The Effect of Insulin on Xylose Uptake by the Intact Hemidiaphragm of the Hypophysectomized Rat (36034)

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Hjalmarson (1) has reported a doubling of xylose incorporation into the intact hemidiaphragm of the hypophysectomized rat by incubation with 100 μU/ml of insulin. These data suggested that xylose incorporation into the intact hemidiaphragm of the hypophysectomized rat might be used to develop an improved bioassay for insulin. Such an assay would differ from standard insulin bioassays (2) in the use of hypophysectomized rats, an intact hemidiaphragm, and radioactive xylose.

Materials and Methods. Conditions described by Hjalmarson (1) were duplicated, except as noted. Only female Sprague–Dawley rats which gained less than 10 g in the 14–19 days after hypophysectomy were used. Radioactive ^{14}C-D-xylose (Amersham) was used with specific activity of 0.2 μCi/μmole at a final concentration of 1.0 m*M*. Porcine insulin (5 times recrystallized, Lilly) was dissolved in acetic acid pH 2.3 and diluted with buffer.

The rats were decapitated and intact hemidiaphragms were dissected (3), preincubated (1) in Krebs–bicarbonate buffer (pH 7.3) with glucose (2.5 mg/ml), and equilibrated with 95% O_2–5% CO_2. Preincubation was for 15 min. Then one intact hemidiaphragm was incubated with insulin and one was used as control. The uptake of ^{14}C-D-xylose was measured after 15 min, because the insulin effect was largest at this time. The dissection from the rib cage, washing, blotting, weighing, homogenization with 10% TCA, and centrifugation were as described by Hjalmarson (1, 4). Scintillation fluid was prepared by dissolving 60 g of naphthalene, 4 g of PPO (2,5-diphenyloxazole), 0.2 g of POPOP {1,4-bis [2-5-(phenyloxazolyl)]benzene} in *p*-dioxane to make 1 liter. All reagents were scintillation grade and counting was done in a Picker Nuclear Liquimat 220 liquid scintillation counter. One hundred microliter aliquots of supernatant were counted in 10 ml of scintillation fluid.

Results. The data in Table I shows that insulin increased the incorporation of ^{14}C-xylose by the intact hemidiaphragm of the hypophysectomized rat. A concentration of 100 μU/ml of insulin produced a 30% increase over control values, while a concentration of 1000 μU/ml of insulin produced a 52% increase over control values. Both differences were significant at the 5% level. The two sets of controls gave different results because the experiments were run at different times. The weight of the

[1] This investigation was supported by a General Research Support Grant from U.S. Public Health Service.

TABLE I. The Effect of Insulin on Xylose Uptake by the Intact Hemidiaphragm of the Hypophysectomized Rat.

	Xylose uptake[a]	Av wt of intact hemidiaphragm (mg)	Av final rat wt (g)
Control (for 100 μU/ml of insulin)	56 ± 3.3 (13)[b]	69	66
100 μU/ml of insulin	73 ± 4.7 (13)	74	69
Control (for 1000 μU/ml of insulin)	73 ± 5.0 (13)	84	77
1000 μU/ml of insulin	111 ± 12.8 (15)	77	73

[a] Counts per minute per milligram of wet weight of hemidiaphragm ± standard error of mean.
[b] Number in parentheses is number of intact hemidiaphragms used.

intact hemidiaphragms and the weight of the rats were similar in control and experimental groups.

Discussion. Our results confirm the conclusion of Hjalmarson that insulin increases xylose incorporation into the intact hemidiaphragm of the hypophysectomized rat. However, we could not obtain as large an effect as Hjalmarson. Furthermore, since 100 μU/ml of insulin produced a 30% increase in xylose uptake while 1000 μU/ml produced a 52% increase, it is apparent that small increments of insulin cannot be readily measured with this method.

Our data are similar to those obtained in standard insulin bioassays (2). However, more effort is expended because of difficulties in caring for hypophysectomized rats, because of the need to dissect an intact hemidiaphragm, and because of the use of radioactive xylose. We therefore conclude that this procedure is not suitable for an improved bioassay for insulin.

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Received June 18, 1971. P.S.E.B.M., 1971, Vol. 138.