Vol. 114, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 29, 1983 Pages 505-510

Inhibition of Adenosylmethionine Decarboxylase And Perturbation of Polyamine Metabolism by 3-Deaza-(±)aristeromycin

Richard K. Gordon, Nesbitt D. Brown and Peter K. Chiang

Division of Biochemistry, Walter Reed Army Institute of Research, Washington, D.C. 20307

Received June 10, 1983

3-Deaza-(±)aristeromycin, previously known mainly as a potent inhibitor of adenosylhomocysteine hydrolase, can also inhibit the activity of adenosylmethionine decarboxylase. The release of [^{14}C]CO_2 from HeLa cells labeled with [carboxyl-^{14}C]methionine was inhibited by more than 70% after 4 hours in the presence of 4 μM 3-deaza-(±)aristeromycin. Concomitant with this inhibition, there was a significant increase in the amount of putrescine in the HeLa cells. Adenosylmethionine decarboxylase isolated from HeLa cells could also be inhibited by 3-deaza-(±)aristeromycin and 3-deazaadenosine, 3-deazaadenosylhomocysteine, and 3-deaza-(±)aristeromycinylhomocysteine.

The 3-deaza analogs of adenosine, such as 3-deaza-(±) aristeromycin (3-deazaAri) and 3-deazaadenosine (3-deazaAdo), have been shown to have a variety of biological effects in vivo and in vitro (1-13). The traditional concept regarding the mode of action of these 3-deazapurine nucleosides is that they specifically inhibit AdoHcy hydrolase. Depending on the nucleosides and the cell types used, nucleosidylhomocysteine (NucHcy) analogs are also formed because of the ability of the 3-deazapurine nucleosides to serve as alternative substrates for AdoHcy hydrolase(2-7). For example, 3-deazaAri can form 3-deazaAriHcy, and 3-deazaAdo can form 3-deazaAdoHcy. The inhibition of AdoHcy hydrolase will lead to a large increase of AdoHcy in cells. The accumulation of AdoHcy and/or NucHcy will inhibit transmethylation reactions (2-7), and generally the cellular level of AdoMet increases as a consequence. Therefore, there is an ensuing alteration in the cellular ratio of AdoHcy/AdoMet or NucHcy/AdoMet.

Abbreviations: AdoMet, adenosylmethionine; AdoHcy, adenosylhomocysteine; 3-deazaAdo, 3-deazaadenosine; 3-deazaAri, 3-deaza-(±)aristeromycin; HEPES, N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid; NucHcy, nucleosidyl-homocysteine.

0006-291X/83 $1.50

The biological effects observed with 3-deazaAri or 3-deazaAdo so far have been chiefly attributed to the inhibition of transmethylation reactions. However, an hitherto overlooked aspect is the effect of 3-deazaAri or 3-deazaAdo on the disposition of AdoMet via decarboxylation by AdoMet decarboxylase (EC 4.1.1.50) to S-methyladenosylhomocysteamine (decarboxylated AdoMet). The latter donates a propylamino group toward the biosynthesis of polyamines, and ends up as methylthioadenosine. In this paper, we demonstrate for the first time that both 3-deazaAri and 3-deazaAdo can also inhibit AdoMet decarboxylase of HeLa cells, resulting in an accumulation of putrescine.

MATERIALS AND METHODS

Materials And Cell Culture: 3-DeazaAdo, 3-deazaAri, 3-deazaAdoHcy, and 3-deazaAriHcy were synthesized at the Southern Research Institute (2, 14). L-[Carboxyl-^{14}C]methionine (51 mCi/mmol) and adenosyl-L-[carboxyl-^{14}C]methionine (62 mCi/mmol) were purchased from Amersham. I-125 protein columns were obtained from Waters Associates. HeLa cells were grown in minimal essential spinner medium (HEM Research, Rockville, MD) containing 100 µg penicillin/ml, 100 meq streptomycin/ml and 5% horse serum at 37° in 5% CO_2.

Enzyme Assays: The activity of AdoMet decarboxylase in the HeLa cells was assayed at 37° in 2 ml of Dulbecco's minimal essential medium, containing 30 mM HEPES (pH 7.3), 5% horse serum and 25.5 µM [^{14}C]methionine (2.6 µCi). The enzymatic activity of AdoMet decarboxylase in the extracts of HeLa cells was assayed in 2 ml of 100 mM sodium phosphate (pH 7.2), 2.5 mM putrescine, 1 mM dithiothreitol and 2.4 µM [^{14}C]AdoMet. The $^{14}CO_2$ released from the cells or extracts was linear up to 90 min. The reactions were terminated by adding 0.4 ml of 2.5 M H_2SO_4 to the reaction vessel, and the $^{14}CO_2$ evolved was collected by adding 0.2 ml of hyamine into a propylene well suspended in the reaction vessel. After a further incubation of 60 min, the well along the contents were placed in a vial containing 10 ml of scintillation fluid and 500 µl of 2.5 M H_2SO_4. Blanks were treated with H_2SO_4 at the initiation of the assay. All assays were done in duplicates or triplicates.

Purification of AdoMet Decarboxylase From HeLa Cells:

Crude extracts: HeLa cell extracts were prepared by sonicating the cells in 0.03 M potassium phosphate (pH 6.9), followed by centrifugation at 48,000 x g for 60 min (15). The supernatant was saved for the next step.

$(NH_4)_2SO_4$ Fractionation: $(NH_4)_2SO_4$ was added to the supernatant; the protein precipitated between 35%-65% was dissolved in a buffer consisting of 2.5 mM putrescine, 1 mM dithiothreitol, 0.1 mM EDTA, and 10 mM Tris-HCl (pH 7.4).

High Pressure Liquid Chromatography: Two I-125 protein columns in tandem were equilibrated with the same buffer. After filtration through a 0.22 µm filter, the supernatant was injected into the columns. The enzyme was eluted with a flow rate of 1 ml/min at room temperature, and used for assays immediately. At this stage, the enzyme was purified about 16-fold, with a specific activity of 0.4 nmol of $^{14}CO_2$ released per 30 min per mg. The estimated molecular weight of AdoMet decarboxylase was 54,000, and it agrees with that determined for AdoMet decarboxylase isolated from rat liver and sea urchin eggs (16).

Determination of Polyamines: HeLa cells (10^6) were extracted with 0.5 ml of 2 N perchloric acid. After 30 min in ice, the pH of the extract was adjusted to about 6.0 with 10 N KOH. After centrifugation, the volume of the

supernatant was adjusted to 1.0 ml. The supernatant was next assayed for polyamines by high pressure liquid chromatography after derivatization with dansyl chloride (17).

RESULTS

When the HeLa cells were treated with a noncytotoxic concentration of 4 μM 3-deazaAri, the release of $^{14}CO_2$ from the cells labeled with [^{14}C]-methionine (which forms adenosyl-[^{14}C]methionine) was inhibited about 10% in the first h, and 70% after 4 h (Fig. 1). This new finding was indicative of an inhibition of the activity of AdoMet decarboxylase in the HeLa cells. The effect of 4 μM 3-deazaAri on the metabolism of polyamines was next examined in the HeLa cells (Fig. 2). After 4 and 5 h of incubation, the treated cells exhibited a significant 35% increase in putrescine. In contrast, the levels of spermidine and spermine were unaffected. However, a perturbation in the total amounts of spermidine and spermine by 3-deazaAri would be unlikely to be observed in 5 h, since putrescine has a much faster turnover rate in cells than spermidine or spermine (18). The half-lives of putrescine, spermidine and spermine are 1.5, 15 and 24 h, respectively (18).

As expected, the partially purified AdoMet decarboxylase was sensitive to the inhibition by and 3-deazaAri and 3-deazaAdo (Fig. 3). At 400 μM of both analogs, AdoMet decarboxylase was inhibited about 30%. The homocysteine conjugates, 3-deaza-AdoHcy and 3-deazaAriHcy, were also capable of inhibiting the enzyme, and the inhibition was higher by about 10% (Fig. 3). The inhibition of the enzyme activity by all four analogs was not time-dependent.

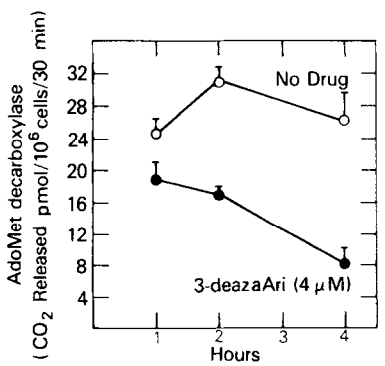
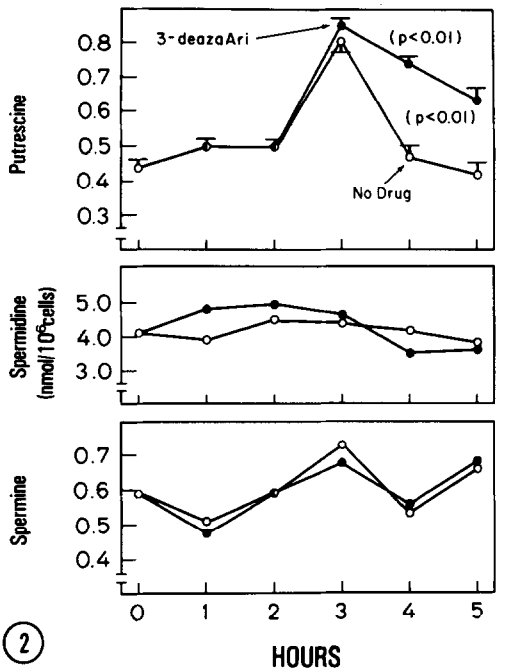


Fig. 1. Inhibition of the activity of AdoMet decarboxylase by 4 μM 3-deazaAri in HeLa cells. Cells were incubated in 2 ml of Dulbecco's minimal essential medium, containing 30 mM HEPES (pH 7.3), 5% horse serum, and 25.5 μM [^{14}C]-methionine (2.6 μCi). The $^{14}CO_2$ released was trapped as described in Methods.



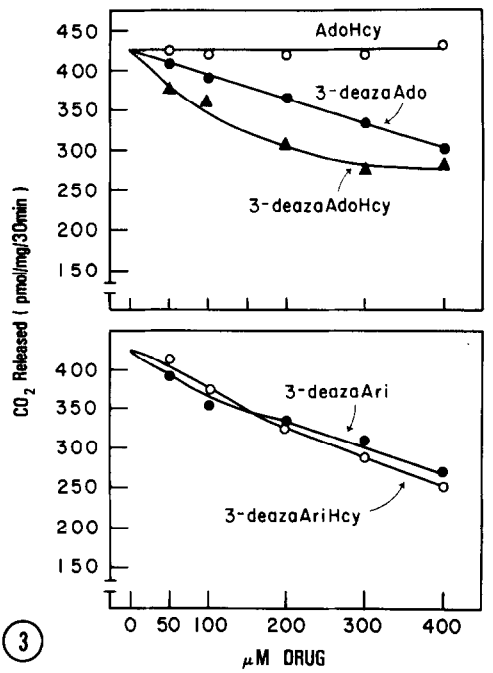


Fig. 2. Metabolic profiles of polyamines in HeLa cells treated with 4 μM 3-deazaAri. The polyamines were determined by high pressure liquid chromatography after derivatization with dansyl chloride.

Fig. 3. Effect of 3-deazapurine nucleosides on the activity of AdoMet decarboxylase isolated from HeLa cells. The activity of AdoMet decarboxylase was assayed as described in Fig 1, and the duration of the assay was 10 min.

Surprisingly, AdoHcy itself was completely inactive even in the presence of 1 μM 2'-deoxycoformycin, a potent inhibitor of adenosine deaminase and AMP deaminase. Neither (±)aristeromycin (carbocyclic adenosine) nor adenosine, at 400 μM of each, had any effect. In contrast to their effects on AdoMet decarboxylase, 3-deazaAri, 3-deazaAdo, 3-deazaAriHcy and 3-deazaAdoHcy were devoid of any effect on the activity of ornithine decarboxylase in the HeLa extracts (not shown).

DISCUSSION

The present findings show that 3-deazaAri, 3-deazaAdo and their corresponding homocysteine conjugates can inhibit AdoMet decarboxylase. The inhibition of AdoMet decarboxylase probably led to a significant decrease of S-methyladenosylhomocysteamine in the HeLa cells. Thus, a drop in the latter compound would lead to a shortage of propylamine groups for putrescine to form spermidine. As a result, putrescine accumulated in the HeLa cells (Fig. 2).

The lack of effect of 3-deazaAri on the cellular levels of spermidine and spermine within 5 h was most likely due to their slower turnover rates. More pronounced effects on all of the polyamines could be expected after long-term treatment with these inhibitors.

The isolated AdoMet decarboxylase was not as sensitive to 3-deazaAri and 3-deazaAdo (Fig. 3) as in the cells (Fig. 1). However, an inhibition of the activity of AdoMet decarboxylase in the cells even by a small proportion would result in a substantial decrease in the amount of $^{14}CO_2$ released from [^{14}C]methionine over a prolonged period (Fig. 1). The inability of adenosine, adenosylhomocysteine, and (±)aristeromycin to inhibit AdoMet decarboxylase points to a sensitivity of the enzyme to inhibition by a 3-deazapurine moiety. This sensitivity to a 3-deazapurine nucleoside is a unique feature shared between AdoHcy hydrolase and AdoMet decarboxylase, thus suggesting some evolutionary homologies between the two enzymes. The latter possibility should not be surprising because both enzymes are involved in the metabolic disposition of AdoMet.

The present investigation showed that the biochemical and biological effects caused by 3-deazaAri and 3-deazaAdo may not be confined to the inhibition of transmethylation reactions alone. Therefore, the perturbation of polyamine metabolism by the 3-deazapurine nucleosides can be a contributing factor in terms of their biological effects.

REFERENCES

1. Chiang, P. K. (1981) Science 211, 1164-1166.
2. Montgomery, J. A., Clayton, S. J., Thomas, H. J., Shannon, W. M., Arnett, G., Bodner, A. J., Kim, I.-K., Cantoni, G. L., and Chiang, P. K. (1982) J. Med. Chem. 25, 626-629.
3. Garcia-Castro, I., Mato, J. M., Vasanthakumar, G., Wiesmann, W. P., Schiffmann, E. , and Chiang, P. K. (1983) J. Biol. Chem. 258, 4345-4349.
4. Zimmerman, T. R., Schmitges, C. G., Wolberg, G., Deeprose, R. D., Duncan, G. S. Cuatrecasas, P., and Elion, G. B. (1980) Proc. Natl. Acad. Sci. USA 77, 5639-5643.
5. Schanche, J.-S., Schanche, T., Ueland, P. M. (1982) Biochim. Biophys. Acta 721, 399-407.
6. Chiang, P. K., Richards, H. H., and Cantoni, G. L. (1977) Mol. Pharmacol. 13, 939-947.
7. Guranowski, A., Montgomery, J. A., Cantoni, G. L., and Chiang, P. K. (1981) Biochemistry 20, 1110-115.
8. Bader, J. P., Brown, N. R., Chiang, P. K., and Cantoni, G. L. (1979) Virology 89, 494-505.

9. Trager, W., Tershakovec, M., Chiang, P. K., and Cantoni, G. L. (1980) Exp. Parasitol. 50, 83-89.
10. Leonard, E. J., Skeel, A., Chiang, P. K., and Cantoni, G. L. (1978) Biochem. Biophys. Res. Commun. 84, 102-109.
11. Braun, J., Rosen, F. S., Unanue, E. R. (1980) J. Exp. Med. 151, 174-183.
12. Morita, Y., Siraganian, R. P., Tang, C. K., and Chiang, P. K. (1982) Biochem. Pharmacol. 31, 2111-2113.
13. Hyman, B. T., Stoll, L. L., and Spector, A. A. (1982) Biochim. Biophys. Acta 713, 375-385.
14. Montgomery, J. L., Thomas, H. J., Thorpe, M. C., and Chiang, P. K. (1981) J. Med. Chem. 24, 1514-1516.
15. Sturman, J. A., and Gaull, G. E. (1974) Pediat. Res. 8, 231-237.
16. Manen, C.-A., and Russell, D. H. (1974) Biochemistry 13, 4729-4735.
17. Brown, N. D., Strickler, M. P., and Whaun, J. M. (1982) J. Chromatog. 245, 101-108.
18. McCormick, F. (1978) Biochem. J. 174, 427-434.