Modifiers of Sugar Transport Under the Influence of Muscular Contraction (36603)

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Several investigators have observed that muscle contractile activity (MC) increases the uptake of glucose and related nonutilizable sugars in various isolated muscle preparations (2, 9, 13, 14). Other studies (9, 10) have suggested that humoral factors released by contracting muscle can enhance the entry of sugar into resting muscle. Holloszy and Narahara (11) suggested that the effects of MC and of insulin probably act on the same sugar transport system but produce their modifications of sugar uptake via different mechanisms.

The current study on perfused diaphragm muscle is an attempt to elucidate the nature of the processes controlling transmembrane sugar transport in contracting muscle and the insulin-like effect of MC on sugar uptake (6, 10). Two insulin inhibitory compounds, phlorizin and maleimide, were employed. Phlorizin is known to inhibit the passage of certain monosaccharides into the cells of various tissues as well as the effects of insulin on this transport process (1, 3, 12). Maleimide, a sulfhydryl binding agent, is believed to compete with insulin for its physiological receptor site (5, 7).

Materials and Methods. Isolated perfused diaphragm preparation. The intact muscle preparation has been described by Wermers *et al.* (15). The rat diaphragm was perfused retrograde through the inferior vena cava, cannulated above the diaphragm, at a rate of 1–3 ml/min. Contractions at 7.5/sec were elicited and maintained throughout a 30-min experimental period by stimulation through silver clip electrodes attached to opposite sides of the rib cage. A stimulus strength of 8–15 V with a duration of 0.5 msec was generated by a Grass Model S-4 stimulator.

Experimental procedures. The aerated perfusing medium consisted of a Krebs–Henseleit solution. The muscle was preperfused for 15 min in a medium containing insulin, phlorizin, or maleimide when these substances were used in a subsequent perfusion period. Following preperfusion the muscle was perfused for 30 min with fluid containing the labeled (^{14}C) test sugar at a concentration of 7.3 m*M* with or without one or more of the modifying factors. Electrical stimulation of the muscle (when employed) was begun at the beginning of the 30-min perfusion period. After perfusion, the labeled test sugar was extracted from the right hemidiaphragm and counted in a scintillation counter. Total tissue water was measured by drying a tared sample of diaphragm at 100° for 24 hr.

Extracellular space. In several experiments the uptake of trace amounts of inulin-^{14}C was employed as an indicator to determine the ECS in the presence of each of the modifying factors employed. Subsequent extraction of the indicator showed no significant alteration in the space under any of the experimental conditions. The average value for ECS used in the data calculations of this study was 16.8% of the total tissue water.

The data are presented as a distribution ratio, the fraction of the intracellular water space occupied by the radioactively labeled test sugar at the concentration in the perfusion fluid assumed to be that of the ECS. This fraction, expressed as a percentage, is denoted by *f*.

Results. Findings on the effects of MC and of insulin on the transport of D-xylose, L-arabinose and 3-*O*-methyl-glucose are shown in Fig. 1. Both D-xylose and L-arabinose showed measurable transmembrane transport in the

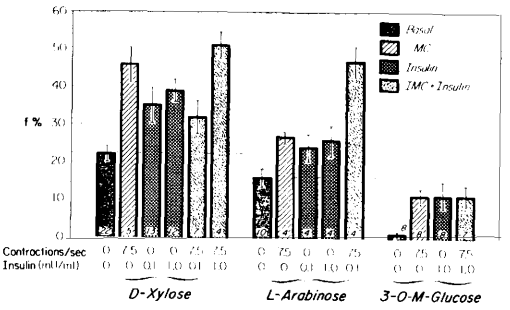

FIG. 1. Effects of muscle contractile activity (MC) and insulin on the intracellular penetration of D-xylose, L-arabinose and 3-*O*-M-glucose in the perfused rat diaphragm. f = fraction of the intracellular water space occupied by the labeled sugar assuming a concentration equal to that existing in the medium. The SEM and the number of experiments are shown for each bar. (- -) Control values.

absence of electrical stimulation or exogenous insulin, *i.e.*, at basal conditions. Under these same circumstances 3-*O*-M-glucose showed no significant cellular uptake.

Preliminary studies showed that stepwise increments in rates of repetitive contraction produced a progressive rise in the intracellular penetration of all three sugars, reaching a plateau at 7.5 contractions/sec. The distribution ratio f increased only slightly as the insulin concentration was elevated from 0.1 to 1.0 mU/ml. Other preliminary studies showed that insulin concentrations above 1.0 mU/ml did not cause a significant additional increase of the f values. Summation of the influences of MC (7.5 contractions/sec) and insulin (0.1 or 1.0 mU/ml) occurred in the case of L-arabinose transport ($p < .005$), but was not apparent in the transport of D-xylose

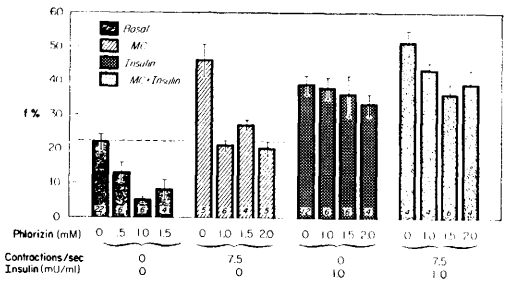

FIG. 2. Modification by phlorizin of the effect of MC and insulin on the percentage of intracellular penetration of D-xylose. Symbols as in Fig. 1.

or of 3-*O*-M-glucose ($p > .10$).

The effects of increasing concentrations of phlorizin on the basal transport of D-xylose, L-arabinose, and 3-*O*-M-glucose, are shown in Figs. 2, 3, and 4, respectively. The data in the left panel of Fig. 2 demonstrate that the intracellular penetration of D-xylose was significantly inhibited at concentrations of the inhibitor up to 1.5 mM ($p < .01$). By contrast, basal transport of L-arabinose, depicted on the left side of Fig. 3, showed no significant alteration at any concentration of phlorizin used ranging from 0.5 to 2.0 mM ($p > .10$). Since 3-*O*-M-glucose did not significantly enter perfused diaphragm muscle cells under basal conditions, no depression of f could be expected for that substance under the influence of phlorizin.

Increasing concentrations of phlorizin were found to diminish the MC effect on the transport of all three sugars studies, as shown

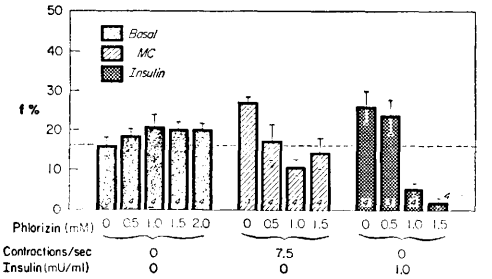

FIG. 3. Modification by phlorizin of the effects of MC and insulin on the percentage of intracellular penetration of L-arabinose. Symbols as in Fig. 1.

in the second panels of Figs. 2, 3, and 4. Inhibition reduced the contraction-related component of sugar uptake to levels which were not significantly different from basal conditions. This inhibitory effect occurred even at the lowest concentration of phlorizin used, 0.5 mM. The modification by phlorizin of the insulin (1.0 mU/ml) stimulated component of sugar uptake, as shown in the third panels of Figs. 2, 3, and 4, appeared to be substantially different from the depression of the MC component. Increasing the concentration of phlorizin to 2.0 or 1.5 mM in the presence of added insulin did not cause a statistically significant inhibition of D-xylose or 3-*O*-M-glucose ($p > .10$) transport. On

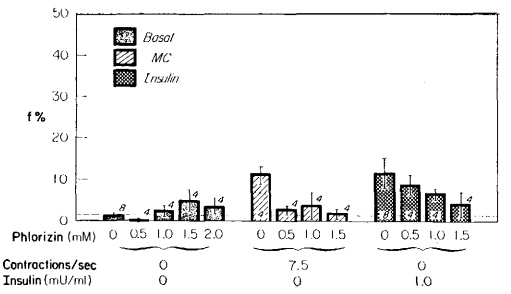

FIG. 4. Modification by phlorizin of the effects of MC and insulin on the percentage of intracellular penetration of 3-*O*-M-glucose. Symbols as in Fig. 1.

the other hand, the insulin-stimulated component of L-arabinose transport was decidedly reduced, even to subbasal levels ($p < .0005$), at phlorizin concentrations of 1.0 and 1.5 m*M* (right portion of Fig. 3).

In studies of the effects of maleimide upon the activity of MC or of insulin in the isolated, perfused rat diaphragm the preperfusion procedure was modified when insulin was used. In this case the preperfusion fluid delivered for 5 min contained maleimide without insulin. It was assumed that this period would allow time for potential insulin binding sites to be occupied by maleimide prior to the introduction of insulin into the preperfusion fluid (8, 16).

Modifications of the effects of MC or of insulin on the transport of the three test sugars by 1.0 m*M* maleimide are shown in Fig. 5. The inhibitor reduced the effects of both MC and insulin on the tranport of D-xylose and 3-*O*-M-glucose, lowering intracellular sugar concentrations to levels which were not significantly different from those of the basal control ($p > .40$). The decrease in the transport of D-xylose produced by this inhibitor was not significant ($.10 > p > .05$). The stimulation of L-arabinose transport by either MC or by insulin did not appear to be significantly altered by the presence of maleimide ($p > .40$).

Discussion. The data reported in these studies provide evidence indicating that MC and insulin produce their effects by different mechanisms (11). Specifically, phlorizin inhibited only the MC, but not the insulin stimulatory effect on the transport of D-xylose and 3-*O*-M-glucose (Figs. 2 and 4). In the presence of maximal MC and insulin stimulation, phlorizin (1.5 m*M*) appeared to inhibit only the MC stimulated component of D-xylose transport, thereby reducing sugar uptake to levels comparable to those observed with insulin alone (Fig. 2, right panel). Then, too, the summation of the maximal effects of MC and insulin, occurring in the case of L-arabinose transport (Fig. 1), lends further support to the above premise.

The results of these studies appear to show that there are at least two different types of transport mechanisms for sugars across striated muscle cell membranes. Specifically, in the rat diaphragm, L-arabinose may be transported by a system separate from that which transports 3-*O*-M-glucose and D-xylose. In support of this premise, Figs. 2 and 3 clearly demonstrate that phlorizin inhibits the basal transport of D-xylose, but not that of L-arabinose. Phlorizin inhibited the action of insulin on the uptake of L-arabinose, but not on the uptake of D-xylose or 3-*O*-M-glucose. Furthermore, maleimide had no discernible effect on the stimulation of L-arabinose transport by MC or insulin, although it inhibited the stimulated transfer of D-xylose and of 3-*O*-M-glucose.

The results of the experiments employing maleimide suggest strongly that the stimulation of the 3-*O*-M-glucose–D-xylose transport system by either MC or insulin involves a thiol–disulfide interchange reaction. In reference to insulin, this result has been observed by several groups of investigators using vari-

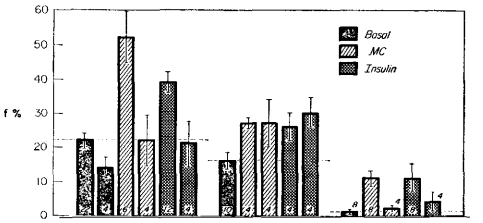

FIG. 5. Modification by maleimide of the effects of MC and insulin on the percentage of intracellular penetration of D-xylose, L-arabinose and 3-*O*-M-glucose. Symbols as in Fig. 1.

ous tissue preparations (4, 7, 16). The competitive nature of this binding process is supported by the studies of Cadenas *et al.* (7) and Whitney, Cutler and Wright (16), who found that pretreatment of the rat heart and the rat diaphragm with insulin prevented subsequent inhibition by maleimide. It can be inferred, therefore, that maleimide prevents the biological action of MC or insulin by interfering with the initial interaction between the modifying agent and the receptor site(s).

Summary. Isolated, perfused rat diaphragm has been studied with respect to changes in the rate of uptake of D-xylose, L-arabinose and 3-*O*-methyl-glucose as influenced by insulin and repetitive muscular contraction in the presence or absence of two inhibitors with differing modes of action, in order to elucidate the question of whether one or more than one mechanism exists for accelerating sugar transport. Evidence from phlorizin and maleimide inhibition of transport of the above three sugars indicates that muscular contraction and insulin accelerate sugar transport by different mechanisms.

The authors thank Dr. M. B. Visscher for his helpful assistance in the preparation of this paper. Crystalline glucagon-free insulin used in these experiments was obtained as a gift from Dr. W. R. Kirtley of Eli Lilly Company, Indianapolis, IN.

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Received Feb. 25, 1972. P.S.E.B.M., 1972, Vol. 140.