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Effect of puromycin on sugar transport in isolated rat adipocytes

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This study was performed to determine whether puromycin effects sugar transport into isolated rat adipocytes. Time-course and kinetic studies demonstrated that puromycin competitively inhibited 3-*O*-methylglucose transport. The data indicate that there is an acute effect of puromycin on sugar transport via competitive inhibition that is independent of the inhibition of protein synthesis.

Puromycin, an inhibitor of protein synthesis [1], has been used to determine whether inhibition of protein synthesis decreases amino acid and sugar transport [2–8]. Amino acid transport in rat diaphragm has been shown to be inhibited by puromycin by Elsas et al. [3]. These authors showed in time-course and kinetic studies that this effect was a consequence of the inhibition of protein synthesis by puromycin. Previously Fain [4] showed that both puromycin and puromycin aminonucleoside (which has no effect on protein synthesis) inhibited glucose metabolism in adipose tissue suggesting that puromycin inhibited glucose metabolism independent of protein synthesis. It was not possible to determine from this study whether altered glucose transport or alterations in steps of glucose metabolism beyond glucose transport were responsible for this inhibition by puromycin. Recently, however, Yu et al. [5] indicated that the inhibitory effect of puromycin on xylose uptake was not due to the inhibition of protein synthesis, and suggested that it was due to some effect directly related to sugar transport. The present study was designed to determine whether sugar transport in isolated rat adipocytes is also inhibited by puromycin by a mechanism that is independent of protein synthesis.

Isolated adipocytes were prepared as described previously from epididymal fat pads of ad libitum fed male Sprague-Dawley derived rats weighing 150–180 g [9]. Hepes buffer (pH 7.4 at 37°C) with or without bovine serum albumin (10 mg/ml) was prepared as described previously [10]. Collagenase (Type 1) was from Worthington, bovine serum albumin (fraction V) from Armour Pharmaceuticals, porcine insulin from Eli Lilly, phloretin from ICN Pharmaceuticals, 3-*O*-methylglucose from Aldrich, and puromycin dihydrochloride from Sigma. 3-*O*-Methyl-D-[U-^{14}C]glucose (300–360 mCi/mmol) was from New England Nuclear. Other chemicals were analytical grade.

The uptake of 3-*O*-methyl-D-[U-^{14}C]glucose was measured at 37°C as described by Whitesell and Gliemann [10] with some modifications [9]. Briefly, 12 μl of 3-*O*-methyl-D-[U-^{14}C]glucose was placed in a 3-ml polypropylene tube, then 40 μl of a 40% (v/v) cell suspension of rat adipocytes was squirted onto the isotope. Transport was stopped by the addition of 400 μl of phloretin (0.1 mM) and a 400-μl aliquot of the mixture was added to a microfuge tube (550 μl) containing 100 μl of silicone oil ($D = 0.99$). The tube was centrifuged in a

Abbreviations: methylglucose: 3-*O*-methylglucose; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

microfuge (Beckman Model B) for 30 s and the cell pellet was separated from the infranatant by cutting through the tube at the oil layer. The cell pellet was then added to a 3-ml vial containing 2.5 ml of scintillation fluid and the radioactivity was measured. Extracellularly trapped methylglucose was determined by adding 400 μl of phloretin solution to the isotope solution before adding cells. In kinetic experiments cells were pulsed with labelled and unlabelled methylglucose (to concentrations of 0, 1, 3, 5, 7 and 10 mM) for 1 to 6 s in the absence or presence of puromycin. K_i was determined using Hanes plots as described previously [11].

The time-course of the effect of puromycin on insulin stimulated methylglucose transport in isolated rat adipocytes is shown in Fig. 1. Puromycin maximally inhibited methylglucose transport within 1 min. In order to determine whether puromycin inhibition could be detected by 1 s, puromycin was added directly to isotope solution of a 1-s pulse. Methylglucose uptake for 1 s in the absence of puromycin was 272 ± 30 cpm, whereas the uptake in the presence of puromycin was 51 ± 10 cpm (means ± S.D. of quadruplicates). Therefore, puromycin inhibited methylglucose transport even after 1-s. Puromycin inhibition of methylglucose transport was also observed in the absence of insulin to the same extent as in the presence of insulin (data not shown). Fig. 2 demonstrates the concentration dependence of inhibition in insulin-stimulated methylglucose transport, a

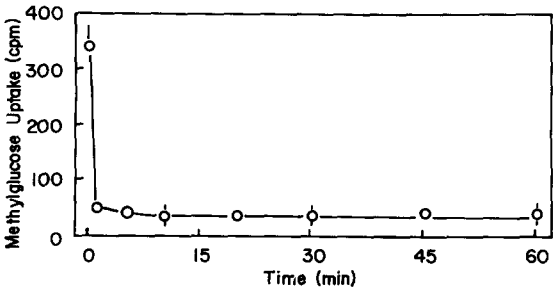

Fig. 1. A typical time-course of the effect of puromycin on insulin-stimulated methylglucose transport in isolated rat adipocytes. Puromycin (1 mM) was added to the cells at zero time. Methylglucose transport was measured by performing a 1-s uptake pulse at each time point shown as described in the text. The points represent means ± S.D. of quadruplicate values.

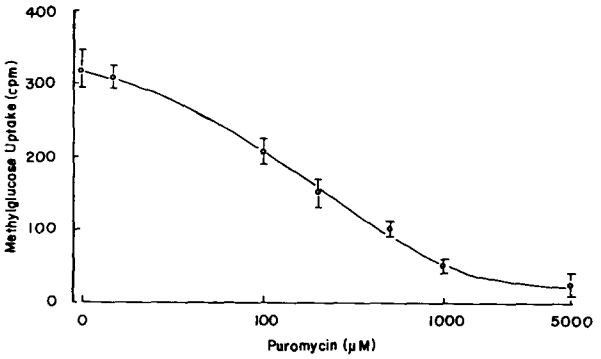

Fig. 2. A typical dose-response relationship of the effect of puromycin on insulin-stimulated methylglucose transport in isolated rat adipocytes. Incubations were carried out in the presence of 10 μM, 100 μM, 200 μM, 500 μM, 1 mM and 5 mM puromycin for 30 min followed by measurement of methylglucose transport using 1-s pulses. The points represent means ± S.D. of quadruplicate values.

half-maximal inhibition occurred at a puromycin concentration of 0.20 mM. Since these results strongly suggested that puromycin inhibited methylglucose transport at the level of methylglucose binding to the carrier, kinetic experiments on insulin stimulated methylglucose transport with or without puromycin were subsequently conducted. Puromycin appeared to inhibit methylglucose transport in a competitive manner with $K_i = 0.36$ mM (Fig. 3).

The results show that puromycin's very rapid

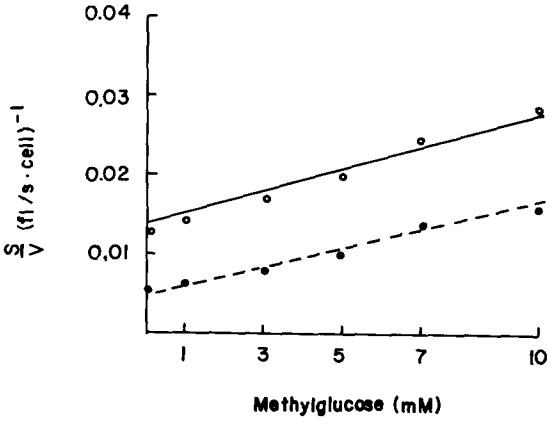

Fig. 3. Kinetic analysis of insulin-stimulated methylglucose transport with (○——○) or without (●-----●) puromycin (0.5 mM) in isolated rat adipocytes. Cells were pulsed with labelled and unlabelled methylglucose (to concentrations of 0, 1, 3, 5, 7 and 10 mM) for 1 to 6 s in the presence or absence of puromycin. The points represent means of quadruplicate values.

(< 1 s) inhibition of sugar transport suggests that the effect is independent of puromycin's inhibition of protein synthesis. In addition, puromycin decreased the affinity of the transport system for methylglucose without affecting the maximal transport velocity. Therefore, puromycin appeared to be a competitive inhibitor of the sugar transport system. The most probable explanation for this competitive inhibition may be the similarity in the ring structure between sugars transported by the glucose transport system and puromycin.

Recently, Yasmeen et al. [8] studied the role of protein synthesis on concanavalin A-stimulated methylglucose transport in rat thymus lymphocytes. They showed that the enhancement of methylglucose transport by concanavalin A was prevented by puromycin but was not prevented by cycloheximide. The difference between the effects of puromycin and cycloheximide suggested that the inhibition of methylglucose transport by puromycin was not due to the inhibition of protein synthesis. This observation is similar to that observed by Yu et al. [5], who demonstrated that cycloheximide, unlike puromycin, did not inhibit xylose uptake in rat soleus muscle. They concluded that the inhibitory effect of puromycin on xylose transport seemed to be attributed not to the inhibition of protein synthesis but rather to some more direct effect on sugar transport itself. These observations previously reported are easily explained by our finding in the present study that puromycin is a competitive inhibitor of the sugar transport system.

On the other hand, puromycin has been shown to decrease amino acid transport in a variety of cells [3,6,7]. This inhibition characteristically occurs only after a suitable period (1–2 h) of exposure to puromycin. In studies of α-aminoisobutyric acid uptake, puromycin decreases the maximal transport velocity without influencing the affinity of the transport system for the amino acid [7]. Therefore, the inhibitory effect of puromycin on amino acid transport appears to be different from that of sugar transport.

In conclusion, the present study provides the first direct evidence that sugar transport can be inhibited by puromycin via competitive inhibition in isolated rat adipocytes. This suggests that studies of the effects of protein synthesis on sugar transport must be interpreted with caution when puromycin is utilized as the inhibitor of protein synthesis.

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