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Anabolic Responses of Diaphragm Muscle to Insulin and to Other Pancreatic Proteins.* (29339)

PETER RIESER AND CATHERINE H. RIESER (Introduced by Thomas G. Scharff)
Department of Physiology and Biophysics, University of Louisville School of Medicine, Louisville, Ky.

No knowledge exists as to the chemical nature of the glucose barrier in the muscle cell, nor is there any real understanding of how this barrier is removed by insulin. The present study was initially undertaken in the hope that partial enzymatic hydrolysis of some cell surface constituent might yield chemical information about this barrier. Alternately, pretreatment of muscle with hydrolases might be expected to abolish a subsequent response to insulin; from this, information concerning the nature of insulin action on the membrane might be obtained. Neither of these objectives was realized. Instead, it was unexpectedly observed that hydrolases, applied to rat diaphragm muscle, acted themselves like insulin. The work reported here characterizes the insulin-like action of several proteins of the exocrine pancreas on sugar uptake, amino acid accumulation, and glycogen formation in rat diaphram.

Methods and procedure. Male rats of the Holtzman strain, weighing 80-120 g, were anesthetized with ether and the "intact" (caged) diaphragms were excised(1). After rinsing in several changes of Krebs bicarbonate buffer(2) they were placed in individual Erlenmeyer flasks in a metabolic shaker at 37°C. Incubation was done in 13 ml Krebs buffer containing the various substrates,† insulin (or enzymes) and the nonpenetrant raffinose, under a water-saturated gas phase (95% oxygen - 5% carbon dioxide). The frequency of shaking was 110 cycles per minute, the amplitude of the stroke 3 cm. After incubation, the diaphragms were removed from the flasks, blotted, dissected free at the insertions, and weighed. Analyses of muscle for the penetrants were made on extracts prepared by boiling the muscle in 2 ml of distilled water for 15 minutes and deproteinizing with zinc hydroxide(3). Samples of medium after incubation were deproteinized in the same way. D-xylose was estimated with the pentose method of Roe and Rice(4). Extracellular water was determined by raffinose in each diaphragm. A value of 77% of the wet weight was taken as the volume of total tissue water. Raffinose was assayed after hydrolysis, using resorcinol (5). Nelson's method(6), with Somogyi's modified reagent(7), was used to determine 3-0-methyl glucose. L-proline was measured with the low pH ninhydrin method(8). Glycogen was estimated with anthrone, according to the procedure of Seifer *et al*(9) for rat

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† D-xylose or 3-0-Methyl Glucose, 33 mM; L-proline, 1 mM.

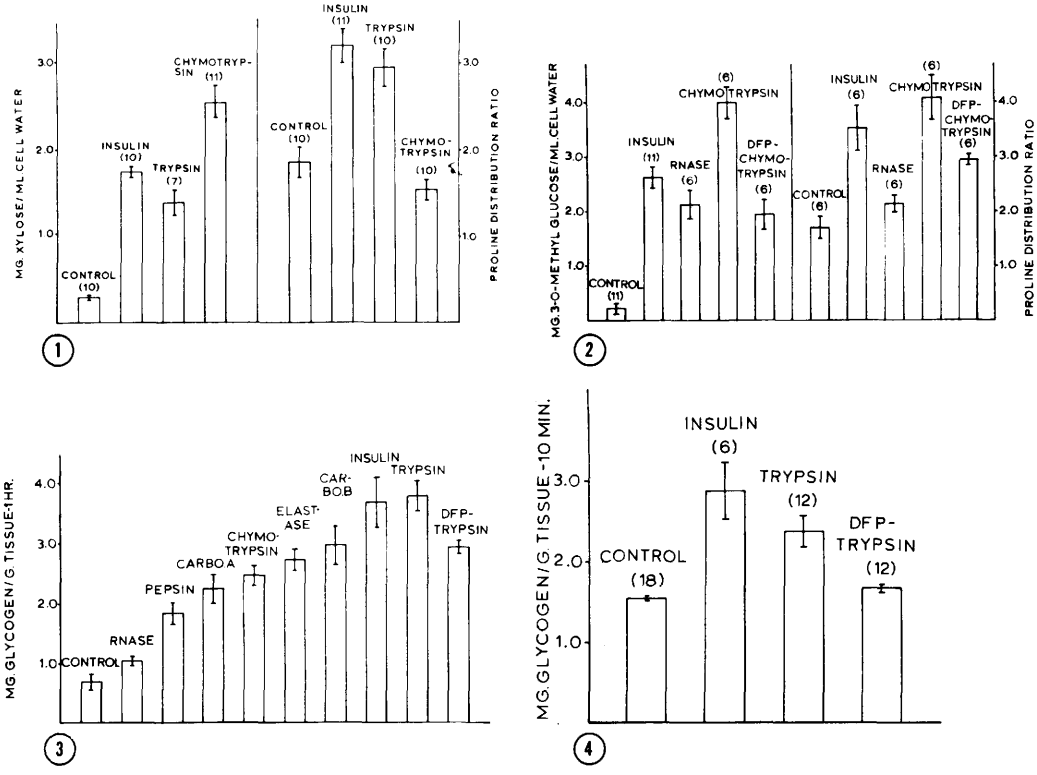

FIG. 1. Intracellular accumulation of D-xylose (left half of figure) and L-proline (right half of figure) in diaphragms incubated for 1 hr; mean and standard error. Figures in parentheses indicate numbers of observations.

FIG. 2. Intracellular accumulation of 3-0-methyl glucose (left half of figure) and L-proline (right half of figure) in diaphragms incubated for 1 hr; mean and standard error. Figures in parentheses indicate numbers of observations.

FIG. 3. Glycogen contents of diaphragms incubated for 1 hr in 5 mg/ml glucose; mean of 6 observations and standard error.

FIG. 4. Glycogen contents of diaphragms incubated for 10 min in 2.8 mg/ml glucose; mean and standard error. Figures in parentheses indicate numbers of observations.

diaphragm. Crystalline beef insulin, a gift from Dr. Mary Root, Eli Lilly and Co., was used at a concentration of 0.5 U/ml (3.67×10^{-6} M). All enzymes, obtained from Worthington Biochemical Corp., were used at 3.67×10^{-6} M.

Results and discussion. Experiments with D-xylose (Fig. 1) and 3-0-methyl glucose (Fig. 2) reaffirm the well known fact that sugar uptake in muscle is minimal in the absence of insulin, and that it proceeds to an appreciable extent only in the presence of the hormone. Ribonuclease, trypsin, and chymotrypsin all stimulate this uptake. The effect of chymotrypsin is particularly striking, being considerably greater than that of insulin. Much of this effect is lost when the diisopropylfluorophosphate-inactivated enzyme is used. The greatly enhanced uptake of xylose in the presence of chymotrypsin is not paralleled by stimulation of proline uptake, which is somewhat less than the control level. This indicates that chymotrypsin does not produce a nonspecific enhancement of cell permeability. There appears to be some antagonism between xylose and proline uptake, as chymotrypsin stimulates the uptake of the amino acid in the presence of 3-0-methyl glucose (Fig. 2). Further selectivity of the enzymes is shown in the case of trypsin (Fig. 1), which particularly favors the uptake of proline. Accumulation of this amino acid in diaphragm is sensitive to insulin(10). Trypsin increases its distribution

ratio to nearly the same level as does insulin. Like insulin, the hydrolases appear to exert a stimulating action not only on membrane transport processes, but also on the intracellular metabolism of glucose. Fig. 3 illustrates this with respect to formation of glycogen from glucose. Appreciable increases in the synthesis of glycogen are obtained with the pancreatic proteases. Trypsin is outstanding in this regard, being as effective as insulin. The DFP (diisopropylfluorophosphate)-derivative of the enzyme is somewhat less potent. Chymotrypsin is not as stimulatory here as is trypsin, despite the much greater encouragement of sugar uptake by the former. These differences suggest a separate action on the intracellular conversion of glucose to glycogen. The rate-limiting step in this synthesis is the transglucosylase reaction(11) whose regulation by insulin has been described(12). The experiments which led Larner and co-workers(11) to suggest an influence of insulin on transglucosylase was their finding that some 90% of the glucose taken up under the influence of insulin in rat diaphragm is converted to glycogen in short periods of incubation. Glucose concentration and incubation time (Fig. 4) were the same as theirs; considerable increases in glycogen formation resulted with trypsin as well as with insulin. DFP-trypsin had no effect. The experiments suggest that trypsin stimulated glycogen formation by an action on the transglucosylase system. Various workers(13,14) have proposed that the catalytic structure in the active sites of chymotrypsin and trypsin involves a histidyl-seryl interaction between an unionized imidazole of histidine and the hydroxyl group of serine. This requires that the serine residue be brought into proximity of a histidine residue. There are opportunities for this to occur in the insulin molecule, where serine B 9 lies next to histidine B 10, and serines A 9 and A 12 lie almost directly opposite histidine B 10. A serine-histidine interaction might thus underlie its physiological action, especially since the integrity of active serine is necessary for full expression of insulin-like behavior of chymotrypsin and trypsin.

Summary. Several pancreatic hydrolases, when applied to rat diaphragm in the same concentration as 0.5 U/ml beef insulin, actually behaved like the hormone. One-hour cellular uptake of D-xylose was enhanced by insulin, chymotrypsin, and trypsin. Uptake of 3-0-methyl glucose was enhanced by insulin, chymotrypsin, and ribonuclease. Accumulation of L-proline, occurring simultaneously with that of sugar, was selectively increased by trypsin or chymotrypsin. DFP-inactivated chymotrypsin was half as effective as the active enzyme in stimulating proline accumulation. Glycogen contents of diaphragms incubated for one hour in 5 mg/ml glucose were increased by trypsin to the same extent as by insulin, and to a lesser extent by carboxypeptidases A and B, elastase, chymotrypsin, and pepsin. Ten-minute incubations in 2.8 mg/ml glucose with insulin or trypsin led to increased glycogen synthesis, while incubation with diiospropylfluorophosphate-inactivated trypsin did not. Attention is focused on the active serine residue of chymotrypsin and trypsin as essential to maximal expression of insulin-like activity of these proteins.

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