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THE INFLUENCE OF A NEW COMPOUND, PATP, ON GLUCOSE TRANSPORT IN THE ISOLATED FAT CELL[1]

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A newly synthesized compound, N-(4-pyridylcarbonyl amino) 1, 2, 3, 6 tetrahydropyridine (PATP) was earlier found to elevate the blood glucose level in rats. This suggested that the compound might be accomplishing this by blocking glucose transport into tissue cells. This hypothesis has now been examined in the isolated fat cell system using a modification of the method to improve the accuracy of measurements made. This study indicated that PATP is a competitive inhibitor of glucose transport and metabolism (K_I = 0.89 mM) but could not unequivocally prove that the effect was on transporter action alone. That the compounds action was at this level, however was shown by its ability to inhibit the uptake of the transported but non-metabolized sugar, 3-O-methyl glucose (K_I = 3 mM). PATP is a non-phenolic inhibitor of glucose transport unrelated in structure to the sugar or to another more potent inhibitor, phloretin.

The facilitated transport of glucose across the plasma membrane of a cell is a complex process made possible by a specific distinct protein named the glucose transporter. A recent review (1) has summarized current knowledge concerning the process and experimental approaches utilized in its investigation. The use of alkylated D-glucose analogs and disaccharides (2) as competitive inhibitors of glucose transport have revealed a number of

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Abbreviations: PATP, N-(4-pyridylcarbonyl amino) - 1, 2, 3, 6, tetrahydropyridine; 3-O-MG, 3-O-methyl glucose.

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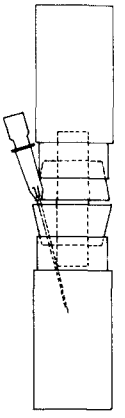
aspects of binding and transfer of the sugar from both sides of the membrane.

A compound of no apparent structural relationship to glucose, namely phloretin has for long been known to be a potent inhibitor of the sugar's transport (3). This triphenol has been definitively shown to competitively inhibit glucose transport in the fat cell (4). Recently the synthesis of PATP and its effect in elevating blood glucose in the rat was described (5). This compound is herein shown to competitively inhibit the uptake and conversion of glucose to CO_2 as well as blocking the uptake of 3-O-MG in the isolated fat cell system. From this it is concluded that a non-phenolic compound, which is unrelated in structure to glucose or phloretin and which blocks glucose transporter action, has been identified.

MATERIALS AND METHODS

The isolated fat cell incubation system described by Rodbell (6) was modified by keeping it 'closed' to increase the accuracy of measurements. All measurements were run in triplicate to reduce the standard error to less than 5% as follows: Two wide-mouth polypropylene scintillation ('Poly-Q') vials (Beckman Instruments, Palo Alto, CA) were attached together through two bored out (no. 3) rubber stoppers with connecting glass tube, as shown just below:



Through the bottom rubber stopper was inserted a #18 hypodermic needle to allow pressure equilibration and acid injection. Into the needle was inserted a 1 ml hypodermic syringe whose barrel was cut off 2 cm. from the tip. Glass wool was inserted into the latter to further slow gas exchange. The lower vial contained 1 ml of incubation media and fat cells. The upper vial contained a 1.8 x 6.8 cm rectangle of Whatman #1 filter paper fitted around the inside of the vial to which 0.23 ml of 1 M hyammine hydroxide in methanol was added. Reaction was initiated by the addition of 0.25 ml of a fat cell suspension. Incubation was for 60 min after which 0.1 ml of 0.63 M H_2SO_4

followed by 0.1 ml of H_2O was injected. This was the amount of acid predetermined to bring the pH of the media to 2.0. Amounts greatly in excess of this released acid (probably HCl) from the media which in turn released collected $^-HCO_3$ from the upper vial as CO_2 resulting in very high standard errors.

The method of measurement of the rate of 3-O-MG uptake in the isolated fat cell is that described by Whitesell and Gliemann (4).

$^{14}C(U)$-glucose and hyammine hydroxide in methanol were obtained from New England Nuclear (Boston, MA). Bovine serum albumin (fraction V) was obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of Analytical Reagent Grade. All water used was purified by reverse osmosis of tap water, then distillation deionization and charcoal treatment (Millipore/Continental Water Systems, Bedford, MA).

RESULTS

In Figure 1 is presented experiments in which the uptake and metabolism of $^{14}C(U)$-glucose to $^{14}CO_2$ is measured as a function of glucose concentration in the absence and presence of two concentrations of PATP. The method of Rodbell (1) for fat cell preparation and measurement was modified to increase precision. The rate of conversion of $^{14}C(U)$-glucose to carbon dioxide followed a sigmoidal pattern. That the latter pattern could not be a rectangular hyperbola as expected was indicated by the same data plotted in a double reciprocal form which is shown in the inset in the

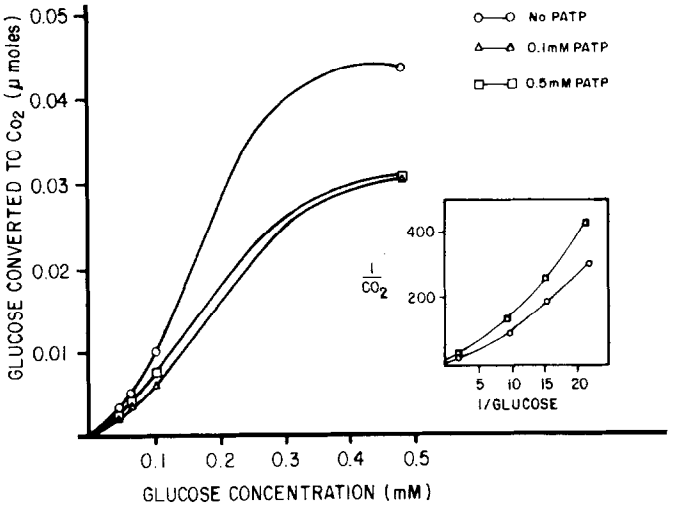


Figure 1. Conversion of glucose to CO_2 by isolated fat cells of the rat in the presence and absence of PATP. Incubation was for 1 hr with $^{14}C(U)$ glucose (0.1 μCi) with the indicated concentrations of unlabelled glucose. $^{14}CO_2$ generated was collected as described in Materials and Methods. Inset: Plotting of the same data in double reciprocal form.

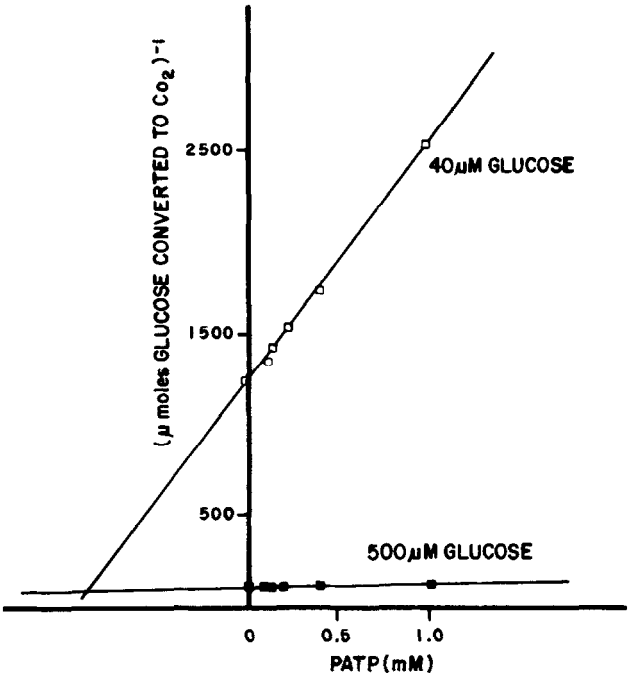


Figure 2. Conversion of two concentrations of glucose plus $^{14}C(U)$ glucose (0.1 μCi) to $^{14}CO_2$ in the presence of varying concentrations of PATP. Isolated fat cells were incubated as described in the legend to Figure 1. The reciprocal of the calculated amount of glucose converted to CO_2 is plotted on the ordinate vs. the PATP concentration on the abscissa.

Figure. As can be seen this data is distinctly non-linear in both the absence and presence of PATP. The latter pattern, noted earlier (1), appears to indicate that the curves intersect on the ordinate and suggests that PATP might be a competitive inhibitor of glucose utilization.

In Figure 2 is shown an experiment similar to that in Figure 1, but one in which PATP concentration was varied at two different concentrations of glucose. The data is presented as described by Dixon and Webb (7) ($1/CO_2$ produced vs. PATP concentration) and obeys a linear relationship at both inhibitor concentrations. The lines cross above the abscissa and thus this result further supports the conclusion that glucose utilization is inhibited competitively by PATP (7). From the point of intersection the K_I was determined to be 0.89 mM and the Vm, 0.010 μmoles/hr.

The results of Figures 1 and 2 suggests that PATP blocks glucose uptake and conversion to CO_2 in a competitive manner, but does not

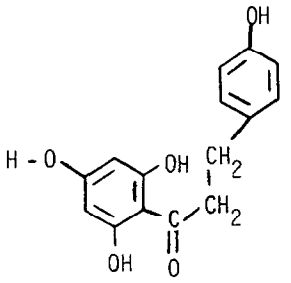
answer the question as to the step at which utilization is blocked. The latter problem was investigated using 3-O-MG, a glucose derivative that is transferred across the plasma membrane by the glucose transporter system, but is not phosphorylated or metabolized further. Using the method of Whitesell and Gliemann (2) the transport of 3-O-MG after 10 sec of incubation at room temperature (24°) was 52.3 $\pm$ 4.4% inhibited in the presence of 3.2 mM PATP. An inhibition constant can be estimated from this data and is 3 mM.

DISCUSSION

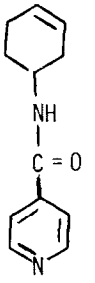
It was earlier reported that PATP can elevate blood glucose levels (5). In the present work it was found to inhibit glucose conversion to CO_2 in a competitive manner. This, however, does not determine by itself that its effect is on glucose uptake alone. However, it provides considerable support for it. The fact that the uptake of the non-metabolized glucose derivative, 3-O-MG, is also inhibited provides confirmatory evidence that the effect is most probably solely on this step.

A much earlier aspect of glucose transporter action was that indicated by its inhibition by phloretin. Le Fevre using human red blood cells (8) tested a number of compounds whose structures were analogous to parts of the phloretin molecule, as well as the diphenolic compounds, diethylstilbestrol and hexesterol. None of the phloretin part-structures inhibited glucose transport appreciably, but diethylstilbesterol and hexesterol were 1.6 times and 0.7 times as potent as phloretin, respectively. Le Fevre concluded (8) that appropriate spacing of phenolic hydroxyl groups is required for efficacy. More recently, however, two nonphenolic compounds, cytochalasin B (9, 10) and furosemide (11) have been shown to inhibit glucose transporter action in the fat cell system.

A comparison of the structures of PATP and phloretin given below shows no obvious relationship to each other. The four aromatic hydroxyl groups in the phloretin-structure suggest a slight relationship to the hydroxyl groups of glucose possibly permitting hydrogen bonding to the transporter active site.



Phloretin



PATP

PATP on the other hand lacks such groups. The inhibition constant for phloretin (.13 μM - ref. 4) is more than 20,000 fold greater than that for PATP (3 mM) and the K_M for 3-O-MG transport (3.5 mM - ref. 4). It is uncertain, therefore, to what part of the glucose transporter molecule PATP binds, but its difference in structure suggests it is other than that of phloretin. From a comparison of the K_I calculated for the two different types of measurements (0.89 vs 3.0 mM) one may conclude that PATP is a slightly better inhibitor of the transport of glucose than of 3-O-MG.

PATP, therefore, may prove to be another valuable tool in the study of glucose transporter action.

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