Extracellular Cation Concentrations and Sugar Transport Under the Influence of Muscular Contraction and Insulin (36925)

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It has been reported that the transport of noncharged molecules through the biological membranes of various tissues is coupled, by some means, to the movements of one or more ions (1–3). Although this concept is based primarily upon studies of the active transport of sugars across the intestinal mucosa (4, 5), it has been suggested that a similar relationship of sugar to ion transport may apply to facilitated carrier-mediated diffusion into muscle (3, 6).

The stimulatory mechanism(s) of muscle contraction and of insulin on sugar transport remains unclear. In reference to ionic characteristics the literature appears to support the premise that the basal transport of certain sugars and subsequent stimulation by insulin in the rat diaphragm is essentially independent of the distributions of Na^+ and K^+ and of the presence of ouabain (6–9). No studies have been found concerning an ionic influence upon the insulin-like effects of muscle contraction on sugar transport.

This paper describes the influence of ionic alterations of the perfusion medium and of ouabain on the basal sugar transport and on the transport stimulated by muscle contraction or insulin in the perfused rat diaphragm.[1]

Materials and Methods. Isolated perfused diaphragm preparation (10): The rat diaphragm was perfused by retrograde flow through the inferior vena cava, cannulated above the diaphragm, at a rate of 1–3 ml/min. Contractions were maintained throughout a 30 min experimental period at the rate of 7.5/sec by stimulation through silver clip electrodes attached to opposite sides of the rib cage. Stimuli of 8–15 V with a duration of 0.5 msec were generated by a Grass Model S-4 stimulator.

Experimental procedures. The perfusing medium consisted of aerated Krebs–Henseleit solution. Ionic alterations were accomplished by replacing either Na^+ with an equivalent amount of choline$^+$, or K^+ with an equivalent amount of Na^+. Following 15 min of preperfusion with a medium containing insulin, ouabain or the altered ionic species, the muscle was perfused for 30 min with the same fluid, containing D-xylose-^{14}C at a concentration of 7.3 m*M*. Electrical stimulation of the muscle (when employed) was started 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 and extracellular space (ECS) were determined and found to be 79.5 and 16.8% of the wet tissue weight, respectively (10).

The data are presented as a distribution ratio of the calculated concentration of D-xylose in the intracellular water divided by the concentration of D-xylose in the extracellular water. The sugar concentration in the extracellular water is assumed to be equal to that of the perfusion fluid.

Results. The basal level of D-xylose penetration is not significantly altered in the perfused rat diaphragm when extracellular Na^+

[1] Previous studies (10) showed that stepwise increments in rates of repetitive contraction produced a progressive rise in the intracellular penetration of D-xylose reaching a plateau at 7.5 contractions/sec. These studies also showed that insulin concentrations above 1.0 mU/ml did not cause a significant increase in the cellular uptake of D-xylose over that at 1.0 mU/ml.

is entirely replaced with an equivalent amount of choline+ ($p > .40$, Fig. 1). Similar ionic alterations, however, not only abolished the usual muscular contraction effect of increasing D-xylose transport, but actually resulted in a great decrease in sugar movement, as may be seen in the middle portion of Fig. 1. Statistical treatment of the data shows the difference to be highly significant ($p < .0005$). It should be noted that there was no discernible change in muscular activity associated with complete replacement of sodium by choline in the perfusion medium. In contrast the stimulation of D-xylose transport by insulin was raised in the absence of sodium ion ($.05 > p > .025$).

The effect of a K^+-free extracellular fluid on the transport of D-xylose is shown in Fig. 2. The absence of K^+, in contrast to the absence of Na^+, caused a highly significant depression of the basal level of D-xylose transport ($.025 > p > .01$). K^+ depletion, like Na^+ depletion, produced a complete inhibition of the contraction stimulated component of D-xylose transport ($p < .0005$). Stimulation of D-xylose uptake by insulin occurred both in the presence and absence of K^+ ($p < .0005$). The sugar uptake was marginally greater in the absence of K^+ than in the presence of K^+ at the concentration of 5.9

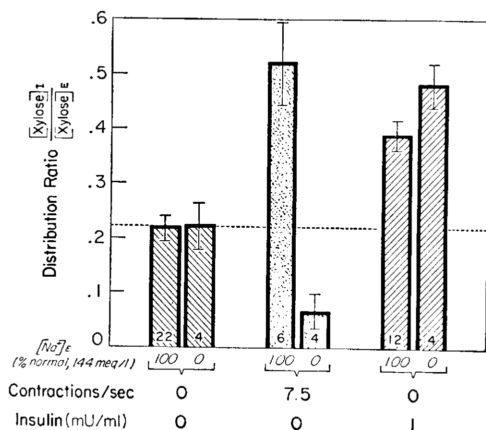

FIG. 1. Effects of extracellular [Na^+] on the intracellular penetration of D-xylose (7.3 mM) stimulated by muscle contractile activity or insulin in the perfused rat diaphragm. The SEM and the number of experiments are shown for each bar. (- - -) Control values.

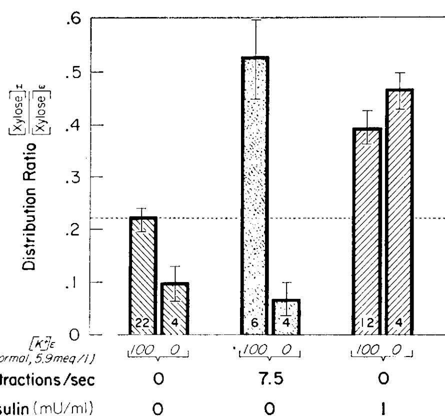

FIG. 2. Effects of extracellular [K^+] on the intracellular penetration of D-xylose (7.3 mM) stimulated by muscle contractile activity or insulin. Symbols as in Fig. 1.

mEq/liter ($.05 > p > .025$).

Figure 3 shows the effects of ouabain on D-xylose transport. When 1 mM ouabain was introduced into a medium containing the normal complement of ions it produced a significant decrease in the basal uptake of D-xylose ($.05 > p > .025$). It also very substantially reduced both the contractile enhancement ($p < .0005$) and the insulin-stimulated ($.005 > p > .0005$) components of D-xylose uptake.

Discussion. Ouabain is known to inhibit the Na^+–K^+ pump (7, 11, 12). The absence of K^+ has also been reported to impede the ionic transfer mechanism (11, 12). On the other hand, an equivalent replacement of Na^+ by choline+ apparently causes little interference with the pump (11). Basal D-xylose uptake is unimpaired only in the latter situation (compare Figs. 1, 2 and 3), a fact that suggests some relationship between a functional ionic pump and sugar transport in skeletal muscle.

The role of ions in the stimulation of D-xylose uptake by muscle contraction or insulin appears to be more complex. Insulin seems to stimulate sugar transport either in the presence or in the absence of Na^+ and K^+ (Figs. 1 and 2). This difference in ionic requirement between muscle contraction and insulin, suggests that these transport modifiers have basically different mechanisms of action (10, 13).

Summary. There appears to be some rela-

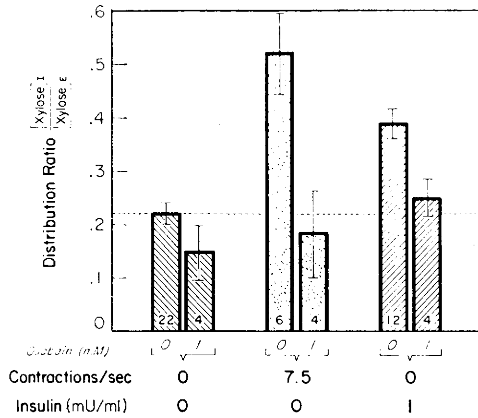

FIG. 3. Effects of 1.0 m*M* ouabain on the intracellular penetration of D-xylose (7.3 m*M*) stimulated by muscle contractile activity or insulin. Symbols as in Fig. 1.

tionship between a funtional Na^+–K^+ pump and basal sugar transport in skeletal muscle. Insulin seems able to stimulate sugar transport either in the presence or absence of Na^+ and K^+. Muscle activity, on the other hand, loses its stimulatory effect on such transport in the absence of either Na^+ or K^+.

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