MUTANT MOUSE CELLS WITH NITROBENZYLTHIOINOSONE-INSENSITIVE NUCLEOSIDE TRANSPORT FUNCTIONS

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SUMMARY

From a mutagenized population of wildtype S49 T lymphoma cells, clones were generated that were resistant to the physiological effects of the potent inhibitor of nucleoside transport, 4-nitrobenzyl-6-thioinosine (NBMPR). NBMPR protected wildtype cells from the cytotoxic effects of a spectrum of nucleosides, whereas two mutant clones, KABI and KAB5, were still sensitive to nucleoside-mediated cytotoxicity in the presence of NBMPR. In addition, NBMPR prevented wildtype cells from surviving in hypoxanthine-amethopterin-thymidine containing medium, whereas KABI and KAB5 cells grew normally. Rapid sampling transport studies indicated that mutant cells, unlike wildtype parental cells, had acquired a substantial NBMPR-insensitive nucleoside transport component. Binding studies with \[^{3}\text{H}]\text{NBMPR} indicated that KAB5 cells were 70-75\% deficient in the number of NBMPR binding sites, whereas KABI cells possessed a wildtype complement of NBMPR binding sites. The characterization of the KABI and KAB5 cell lines suggested that the NBMPR binding site in wildtype S49 cells is genetically distinguishable from the nucleoside carrier site.

INTRODUCTION

In order to exert many of their physiological and cytotoxic effects in mammalian cells, nucleosides must first permeate across their plasma membranes. The study of nucleoside transport in mammalian cells has been greatly enhanced by the existence of specific high affinity inhibitors of the nucleoside transporter in mammalian cells, including the 6-substituted thiopurine ribonucleosides (1) and dipyridamole (2). 4-Nitrobenzyl-6-thioinosine (NBMPR) is a highly specific competitive inhibitor of nucleoside transport which binds to animal cell surfaces with an apparent Kd around 0.1-1.0nM (3,4). Not all mammalian cells, however, possess high affinity NBMPR binding sites or nucleoside transporters which are completely sensitive to NBMPR (5-10). Certain mammalian cell lines possess NBMPR-insensitive components ranging from 2\% to 100\% of the total cellular nucleoside transport capacity (6-10). Furthermore, a nucleoside transport-deficient cell line derived from S49 cells lacks high affinity NBMPR binding sites (11). Whether the NBMPR-sensitive and NBMPR-insensitive nucleoside transporters are genetically identical or distinct is not clear.
To attempt to resolve whether or not two structurally distinct transporters exist we have taken advantage of this high affinity interaction of NBMPR with the nucleoside transport function in S49 murine lymphoma cells to isolate mutants which were resistant to the physiological effects of NBMPR. Growth rate determinations, rapid transport measurements, and binding site quantitations indicated that mutant cells were not completely responsive to NBMPR and had gained an NBMPR-insensitive nucleoside transporter function. The results of these studies may ultimately have important chemotherapeutic implications in the clinical utilization of transport inhibitors to modulate nucleoside cytotoxicity.

EXPERIMENTAL PROCEDURES

Cell culture The growth characteristics and lymphocytic properties of wildtype S49 cells have been described previously in great detail (12). The KABI and KAB5 clones were isolated from approximately 10^6 mutagen-treated wildtype cells in soft agarose containing 0.5mM hypoxanthine, 0.4μM methotrexate, 30μM thymidine, 30μM deoxycytidine, and 30μM NBMPR. To determine the growth sensitivities of wildtype and mutant cells to various nucleosides and nucleoside analogs in the presence and absence of inhibitors of nucleoside transport, cells were incubated in Costar multiwell (24 well) tissue culture plates and enumerated on a Model ZB1 Coulter Counter as described previously (13).

Nucleoside transport measurements Nucleoside transport was measured by the rapid sampling technique described by Aronow et al. through a layer of inert oil (14).

Measurements of 4-nitrobenzylthioinosine binding. The binding of [3H]NBMPR to wildtype and mutant cells was measured by the procedure of Aronow et al. (14). The differences between the amount of radiolabeled NBMPR associated with the cells in the presence and absence of 20μM nonradiolabelled NBMPR were considered to be a measure of the specifically bound NBMPR.

RESULTS

Selection and isolation of mutant clones. In order to dissect genetically the high affinity interactions of inhibitors of nucleoside transport with the nucleoside transporter, we attempted to devise selective procedures to isolate variants resistant to the physiological effects of these inhibitors. Since the cytotoxic effects of 0.4μM methotrexate in the presence of 0.5mM hypoxanthine can be reversed by thymidine, a pyrimidine nucleoside, the ability of NBMPR to prevent thymidine rescue of methotrexate toxicity was determined. Wildtype cells in the presence of 10μM NBMPR did not survive in hypoxanthine-methotrexate regardless of the thymidine concentration (HAT medium). Two clones, KABI and KAB5, were isolated from semi-solid agarose as described (14) and grew normally in HAT medium containing 10μM NBMPR (Figure 1). To determine whether the altered apparent sensitivities of KABI and KAB5 cells to NBMPR were specific for thymidine or general for a spectrum of nucleosides, the ability of NBMPR to prevent thymidine rescue of methotrexate toxicity was determined. Wildtype cells in the presence of 10μM NBMPR did not survive in hypoxanthine-methotrexate regardless of the thymidine concentration (HAT medium). Two clones, KABI and KAB5, were isolated from semi-solid agarose as described (14) and grew normally in HAT medium containing 10μM NBMPR (Figure 1). To determine whether the altered apparent sensitivities of KABI and KAB5 cells to NBMPR were specific for thymidine or general for a spectrum of nucleosides, the ability of NBMPR to protect wildtype and mutant cells from the cytotoxic effects of deoxyguanosine, 5-fluorouridine, adenosine-EHNA, 6-thioguanosine, and deoxyadenosine-EHNA were examined. Wildtype and mutant cells were approximately equally sensitive to any of the five nucleosides in the absence of NBMPR. For all five nucleosides, NBMPR protected wildtype cells from the nucleoside-mediated cytotoxicity, whereas the KABI and KAB5 cells were unprotected or only slightly protected from nucleoside toxicity by NBMPR (data not shown). Conversely, dipyridamole protected wildtype and mutant