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INHIBITION OF 2-DEOXY-D-GLUCOSE TRANSPORT BY THEOPHYLLINE, CAFFEINE AND PAPAVERINE IN ALVEOLAR MACROPHAGES

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Alveolar macrophages, pulmonary defense cells, play an important role in bacterial clearance from the lung (1). Theophylline, prostaglandin E_1 and dcAMP, all of which are presumed to elevate intracellular cyclic AMP levels, inhibit the glucose conversion to CO_2 in alveolar macrophages* (2,3). In a previous study of the effects of various pharmacological agents on glucose transport in alveolar macrophages, we reported that a phosphodiesterase inhibitor, theophylline, inhibits 2-deGlu transport (4). This report suggests that theophylline inhibits 2-deGlu transport in alveolar macrophages by a mechanism not related to its inhibition of phosphodiesterase. Our findings also indicate that cyclic AMP may not play a role in hexose transport by alveolar macrophages.

Materials and Methods: [^{3}H]2-deoxy-D-glucose and [^{14}C]sucrose were purchased from New England Nuclear; caffeine, papaverine and dcAMP from Sigma Chemical; dcGMP from Boehringer Co.; and theophylline from Calbiochem. Co. Prostaglandin E_1 was donated by Dr. John Pike of Upjohn Co., Kalamazoo, Michigan.

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*Abbreviations used: 2-deGlu, 2-deoxy-D-glucose; KRPS, Krebs Ringer Phosphate solution; dcAMP, $N^6,O^{2'}$-dibutyryl adenosine 3',5' cyclic monophosphate; dcGMP, $N^6,O^{2'}$-dibutyryl guanosine 3',5' cyclic monophosphate.

Alveolar macrophages from New Zealand white rabbits were obtained by pulmonary lavage with Krebs-Ringer Phosphate solution, washed once with the same solution at 4°C and the aliquots prepared as previously described (5).

Approximately 20 x 10^6 cells were suspended in KRPS, pH 7.4. At time zero 0.03 mCi of [^{3}H]2-deGlu and 0.1 µCi of [^{14}C]sucrose were added. After adding the required amounts of cold 2-deGlu and inhibitors, the volume was adjusted to 2.0 ml with KRPS. The cells, with air as the gas phase, were incubated for up to an hour at 37°C in a Dubnoff metabolic shaker, and the cells then recovered by centrifugation at 750 x g for 10 minutes at 4°C. Since the 2-deGlu uptake was linear for 30 minutes (4), 15 minute incubation periods were used to determine the initial rates of 2-deGlu uptake for kinetics experiments (V_i = nmoles 2-deGlu transported/min/10^6 AM). Aliquots of both the supernatants and the cell sonicates were counted in a Packard Tri-Carb liquid scintillation spectrometer. Since sucrose does not enter the cell (4,6), [^{14}C]sucrose counts in both the cell sonicate and the supernatant were used to calculate the trapped extracellular 2-deGlu as follows:

$$\text{Trapped extracellular } [^3\text{H}]\text{2-deGlu} = \frac{[^{14}\text{C}]\text{ pellet}}{[^{14}\text{C}]\text{ supernatant}} \times [^3\text{H}]\text{2-deGlu supernatant}$$

The amount of intracellular 2-deGlu was then calculated by deducting the trapped extracellular 2-deGlu from the pellet 2-deGlu. The transported sugar was fractionated using the Barium-Zinc reagent and the amounts of free and phosphorylated 2-deGlu determined as previously reported (4).

Results and Discussion: The kinetics of 2-deGlu transport inhibition were studied by determining the initial rates of 2-deGlu uptake (V_i), in the presence of 3, 5, 7 and 9 mM theophylline over an external 2-deGlu concentration range of 0.1 to 5.0 mM. The high concentration (9mM) of theophylline did not affect the cell viability as judged by eosin Y exclusion. The data presented as Lineweaver-Burke plots (Fig. 1) show that (1) the inhibition of 2-deGlu uptake by theophylline is dose-related and (2) since both theophylline and the control cell plots have a common ordinal intercept, theophylline in concentration range of 3-9 mM acts as a competitive inhibitor of 2-deGlu transport. The Dixon plots

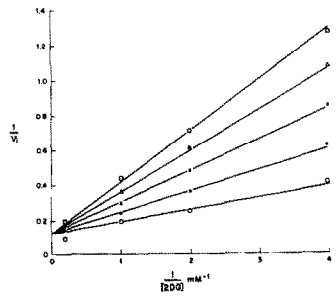

Fig. 1. Lineweaver-Burke plots of inhibition of 2-deGlu transport by various concentrations of theophylline. The lines are drawn by the least square method, and the points represent an average of four flasks. (O) control cells, (●) theophylline 3 mM, (x) theophylline 5 mM, (Δ) theophylline 7 mM and (□) theophylline 9 mM.

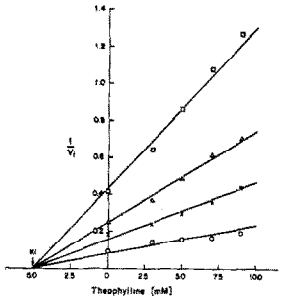

Fig. 2. Dixon plots of inhibition of 2-deGlu transport by various concentrations of theophylline. The lines are drawn by the least square method and the points represent an average of four flasks. The concentration of 2-deGlu was (□) 0.25 mM, (Δ) 0.50 mM, (x) 1.0 mM and (O) 5.0 mM.

(Fig. 2) depict initial rates of 2-deGlu transport against various concentrations of theophylline. The common abscissal intercept confirms the competitive nature of the inhibition and shows the apparent dissociation constant for the inhibitor to be 5.0 mM. Since theophylline (1,3-dimethylxanthine) is not a structural analogue of 2-deGlu, it is surprising to find that it is a competitive inhibitor of the sugar transport. The linearity of the Dixon plots further indicates that the inhibition does not conform to a classical allosteric type of inhibition (7). In these respects, the inhibitory effect of theophylline is similar to that of cytochalasin B, which also acts as a competitive inhibitor of 2-deGlu transport in alveolar macrophages (4).

The effects of several other agents presumed to act via elevation of intracellular cyclic AMP levels on 2-deGlu uptake and phosphorylation by alveolar macrophages are presented in Table 1. Theophylline, caffeine and papaverine caused 37%, 41% and 57% diminution in the 2-deGlu uptake, respectively, without affecting the phosphorylation of the transported sugar. However, two cyclic

TABLE 1.

Effects of Drugs Affecting Cyclic AMP Levels on 2-deGlu Uptake[a]

	Concentration mM	n[b]	nmoles 2-deGlu transported/min/ per 10^6 alveolar macrophage	% Phosphorylated	% Free
Control		10	0.135 ± 0.013	82.9 ± 2.6	17.1 ± 2.6
Theophylline	3.0	10	0.087 ± 0.016*** [c]	80.0 ± 4.1	20.0 ± 4.1
Caffeine	3.0	4	0.080 ± 0.020**	85.2 ± 3.6	14.8 ± 3.6
Papaverine	0.1	7	0.056 ± 0.006***	78.4 ± 5.5	21.6 ± 5.5
dcAMP	3.0	8	0.139 ± 0.028	80.3 ± 3.5	19.7 ± 3.5
dcGMP	3.0	4	0.123 ± 0.018	79.9 ± 3.6	20.1 ± 3.6
PGE_1	0.05	4	0.115 ± 0.014	76.2 ± 4.5	23.8 ± 4.5

[a] Values represent average of given number of experiments ± 1 S.E. The incubations were carried out at 37°C for one hour in the presence of 8 mM 2-deGlu.

[b] Number of experiments performed in triplicate.

[c] ** $p = < 0.01$
*** $p = < 0.001$.

nucleotide analogues, dcAMP and dcGMP, failed to affect 2-deGlu transport (Table 1). This failure was unexpected since these agents, along with the phosphodiesterase inhibitors, may reasonably be presumed to elevate intracellular levels of cyclic nucleotides as shown with other phagocytic cells, e.g. polymorphonuclear leukocytes (8). Furthermore, prostaglandin E_1, which increases cyclic AMP levels in polymorphonuclear leukocytes by activating adenyl cyclase (8-11), also failed to affect the 2-deGlu transport. Three possible explanations for these apparently conflicting results have been considered. First, the three phosphodiesterase inhibitors might affect substrate and ATP metabolism in alveolar macrophages, thereby decreasing the available energy from oxidative phosphorylation, an essential requirement for 2-deGlu transport (4). This explanation can probably be excluded since these three drugs did not affect the

phosphorylation of the transported sugar (Table 1). Second, dcAMP, dcGMP and prostaglandin E_1 may not enter the cell in sufficient quantities to raise the intracellular level of cyclic AMP. This explanation is unlikely since these same concentrations of dcAMP (3mM) and prostaglandin E_1 (0.05mM) significantly affected glucose conversion to CO_2 in both resting and phagocytosing alveolar macrophages (2). Further, other investigators (8,10,12,13) have shown that, in polymorphonuclear leukocytes, dcAMP and prostaglandin E_1 affect leukocyte functions and elevate cyclic AMP levels. Third, and the most likely, theophylline, caffeine and papaverine may exert their inhibitory effect on 2-deGlu transport in alveolar macrophages by an unknown mechanism independent of phosphodiesterase inhibition. This explanation is strengthened by observations that the effect of theophylline on adipose tissue metabolism is unrelated to its activity as a phosphodiesterase inhibitor (14).

Elucidation of the role of cyclic AMP in 2-deGlu transport by alveolar macrophages must await direct measurements of intracellular cyclic AMP levels. However, the inhibition of 2-deGlu transport by theophylline, caffeine and papaverine is clear and is not produced by cyclic nucleotide analogues or prostaglandin E_1. This suggests the inhibition of 2-deGlu transport in alveolar macrophages by theophylline, caffeine and papaverine is independent of cyclic AMP levels.

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