COMPETITIVE INHIBITION OF THE TRANSPORT OF NUCLEOSIDES, HYPOXANTHINE, CHOLINE AND DEOXYGLUCOSE BY THEOPHYLLINE, PAPAVERINE AND PROSTAGLANDINS

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SUMMARY

Theophylline, papaverine and prostaglandins E_1 and $F_{2\alpha}$ competitively inhibit the transport of uridine, thymidine, hypoxanthine, choline and deoxyglucose by cultured Novikoff rat hepatoma cells. The transport processes are maximally inhibited immediately upon addition of the drugs and the effects are readily reversed by their removal.

Theophylline, papaverine and aminophylline, inhibitors of cyclic AMP phosphodiesterase, and prostaglandin E_1 (PGE_1) are frequently used alone or in combination with dibutyryl cyclic AMP to assess the physiological functions of cyclic AMP in various cellular processes, including the transport of nutrients into the cell (1-7). Our present study, however, indicates that caution must be exercised in the latter studies because these substances themselves, competitively inhibit the transport of a wide variety of precursors into the cell.

MATERIALS AND METHODS

Novikoff rat hepatoma cells (subline N1S1-67) were propagated in suspension culture in Swim's medium 67 as described previously (8,9). Cells were harvested by centrifugation from cultures in the late exponential phase of growth and suspended to 2×10^6 cells/ml of various basal media (see legends to Figs.). Samples of the suspensions were supplemented

with the various agents to be studied and ^{3}H-labeled precursors as indicated in the appropriate experiments and monitored for the incorporation of radioactivity into total cell material (acid soluble plus acid-insoluble) and radioactivity in acid-insoluble material as described previously (10-14).

RESULTS AND DISCUSSION

Fig. 1A illustrates the inhibition of uridine incorporation into total

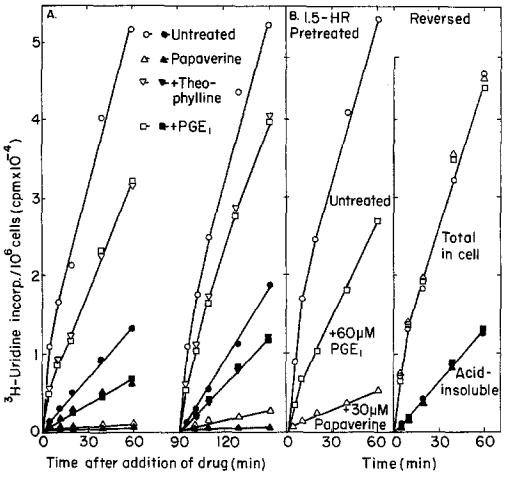

Fig. 1. Effects of papaverine, theophylline and PGE_1 on uridine incorporation into total cell material and acid-insoluble material (A) and reversibility of the effect (B). (A) Duplicate samples of suspension of cells in basal medium 42(BM42; 10) were supplemented as indicated with 270 μM papaverine (Merck, Sharpe and Dohme), 550 μM theophylline (Mann Res. Lab) or 30 μM PGE_1 (0 time). Immediately thereafter, one set of samples received 10 μM ^{3}H-5-uridine (80 cpm/pmole; Schwarz/Mann) and the other set of samples after 1.5 hr of incubation at 37° C. At the indicated duplicate 0.5-ml samples of each suspension were analyzed for radioactivity in total cell material (o-o, Δ-Δ, ▽-▽, □-□) or in acid-insoluble material (●-●, ▲-▲, ▼-▼, ■-■). All points represent averages of the duplicate samples. (B) Samples of a suspension of cells in BM42 were supplemented as indicated with 60 μM PGE_1 or 30 μM papaverine and incubated at 37° C for 1.5 hr. Then one half of each suspension was directly supplemented with 5 μM ^{3}H-5-uridine (80 cpm/pmole), whereas the other half of each suspension was centrifuged and the cells were resuspended to the same cell density in BM42 containing ^{3}H-uridine (reversed). The suspensions were incubated at 37° C and monitored for radioactivity as in (A).

cell material and acid-insoluble material by papaverine, theophylline and PGE_1. The inhibition of uridine incorporation into acid-insoluble material was directly proportional to the inhibition of its incorporation into total cell material and the effects were about the same whether the cells were labeled immediately after addition of the drugs or after preincubation of the cells with the drugs for 1.5 hr. $PGF_{2\alpha}$ had a similar effect. The effects of the inhibitors on uridine incorporation into both total cell material or acid-insoluble material were readily abolished by collecting the cells by centrifugation and suspending them in fresh medium (Fig. 1B).

Chromatographic analyses of acid-extracts prepared from labeled cells (15) showed that most of the radioactivity in the acid-soluble pool was associated with UTP and UDP-sugars whether or not the cells were labeled in the presence of the three drugs. The uridine kinase activity of cell-free preparations of Novikoff cells (16) was not affected by 5 mM theophylline, 300 μM papaverine or 300 μM PGE_1. These results indicate that the inhibition of uridine incorporation occurred at the level of transport into the cell, rather than at the level of phosphorylation or nucleic acid synthesis (see also Refs. 10 and 11).

Uridine transport was inhibited by papaverine, theophylline and the prostaglandins in an apparent competitive manner (Fig. 2). The apparent K_i values for the inhibition by PGE_1 (35 μM) and papaverine (10 μM) were similar to the apparent K_m for uridine transport (about 15 μM). $PGF_{2\alpha}$ was a less efficient inhibitor than PGE_1 and theophylline had a relatively low affinity for the uridine transport system. Because transport is the rate-limiting step in the initial rate of incorporation of uridine into nucleic acids, the latter process also follows normal Michaelis-Menten kinetics and

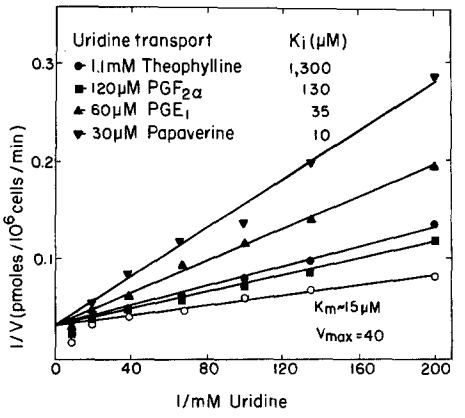

Fig. 2. Competitive inhibition of uridine transport by theophylline, $PGF_{2\alpha}$, PGE_1 and papaverine. Samples of a suspension of cells in BM42 were supplemented with the inhibitors as indicated. Then samples of an untreated suspension and the treated suspensions were supplemented with 5, 7.5, 10, 15, 25, or 40 μM ^{3}H-uridine (33 cpm/pmole) or 110 μM (14 cpm/pmole). The initial rates of uridine transport were estimated by analyzing duplicate 1-ml samples for radioactivity in total cell material after 5 min of incubation at 37° C (10, 11).

the apparent K_m is about the same as that for uridine transport (10, 11). The inhibitions of uridine incorporation into RNA by papaverine, theophylline and PGE_1 were also of the competitive type and the apparent K_i values were similar to those for the inhibition of uridine transport (Table 1). This finding is the same as with other inhibitors of uridine transport, Persantin (10, 11) and Cytochalasin B (17, 18), and other ribo- or deoxyribonucleosides (11, 14).

The data in Fig. 3 illustrate that the three drugs also inhibited the incorporation of hypoxanthine, choline and deoxyglucose into total cell material. The incorporation of all three precursors as well as of uridine and thymidine were inhibited to a similar extent by the drugs. The incorporation of hypoxanthine, choline (Fig. 3) and thymidine into macromolecules was inhibited to about the same extent as their incorporation into total cell material, whereas the intracellular phosphorylation of

ble 1. Apparent Kinetic Constants for the Inhibition of the Transport of Various Precursors and their Incorporation into Macromolecules by Papaverine, Theophylline and PGE_1

ecursor	K_m	K_i for Competitive Inhibition (μM)					
		Transport			Incorp. into Macromolecule		
	(μM)	Papa-verine	Theophyl-line	PGE_1	Papa-verine	Theophyl-line	PGE_1
idine	10-15	10	1,300	35	7	1,400	43
ymidine	0.4-0.6	20	1,600	45	24	1,000	40
poxanthine	3-5	12	400	9	17	800	10
oxyglucose	1,400	85	4,000	>600	--	--	--
oline	4-8	4	6,000	>600	11	>6,000	>600

lls were suspended in BM42 for measuring uridine, thymidine and hypoxanthine corporation and in glucose-free BM42 (13) or choline-free BM42 (12) for easuring deoxyglucose and choline incorporation, respectively. The kinetic alyses were conducted essentially as described in the legend to Fig. 2. mples of cell suspensions were supplemented with 1.1 mM theophylline, μM PGE_1 or 30 μM papaverine. The kinetics of the transport reactions d of the incorporation of the precursors into macromolecules and of ir inhibitions were determined as described elsewhere: thymidine (14), oxyglucose (13), choline (12), and hypoxanthine (Zylka and Plagemann, preparation).

none of the precursors was affected by any of the drugs (not shown). Thus, as with uridine, the inhibitions were at the level of transport into the cell. Transport of all substrates was inhibited by the drugs in a competitive manner and the apparent K_i values are summarized in Table 1. The apparent K_i values for the inhibition of uridine, hypoxanthine, choline and thymidine incorporation into macromolecules were similar to those for the inhibition of their transport into the cell. Results from other experiments have shown that the transport of the various substrates by secondary cultures of mouse embryo fibroblasts (19) was inhibited by papaverine and PGE_1 to about the same extent as the transport systems of Novikoff cells. Papaverine also inhibits competitively the incorporation of adenine by human platelets (20; Ki = 15 μM), though it has not been determined

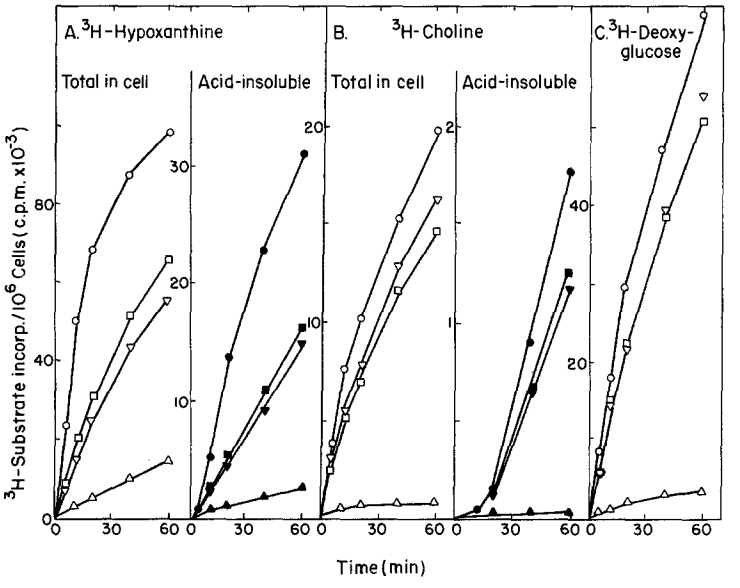

Fig. 3. Inhibition of hypoxanthine (A), choline (B) and deoxyglucose (C) incorporation by theophylline, papaverine and PGE_1. Samples of suspensions of cells in BM42 (A) or choline-free BM42 (B) or glucose-free BM42 (C) were supplemented with 1.1 mM theophylline (□-□, ■-■), 150 μM papaverine (△-△, ▲-▲), or 60 μM PGE_1 (▽-▽, ▼-▼) or remained untreated (o-o, ●-●) Immediately thereafter, the suspensions received (A) 2.5 μM ^{3}H-8-hypoxanthine (100 cpm/pmole; Schwarz/Mann), or (B) 2.5 μM ^{3}H-methyl-choline (80 cpm/pmole; Amersham/Searle), or (C) 50 μM ^{3}H-G-2-deoxy-D-glucose (6 cpm/pmole; New England Nuclear Corp.). All suspensions were incubated at 37° C and at the indicated times, duplicate 0.5-ml samples of each suspension were analyzed for radioactivity in total cell material or in acid-insoluble material. All points are averages of the duplicate samples.

whether the effect is one adenine transport or its phosphorylation.

The molecular basis for the inhibitions of the transport systems by papaverine, theophylline and the prostaglandins is unknown. Structurally these substances have little in common or with the substrates of the various transport systems they inhibit. We are presently investigating whether the inhibitions are due to binding of these substances to the transport sites for substrates, as suggested by the kinetic analyses, or may represent indirect effects, possibly via an increased cellular cyclic AMP level (21).

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