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Studies on the sugar carrier in skeletal muscle

The operation of the insulin-dependent sugar carrier in skeletal muscle does not depend on any direct coupling with energy-producing mechanisms[1], nor does it require the presence of a Na^+ gradient as is the case with the insulin-independent sugar transport system in the intestine[2]. It is possible, however, that the distribution of Na^+, K^+ and also Ca^{2+} may in some way regulate the effectiveness of the sugar transport system in skeletal muscle[3–5]. At present it is not known whether the basis of such an effect would be in the location of the cations at specific, functionally critical sites in the membrane, or whether their transmembrane movements may in some way influence the sugar transport process. We have found that an electrolyte-deficient extracellular environment promotes an increase in the uptake of the nonmetabolizable sugar derivative 3-*O*-methyl-D-glucose by rat hemidiaphragms. Intact hemidiaphragms were obtained from young hooded rats[6]. Both halves of each pair were preincubated for 20 min at 37° in KREBS–HENSELEIT[7] solution. Subsequently one half, the control, was incubated for a further 30 min in Krebs–Henseleit solution to which had been added the appropriate additives as indicated later. The other half, the test, was incubated for 30 min in a solution which was similar in composition to the Krebs–Henseleit solution, except for the isosmolar replacement of a certain fraction of the sodium chloride with a nonionic, nonpenetrating substance such as the hexitol D-mannitol. The incubation mixtures used for the test and for the control contained 5 mM 3-*O*-methyl-D-glucose, 0.25 munits/ml insulin, as well as tracer quantities of [^{14}C]3-*O*-methyl-D-glucose and [^{3}H]mannitol, the latter for the determination of effective extracellular spaces[5]. Where required, ^{45}Ca was added instead of 3-*O*-methyl-D-glucose. Uptake of isotopes by the tissue was determined by standard double-labeling liquid scintillation techniques and was evaluated as the "percentage penetration"[5]. However, comparison of the test with the control was made as P_t/P_c and was appraised statistically by a two-tailed "*t*"-test[8], taking into account possible values larger or smaller than unity for P_t/P_c. This manner of comparison was necessitated by the variation of P_c from one rat to another, probably because of seasonal, environmental and genetic factors. Whenever P_c was high, and close to the maximum value attainable under these experimental conditions[5], then P_t/P_c was close to unity.

Fig. 1 shows that progressive isosmolar replacement of NaCl with D-mannitol caused an increase in the uptake of 3-*O*-methylglucose and at the same time, also, of ^{45}Ca. The ratio P_t/P_c reached a maximum on replacement of about 65 mM NaCl with mannitol but eventually decreased again and approached unity at very low Na^+ levels, at which all the NaCl and also some $NaHCO_3$ had been omitted. There was no significant enhancement of sugar uptake on replacement of 50 mequiv Cl^- with HCO_3^- or of 50 mequiv Na^+ with Tris or choline. In fact, in the case of choline, such a replacement led to an inhibition (Fig. 2). It is known that Tris may affect some cellular processes[10], but whether it would affect the operation of the membrane-located transport processes is uncertain. Absence of insulin caused low values for percent penetration but did not have much effect on the value of P_t/P_c. Replacement of 50 mM NaCl with 100 mM D-mannitol did not counteract the inhibitory effect of phlorizin, and fully competitive inhibition to the extent of 80 % of the augmented

uptake was still observed. Passive permeability was not noticeably increased, for saturation kinetics were maintained, as was the rigorous stereochemical selectivity of the carrier as is seen in the discrimination between D- and L-arabinose, with a distinct

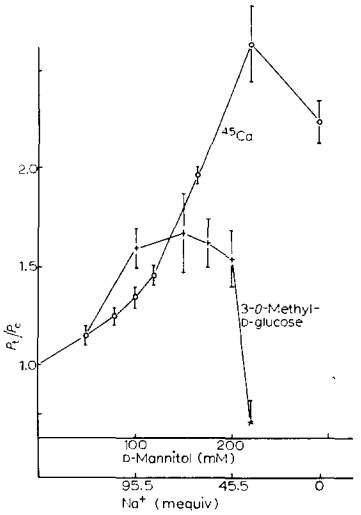

Fig. 1. Isosmolar replacement of NaCl with D-mannitol. Effect on uptake of ^{45}Ca and 3-*O*-methylglucose by rat hemidiaphragms. P_t/P_c = relative stimulation (see text). At least four animals per point, which represents the mean, given with 1 S.E.

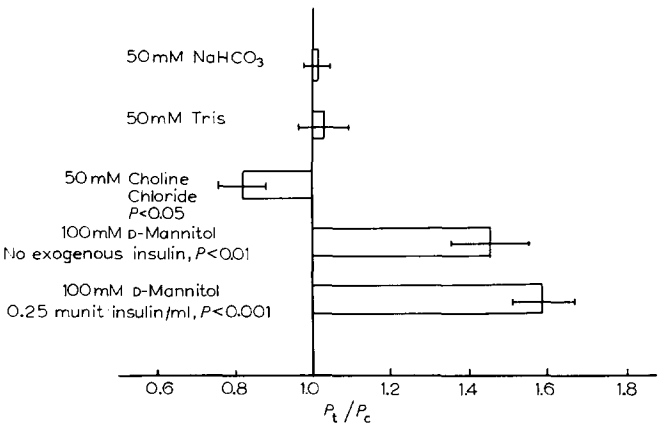

Fig. 2. Effect of NaCl replacement on the uptake of 3-*O*-methyl-D-glucose by rat hemidiaphragms. 50 mM NaCl were replaced with ionic and nonionic substances as indicated. Values given are means ± S.E., for at least six animals per group.

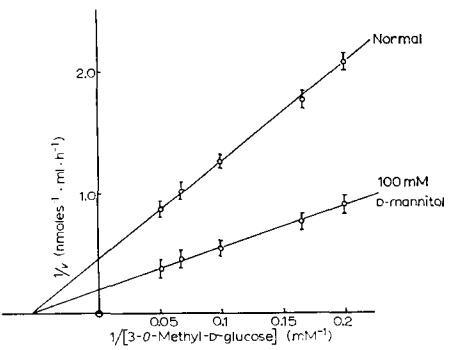

Fig. 3. Lineweaver–Burk plot for the uptake of 3-*O*-methyl-D-glucose by rat hemidiaphragms. At least six animals per point, which gives mean ± S.E.

preference for the latter. The relative uptake, L : D isomer was 8.5 : 1 and for L-arabinose $P_t/P_e = 1.29$, $P<0.01$. Replacement of 50 mM NaCl with 100 mM mannitol led to a change in the apparent maximum velocity of the transport process without any alteration in the apparent affinity of the carrier for 3-*O*-methyl-D-glucose (Fig. 3). The increased value for the apparent v_{max} may reflect an increase in the carrier mobility and is reminiscent of similar changes produced by limited proteolysis[11], or by insulin alone[12], in the uptake of sugar by skeletal muscle. Mechanistically, an increased carrier mobility may be due to a partial reorientation of the stereochemical configuration in the vicinity of the carrier and could be a response to the redistribution of fixed charges in the cell membrane. It is believed that replacement of electrolyte by a nonionic substance could appreciably alter the distribution of fixed charges in the erythrocyte membrane[13]. It has recently been established that a change in the environmental ionic strength does have a considerable effect on the orientation of specific structural regions in the membranes of erythrocytes and mitochondria[14], but similar data for the membranes of skeletal muscle cells are not yet available. Our present data do not unequivocally link the increase in 3-*O*-methylglucose uptake to the decreased availability of Na^+ as such, as is suggested to be the case with adipocytes[9]. However, our data also do not exclude the possibility that the depletion of unielectropositive ions (Na^+) from the membrane may indeed be a contributing factor in the manifestation of the phenomenon described here. For when Tris or choline are used to replace Na^+, there is no increase in sugar uptake as is the case with D-mannitol as Na^+ replacement. The increased influx of ^{45}Ca seen here resembles that observed under similar circumstances with cardiac muscle[15] and with nerve[16], in which the altered Ca^{2+} movement is believed to be due to a change in the character of the Ca^{2+} carrier. While we have established that the altered Ca^{2+} flux accompanies the increased uptake of 3-*O*-methyl-D-glucose under our experimental conditions, the exact role of intracellular and membrane-bound Ca^{2+} in the sugar transport mechanism has still to be determined.

Department of Pharmacology and Therapeutics, D. ILSE
University of Manitoba, Faculty of Medicine, S. ONG
770 Bannatyne Avenue, Winnipeg 3, Manitoba (Canada)

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