Binding of S-adenosylhomocysteine to isolated rat hepatocytes and purified plasma membranes from rat liver

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INTRODUCTION

Extracellular AdoHcy, the endogenous transmethylase inhibitor formed from AdoMet during transmethylation, is not taken up by rat hepatocytes (Aarbakke & Ueland, 1981). On the other hand, accumulation of intracellular AdoHcy is associated with a pronounced export of AdoHcy, suggesting a vectorial transport of AdoHcy (Hoffman et al., 1980). AdoHcy may thus interact with a permease in the cell membrane. Additional observations suggesting interaction of AdoHcy with membrane components, include: inhibition of membrane transport by the AdoHcy analogue, SIBA (Pierre & Robert-Géro, 1979); binding of AdoHcy to the surface of rat hepatocytes (Aarbakke & Ueland, 1981) and membranes from rat cerebral cortex (Fonlupt et al., 1981); and inhibition by AdoHcy of phospholipid methyltransferase of rat liver plasma membrane (Schanche et al., 1981). The present communication reports on the characteristics of AdoHcy binding to isolated rat hepatocytes and purified rat liver plasma membranes.

RESULTS

The binding of [^{14}C]AdoHcy to hepatocytes and membranes is determined by a method based on the separation of free and bound ligand by centrifugation of cells or membranes through oil (Aarbakke & Ueland, 1981). The incubation is performed at 0^{o}C, to avoid enzymatic degradation of AdoHcy. Specific binding of AdoHcy refers to the binding suppressed by high concentrations (2.5 mM) of unlabeled AdoHcy.

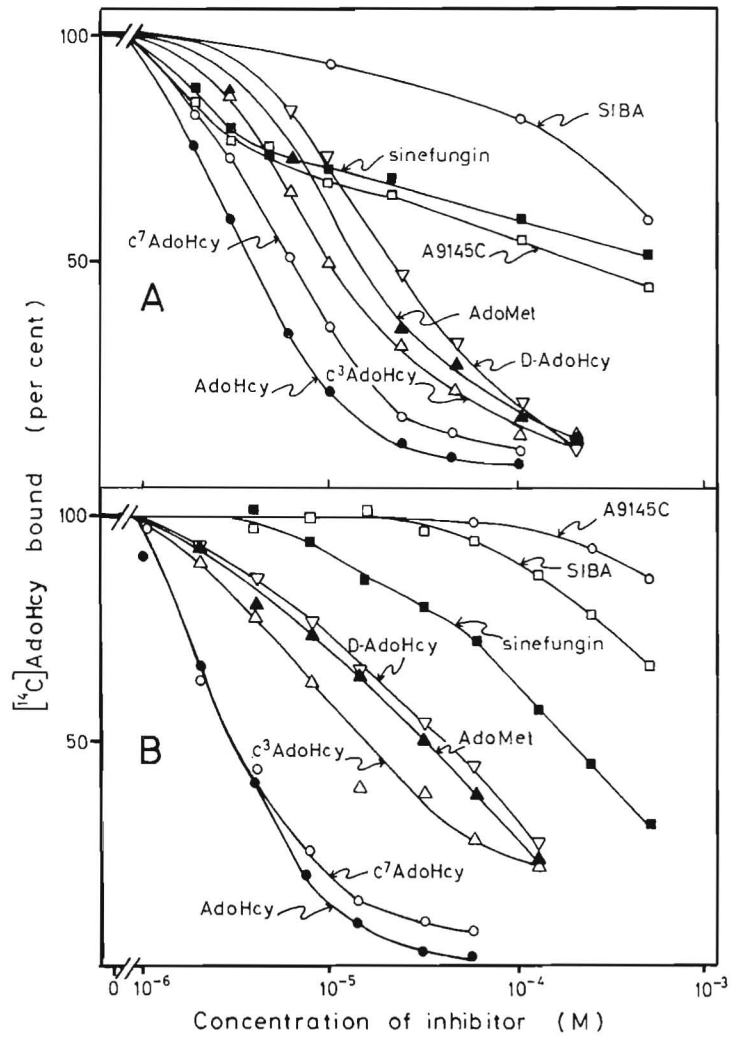

FIG. 1. DISPLACEMENT OF [^{14}C]ADOHCY BINDING BY ADOHCY ANALOGUES

[^{14}C]AdoHcy (1 μm) was incubated with hepatocytes (4.5×10^6 cells/ml (A) or purified plasma membranes (4.8 mg of protein/ml (B) in the presence of increasing concentrations of unlabeled AdoHcy or AdoHcy analogues indicated on the figures. The amount of [^{14}C]AdoHcy bound is plotted versus the concentration of inhibitor.

TABLE 1

INHIBITION OF PHOSPHOLIPID TRANSMETHYLASE BY ADOHCY AND ANALOGUES[x]

Inhibitor	Inhibitor constants, K_i (μM) pH 7.4	pH 10.0
AdoHcy	0.5	7.5
c^7AdoHcy	0.3	5.5
c^3AdoHcy	1.5	20
Sinefungin	20	89
A9145C	NI[a]	NI
D-AdoHcy	15	125
SIBA	NI	NI

[x]From Schanche et al., 1981.

[a]Not inhibitory.

The progress curve for the specific binding of AdoHcy to cells and membranes is characterized by an initial rapid phase and equilibrium is obtained after 5-10 minutes of incubation. AdoHcy binds to high affinity acceptors on the surface of intact hepatocytes (K_d=0.4 μM) and to purified plasma membranes (K_d=0.7 μM). The dissociation rate curves for AdoHcy from both cells and membranes are biphasic, and the second slow phase is consistent with a dissociation rate constant of 0.09 min^{-1}.

Apart from unlabeled AdoHcy, c^7AdoHcy is a particularly effective inhibitor of AdoHcy binding to both cells and membranes, followed by c^3AdoHcy, AdoMet and D-AdoHcy in the order mentioned (Fig. 1). High concentrations of SIBA are required for the inhibition of AdoHcy binding. The effect of sinefungin and its metabolite, A9145C on the binding of AdoHcy to cells differed markedly from their effect on the binding of AdoHcy to purified membranes. Both compounds show a biphasic displacement curve with intact cells. In contrast, A9145C has essentially no effect on AdoHcy binding to purified membranes, whereas sinefungin is effective.

DISCUSSION

AdoHcy binds to a heterogenous population of binding sites on hepatocytes and plasma membranes, as judged by non-linear Scatchard plots, biphasic dissociation rate curves and displacement curves (for sinefungin and A9145C). The affinity for AdoHcy of the membrane acceptors is of the same order of magnitude as the inhibitor constant for AdoHcy of phospholipid methyltransferase (Table 1), the K_m-values for AdoMet being 4.1 and 92 μM at pH 7.9 and 10.0,

respectively. The following observations suggest, however, that phospholipid methyltransferase does not totally account for the membrane binding of AdoHcy. Phospholipid methyltransferase in purified plasma membranes from rat liver is not inhibited by A9145C, and c^7AdoHcy is even more potent than AdoHcy itself (Table 1). Furthermore, the high capacity of the AdoHcy binding sites (12 pmole/10^6 cells, and 100 pmole/mg of membrane protein) contrasts to the low specific activity of phospholipid methyltransferase (Vance & de Kruiff, 1980).

The binding capacity of the AdoHcy acceptors on the surface of hepatocytes (about 12 pmole/10^6 cells) is high relative to the cellular content of AdoHcy in hepatocytes (about 50 pmole/10^6 cells). In addition, one may expect that AdoHcy binds to membrane acceptors facing the interior of the cell. Thus, the possibility exists that a substantial fraction of cellular AdoHcy is bound to membranes.

A membrane receptor for SIBA has been suggested based on the observation that the AdoHcy analogue inhibits membrane transport (Pierre & Robert-Géro, 1979). However, the finding of essentially no effect of SIBA on AdoHcy binding to membrane suggest that SIBA does not mediate its effects through a direct interaction with AdoHcy acceptor(s) of the plasma membrane.

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