukocytes) were resuspended in Krebs-Henseleit bicarbonate buffer, pH 7.4, and the volume adjusted so as to obtain a packed cell volume of 0.01-0.02 ml per ml of suspension. Aliquots of this (generally 5 ml), were transferred to vessels each containing 1 ml of 0.4 M xylose (unless otherwise stated), without or with added insulin at a final concentration of 0.8-1.0 I.U./ml, and incubated with shaking at 37° in a water bath. After the desired time 5 ml of the mixture were transferred to Shevkj-Stafford tubes and incubation stopped by centrifuging at 1200 x g at 0° for 30 min. The supernatant fluid was discarded, the cell pellet wiped dry by careful aspiration through capillary pipettes and finally extracted with 6% $HClO_4$. After filtration, xylose determinations were carried out by the Bial orcinol reaction according to Bonsignore et al. (1952). All the glassware used for blood manipulation and incubation was siliconized.

At 37° xylose equilibrates in 60 min. with about 75% of the packed cell volume, which corresponds to the volume of intracellular water, according to Crane et al. (1957). As shown in

fig. 1 the sugar entry follows a first order kinetics with a

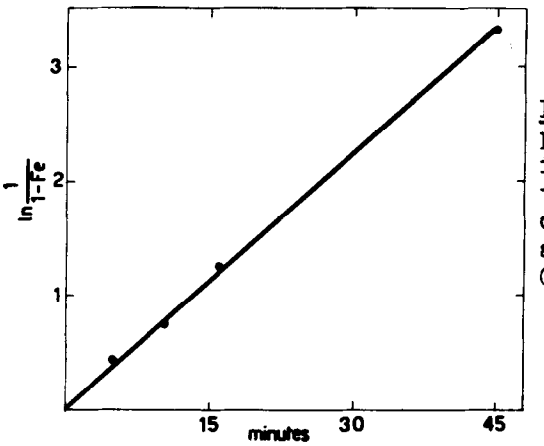

Fig. 1. Time course of xylose penetration. Cells were incubated as described in the text, with xylose at final concentration 0.1 M. Data are plotted according to Crane et al. (1957).

velocity constant $k = 0.073$ min.$^{-1}$ The effect of insulin in vitro was always studied only with a 5 min. incubation time in order to have as many cells as possible in each vessel. Results are summarized in table 1. A highly significant (P 0.01) stimulating effect of the added hormone upon the rate of entry of xylose is clearly seen. A rather broad scattering among the values obtained from different individuals has been observed, which is consistent with the findings by Dumm (1957) on insulin induced increase in glucose utilization. However, in no instance was the accelerating effect of insulin less than 60%. The plasma glucose level (determined by the glucose oxidase method) averaged 88 mgr/100 ml, with a range from 53 to 136, but no correlation could so far be established between this and the entry

Table 1

EFFECT OF INSULIN IN VITRO ON XYLOSE TRANSPORT IN HUMAN LEUKOCYTES

	Xylose entry	
	Mean	Standard deviation
Without insulin	0.15 ±	0.04
With insulin	0.32 ±	0.11
% Acceleration	113 ±	52

Values are obtained from 8 separate experiments and are expressed in µmoles transported xylose/0.01 ml packed cell volume/5 min. Details are described in the text.

rate of xylose[1].

The data clearly indicated an action of insulin on xylose transport in leukocytes, and strongly suggested the effect on glucose consumption (Dumm, 1957) to be due to a similar mechanism. Two further findings are consistent with the latter assumption: (1) the rate of the hexokinase reaction, which has been shown to be the limiting step in glycolysis of homogenized leukocytes (Beck, 1958), measured by the technique of Slein et al. (1950), is not increased by insulin at concentrations up to 1.5 I.U./ml; (2) no insulin effect is detected when glucose consumption by similarly homogenized cells is determined with glucose oxidase after 30 min. incubation at 37° in the presence of 0.05 M glycylglycine buffer, pH 7.6, 0.02 M $MgCl_2$, 0.0025 M ATP and 0.004 M glucose.

Experiments are in progress to obtain direct evidence of the action of insulin on the transport also of utilizable sugars under circumstances in which intracellular accumulation of glucose takes place.

[1] Some experiments were carried out using the leukocyte rich plasma obtained directly after red cell sedimentation. Results were similar to those described in the text with cells re-suspended in Krebs-Henseleit buffer, but were definitely less reproducible: it is felt that this might be due to (a) competition for entry between added xylose and plasmatic glucose and (b) variable plasmatic insulin in different individuals.

REFERENCES

Beck W.S., J. Biol. Chem., 232, 251 (1958).

Bonsignore A., Conte G. and Orunesu M., G. Biochim., 1, 383 (1952).

Crane R.K., Field R.A. and Cori C.F., J. Biol. Chem., 224, 649 (1957).

Dumm M.E., Proc. Soc. Exper. Biol. Med., 95, 571 (1957).

Guensberg E., Diss. Berne, Gerber, Schwarzenburg, 1947.

Lapin J.H., Horonick A. and Lapin R.H., Blood, 13, 1001 (1958).

Levine R., Goldstein M., Klein S. and Huddlestun B., J. Biol. Chem., 179, 985 (1949).

Slein M.W., Cori G.T. and Cori C.F., J. Biol. Chem., 186, 763 (1950).