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THE EFFECT OF DIAMIDE AND GLUTATHIONE ON THE UPTAKE OF α-METHYL-D-GLUCOSIDE BY SLICES OF RAT KIDNEY CORTEX

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SUMMARY

The uptake of α-methyl-D-glucoside was stimulated in slices of rat kidney cortex by pretreatment with reduced glutathione. Diamide, an oxidizing agent with high specificity for GSH, caused an inhibition of α-methyl-D-glucoside uptake. These effects appeared to be related specifically to GSH, since dithiothreitol and mercaptoethanol did not increase α-methyl-D-glucoside uptake, and were not as effective as GSH in reversing the effects of diamide. GSH and diamide had no effect on the uptake of another sugar analog, 3-*O*-methylglucose, which is not actively transported. Kinetic studies indicated that GSH increased the apparent V without affecting K_m. The results are discussed in terms of the possible role of GSH in the process of sugar transport.

INTRODUCTION

α-Methyl-D-glucoside is a non-metabolizable sugar which is actively accumulated by slices of rat kidney cortex, and resembles glucose and galactose in its transport characteristics [1]. In recent years detailed in vitro investigations of sugar transport in renal cortex slices have been reported. This has resulted in delineation of transport characteristics such as pH, extracellular Na^+, cyclic AMP, developmental characteristics and others [1–4].

Diazene dicarboxylic acid bis-(*N*,*N*-dimethylamide), commonly called diamide, has been shown by Kosower and his coworkers to be highly specific for the oxidation of GSH [5], although there is some suggestion that oxidation of NADPH also occurs [6]. Addition of diamide to human red blood cells led to stoichiometric oxidation of intracellular GSH, and this process was very rapid even at low temperature. Diamide has been used to lower GSH levels in the lens of the rat, in Ehrlich ascites tumor cells and in adipose tissue cells [7–9].

In a previous communication from our laboratory it was shown that diamide could be used to lower GSH levels in slices of rat kidney cortex [10]. In addition it was shown that diamide caused inhibition of amino acid uptake in these slices. The present study was undertaken to investigate the effect of diamide on the accumulation of α-methyl-D-glucoside. In view of our findings with amino acids it was of interest to determine whether the effect of diamide was specific for the transport of amino

acids or affected other transport systems as well. A preliminary report of this work has been presented [11].

MATERIALS AND METHODS

Adult female Sprague-Dawley rats were used throughout the investigation and were sacrificed by cervical dislocation. Kidneys were quickly removed and placed in ice-cold saline. After the capsule was removed, the kidneys were bisected; three slices of cortical tissue were made with a Stadie-Riggs microtome and the first slice was discarded. A typical experiment involved three rats; 24 slices were used to make eight samples, each sample containing a cortical slice from each of the rats.

For uptake studies, the slices were placed in 3.0 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, which had been freshly gassed with $O_2 : CO_2$ (95 : 5). The samples were sealed and placed in a shaking ice bath for 15 min (Incubation I). The samples were then transferred to flasks containing 3.0 ml of fresh Krebs-Ringer bicarbonate buffer and were shaken for 15 min at room temperature (Incubation II). The slices were transferred to flasks containing labelled and unlabelled sugar (180 μM and 0.4 μCi) in 2.0 ml of Krebs-Ringer bicarbonate buffer. These samples were incubated in a shaking water bath at 37 °C for various times (Uptake Period). Slices were then transferred to conical glass centrifuge tubes containing 2.0 ml water, sealed and placed in a boiling water bath for 10 min. After cooling, the tubes were spun at 3000 rev./min for 10 min. An aliquot of 0.2 ml was then taken from the supernatant and also from the media used for the uptake period and placed in scintillation vials. 2.8 ml absolute ethanol and 7.0 ml toluene/PPO-POPOP (0.457 % and 0.010 %) were then added and the radioactivity was counted in a Packard tricarb liquid scintillation spectrometer with a counting efficiency of 83 %. Distribution ratios were calculated according to Rosenberg et al. [12].

Experiments were conducted in order to determine if GSH affected the rate at which α-methyl-D-glucoside was released from renal cortical slices [13]. Slices were incubated at room temperature in the presence or absence of GSH and then incubated in labelled and unlabelled sugar for 15 min at 37 °C. The slices were then placed in flasks containing 3.0 ml Krebs-Ringer bicarbonate buffer and incubated at 37 °C for 18 min. Aliquots of 0.2 ml were removed from the flasks at 3 min intervals and sampled for radioactivity. After 18 min the tissue was removed and the amount of radioactivity remaining in the tissue was determined.

α-Methyl D-[^{14}C]glucoside, specific activity 50 mCi/mM, was purchased from Cal Atomic. 3-*O*-Methyl D-[^{14}C]glucose, specific activity 53.9 mCi/mM, was purchased from New England Nuclear.

RESULTS

The uptake of α-methyl-D-glucoside by slices of rat kidney cortex is shown in Fig. 1. Distribution ratios represent the ratio of cpm/ml intracellular fluid to cpm/ml extracellular fluid. The extracellular fluid volume was calculated using [^{14}C]inulin, while the total tissue water was calculated as the difference between wet and dry weight; these values equalled 28 % and 80 % of tissue weight respectively and did

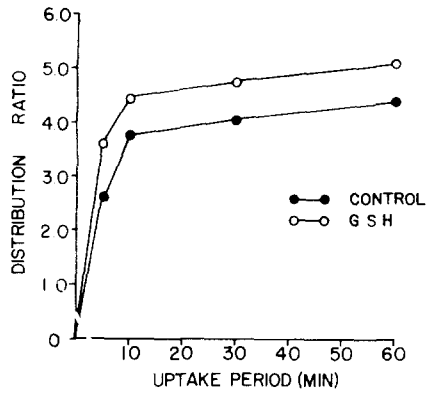

Fig. 1. Accumulation of α-methyl-D-glucoside in control and GSH-treated slices of rat kidney cortex. Three cortical slices, weighing approx. 20 mg each, were incubated in Krebs-Ringer bicarbonate buffer for 15 min at 4 °C. Slices were transferred to flasks containing buffer with or without 20 mM GSH and incubated for 15 min at 25 °C. Samples were then placed in flasks containing α-methyl-D-glucoside (0.4 μCi and 180 μM) in 2.0 ml buffer and incubated at 37 °C for various times. Distribution ratios were calculated as described under Methods. Each point represents the average of triplicate determinations.

not vary significantly with different conditions of incubation. The data in Fig. 1 demonstrate that distribution ratios increase in magnitude as the uptake period is lengthened. Control samples, which are incubated in buffer only before the uptake period, reach a distribution ratio of greater than 1 very rapidly, indicating accumulation of the sugar against a concentration gradient. Samples which were pretreated with 20 mM GSH show distinctly higher levels of sugar uptake: this increase can be noted at the earliest time period tested, indicating that GSH increases the initial rate of entry of α-methyl-D-glucoside as well as the steady state level of accumulation.

Additional work has shown that the release of α-methyl-D-glucoside from GSH-treated slices is similar to release from control samples (unpublished data). The percent of sugar released per 3 min interval for either control or GSH-treated slices was virtually identical over an 18 min period. After 18 min, 88 % of the original concentration of α-methyl-D-glucoside from untreated slices had been released into the medium, while 89 % of the sugar had been released from GSH-treated slices. GSH thus appears to stimulate the accumulation of α-methyl-D-glucoside specifically by increasing the rate of sugar uptake.

In order to determine the specificity of the effect of GSH on sugar transport, we investigated the effect of diamide, a highly specific GSH oxidant, on sugar accumulation. The results shown in Table I indicate that diamide effectively inhibits the uptake of α-methyl-D-glucoside, and that GSH can reverse this effect. These results are very similar to those obtained previously with several amino acids [10]. However, α-methyl-D-glucoside accumulation was stimulated by treatment with GSH alone. Table I also presents similar data on the uptake of 3-*O*-methylglucose, which is not actively accumulated by cortical slices [4] (the distribution ratio remains less than one). It is apparent from these results that neither GSH nor diamide have a significant effect on the accumulation of this sugar.

The concentration of GSH used in the previous experiments was 20 mM. This

figure was arrived at on the basis of the data presented in Fig. 2. In these experiments increasing concentrations of GSH were used to stimulate the uptake of α-methyl-D-glucoside. Maximum stimulation of sugar accumulation occurred at a GSH concentration of 20 mM. At concentrations above 40 mM, sugar accumulation was in-

TABLE I

THE EFFECT OF GSH AND DIAMIDE ON THE UPTAKE OF α-METHYL-D-GLUCOSIDE AND 3-*O*-METHYLGLUCOSE

Slices were incubated in Krebs-Ringer bicarbonate buffer in the absence or presence of $3 \cdot 10^{-2}$ M diamide for 15 min at 4 °C (Incubation 1). Tissues were then placed in buffer with or without GSH ($2 \cdot 10^{-2}$ M) for 15 min at 25 °C (Incubation 2). The tissues were then incubated for 60 min at 37 °C in the presence of labelled and unlabelled sugar (180 μM and 0.2 μCi/ml). Distribution ratios were calculated as under Methods. Values represent mean distribution ratio ± S.E. Number of determinations is given in parentheses.

Incubation 1	Incubation 2	Distribution ratios	
		α-Methyl-D-glucoside	3-*O*-methylglucose
Krebs-Ringer bicarbonate buffer	Krebs-Ringer bicarbonate buffer	3.80±0.12 (8)	0.79±0.03 (4)
Krebs-Ringer bicarbonate buffer	GSH	5.24±0.28 (8)	0.71±0.05 (4)
Diamide	Krebs-Ringer bicarbonate buffer	2.09±0.26 (7)	0.79±0.02 (4)
Diamide	GSH	3.66±0.20 (8)	0.82±0.06 (4)

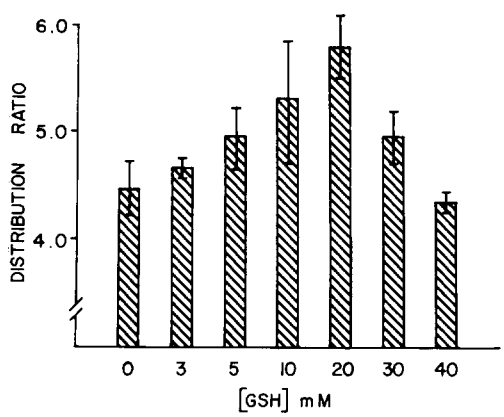

Fig. 2. Effect of GSH concentration on α-methyl-D-glucoside accumulation. Slices were incubated in Krebs-Ringer bicarbonate buffer for 15 min at 4 °C and then transferred to flasks containing increasing amounts of GSH. After 15 min at 25 °C the slices were transferred to flasks containing α-methyl-D-glucoside (0.2 μCi/ml and 180 μM) and incubated for 60 min at 37 °C. Distribution ratios were calculated as under Methods. Each column represents the average of four separate determinations.

TABLE II

EFFECT OF THIOL COMPOUNDS ON THE ACCUMULATION OF α-METHYL-D-GLUCOSIDE

Slices were incubated at 4° C in Krebs-Ringer bicarbonate buffer for 15 min and were then treated with various sulfhydryl compounds at a concentration of 20 mM. α-Methyl-D-glucoside was used at a concentration of 180 μM and 0.2 μCi/ml and the uptake period was 60 min at 37° C. Data represent mean distribution ratios ± S.E. from four separate experiments.

Treatment	Distribution ratio
None	5.49 ± 0.20
GSH	6.73 ± 0.51
Dithiothreitol	5.19 ± 0.54
Mercaptoethanol	5.30 ± 0.57

hibited, and at a GSH concentration of 100 mM, no active accumulation took place.

Another question which remained to be answered was whether or not the results obtained with GSH could be duplicated with other sulfhydryl compounds. Table II shows that neither dithiothreitol nor mercaptoethanol was capable of stimulating the uptake of α-methyl-D-glucoside. In addition, the data in Table III indicate that neither dithiothreitol nor mercaptoethanol was as effective as GSH in reversing the effect of diamide on sugar uptake. These results indicate that GSH is specifically involved in α-methyl-D-glucoside accumulation, and that diamide inhibits the uptake of this sugar by virtue of its reaction with GSH.

The uptake of α-methyl-D-glucoside follows saturation kinetics. Fig. 3 represents a Lineweaver-Burk plot from uptake data and compares control and GSH-

TABLE III

THE EFFECT OF THIOL COMPOUNDS ON THE DISTRIBUTION RATIO OF α-METHYL-D-GLUCOSIDE IN DIAMIDE-TREATED SLICES

Slices were incubated in Krebs-Ringer bicarbonate buffer in the absence or presence of $3 \cdot 10^{-2}$ M diamide for 15 min at 4° C (Incubation 1). Tissues were then placed in buffer with or without thiol compounds ($2 \cdot 10^{-2}$ M) for 15 min at 25° C (Incubation 2). The tissues were then incubated for 60 min at 37° C in the presence of labelled and unlabelled α-methyl-D-glucoside (180 μM and 0.2 μCi/ml). Distribution ratios were calculated as under Methods. Values represent mean distribution ratio ± S.E. from four separate experiments.

Incubation 1	Incubation 2	Distribution ratio
Krebs-Ringer bicarbonate buffer	Krebs-Ringer bicarbonate buffer	4.55 ± 0.26
Diamide	Krebs-Ringer bicarbonate buffer	1.68 ± 0.16
Diamide	GSH	4.38 ± 0.16
Diamide	Dithiothreitol	3.32 ± 0.20
Diamide	Mercaptoethanol	4.04 ± 0.17

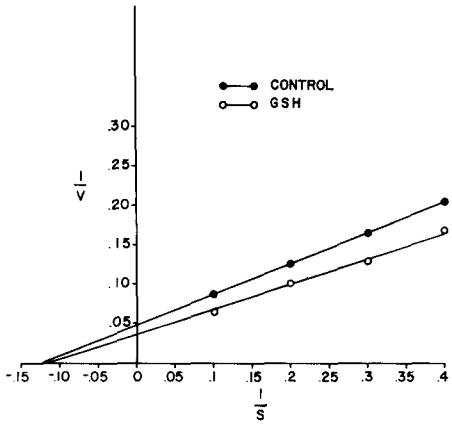

Fig. 3. Lineweaver-Burk plot of the effect of GSH on α-methyl-D-glucoside uptake by slices of rat kidney cortex. Slices were incubated in Krebs-Ringer bicarbonate buffer for 15 min at 4 °C and then incubated in buffer with or without 30 mM GSH for 15 min at 25 °C. Subsequently, tissues were placed in flasks containing 0.4 μCi and various concentrations of unlabelled α-methyl-D-glucoside and incubated for 15 min at 37 °C. The amount of radioactivity in the intracellular space was corrected for diffusion and converted to μmol by using the specific activity of the incubation medium. Velocity was expressed as μmol sugar accumulated/15 min/ml intracellular fluid. Substrate concentration varied from 2.5 mM to 10 mM. Each point represents the average of triplicate determinations.

treated samples. Intracellular radioactivity has been converted to μmol of sugar using the specific activity of α-methyl-D-glucoside in the incubation medium and correcting for non-saturable uptake [14]. The K_m for control samples is 8.1 mM, with a V of 20.8 μmol/15min/ml. GSH-treated slices have a V of 27.0 μmol/15 min/ml while the K_m is virtually unchanged.

DISCUSSION

The results presented in this communication establish the following points: 1) GSH stimulates the uptake of α-methyl-D-glucoside in slices of rat renal cortex while other SH compounds do not; 2) diamide, which oxidizes intracellular GSH, inhibits the uptake of this sugar; 3) GSH can reverse this effect of diamide.

Previous work in our laboratory has indicated that diamide inhibits the accumulation of a wide variety of amino acids, without affecting the uptake of hippuran, which is actively secreted across the brush border of luminal cells. The demonstration that diamide inhibits the transport of α-methyl-D-glucoside, but has no effect on 3-*O*-methylglucose, which is not actively accumulated, suggests that GSH is involved in the process of active transport of sugars and amino acids.

The present studies were carried out in tissue slices and thus may not represent the actual in vivo situation. However, the transport of sugars and amino acids in vivo is presumably accomplished by the action of membrane-bound carrier proteins. Much work has been done on the effect of various sulfhydryl inhibitors on the uptake of amino acids and sugars [15, 16], and it is not unreasonable to assume that such carrier proteins contain reactive sulfhydryl residues [17]. It is our contention that GSH, by virtue of its size, charge, and reducing power, may serve to reduce disulfide

linkages on the carrier proteins. The hypothesis that GSH can reduce disulfide linkages in proteins and affect their transport activity is, of course, speculative. Nonetheless, GSH is a ubiquitous compound that is known to be essential for the maintenance of membrane integrity [5]. The inhibition of sugar and amino acid accumulation caused by diamide indicates that the transport process requires the presence of GSH to function properly.

Further work is needed to test the validity of this hypothesis. Studies are currently underway in our laboratory to determine the effect of GSH and diamide on amino acid and sugar binding to isolated brush border fragments from rat kidney (Mendicino, J., Leibach, F. H., Weiner, C. and Pillion, D. T., in preparation).

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