Biochemical Pharmacology, Vol. 31, No. 8, pp. 1649–1650, 1982.
Printed in Great Britain.

0006-2952/82/081649-02 $03.00/0
Pergamon Press Ltd.

SHORT COMMUNICATIONS

Inhibition of aminopropyltransferases by 5′-*S*-isobutyl-5′-deoxyadenosine *in vitro*

(*Received* 7 *October* 1981; *accepted* 13 *November* 1981)

The polyamines spermidine and spermine appear to play an essential role in cellular metabolism and regulation [1]. The development of specific inhibitors of polyamine biosynthesis has received considerable attention during the past decade. These inhibitors were developed as tools for the study of the function of the polyamines. Four enzymes are known to be involved in the synthesis of polyamines in mammalian tissues, i.e. ornithine decarboxylase, *S*-adenosylmethionine decarboxylase and two aminopropyltransferases, one catalyzing the synthesis of spermidine, the other producing spermine [2–5]. Many inhibitors of the first two enzymes, ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, have been developed. However, only a few studies have been carried out on inhibitors of spermidine and spermine synthases [6–12]. Potent inhibitors of these synthases may have some advantages over the currently available inhibitors of the decarboxylases. The compound described here, 5′-*S*-isobutyl-5′-deoxyadenosine is known to have antimalarial [13] and antiviral activities [14, 15].

5′-*S*-Isobutyl-5′-deoxyadenosine was purchased from Sigma Chemical Co. (St. Louis, MO). *S*-Adenosyl-L-[methyl-^{14}C]methionine (sp. act. 53.6 mCi/mmole) was a product of New England Nuclear Corp. (Boston, MA) Decarboxylated *S*-adenosylmethionine, both unlabeled and labeled in the methyl group was prepared by the action of *S*-adenosylmethionine decarboxylase from *Escherichia coli* and purified by chromatography on Dowex-50-H$^+$ and high voltage electrophoresis [16]. Spermidine and spermine synthases (aminopropyltransferases) were partially purified from rat ventral prostate of male Sprague–Dawley rats (250–300 g) as in [10]. Extracts containing both enzyme activities were fractionated into spermidine synthase and spermine synthase preparations by the treatment with ammonium sulfate followed by DEAE-cellulose and Ultrogel ACA 34 column chromatography. The enzyme preparations used here were 260-fold (spermidine synthase) and 160-fold (spermine synthase) of purification over the specific activity present in the crude ultracentrifugal extracts. The aminopropyltransferase activity was determined by measuring the production of [methyl-^{14}C]methylthioadenosine from decarboxylated *S*-adenosyl[methyl-^{14}C]methionine in the presence of putrescine (spermidine synthase) or of spermidine (spermine synthase) as in [5]. The assay medium contained 100 mM sodium phosphate buffer (pH 7.5), 5 mM dithiothreitol, 40 μM decarboxylated *S*-adenosyl-[methyl-^{14}C]methionine (2 μCi/μmole), 0.5 mM putrescine or spermidine and the enzyme preparation in 0.2 ml of total volume. Assays were triplicately incubated at 37° for 30 min. The methylthioadenosine was produced in stoichiometric amounts with putrescine and spermidine. An adequate amount of 5′-*S*-isobutyl-5′-deoxyadenosine dissolved in distilled water was added to the assay mixture.

Figure 1 shows the dose–response curve for 5′-*S*-isobutyl-5′-deoxyadenosine on the inhibition of spermidine synthase and spermine synthase activities. Spermine synthase was slightly more sensitive to the inhibitor than spermidine synthase. The effect of the concentration of putrescine on the inhibition of spermidine synthase by 5′-*S*-isobutyl-5′-deoxyadenosine is shown in Fig. 2. This inhibition was noncompetitive and the calculated K_i of 5′-*S*-isobutyl-5′-deoxyadenosine was 38 μM; K_m for putrescine was 22 μM. Figure 3 shows the effect of spermidine concentration on the inhibition of spermine synthase by 5′-*S*-isobutyl-5′-deoxyadenosine. This drug showed a mixed type of inhibition for spermine synthase. Dialysis of 5′-*S*-isobutyl-5′-deoxyadenosine-treated enzymes restored the activity to that found in preparations treated similarly except for exposure to the inhibitor, suggesting that the inhibition is a reversible process. Since 5′-*S*-isobutyl-5′-deoxyadenosine was proved to be a strong inhibitor of aminopropyltransferases, its antimalarial and antiviral effect might depend on this activity.

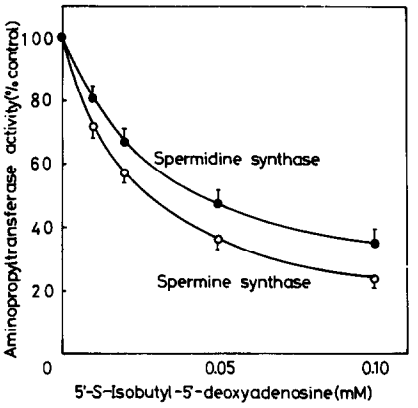

Fig. 1. Inhibition of spermidine synthase and spermine synthase by 5′-*S*-isobutyl-5′-deoxyadenosine. The results are expressed as percentages of the control activity measured in the absence of 5′-*S*-isobutyl-5′-deoxyadenosine. Putrescine and spermidine were 0.5 mM. Each point represents mean ± S.D. of triplicate determinations.

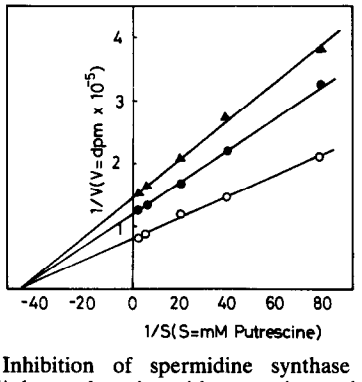

Fig. 2. Inhibition of spermidine synthase by 5′-*S*-isobutyl-5′-deoxyadenosine with putrescine as the variable substrate. Spermidine synthase activity was assayed in the absence (○) or presence of 25 μM (●) or 50 μM (▲) 5′-*S*-isobutyl-5′-deoxyadenosine, 40 μM decarboxylated *S*-adenosylmethionine and 0.0125–0.5 mM putrescine and 83 μg enzyme protein.

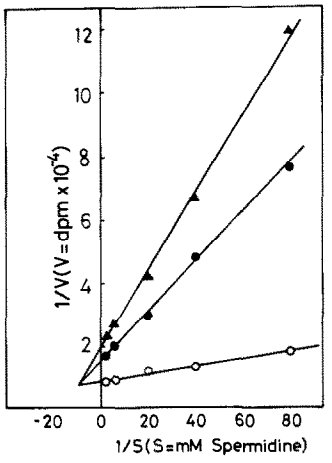

Fig. 3. Inhibition of spermine synthase by 5′-*S*-isobutyl-5′-deoxyadenosine with spermidine as the variable substrate. Spermine synthase activity was assayed in the absence (○) or presence of 25 μM (●) or 50 μM (▲) 5′-*S*-isobutyl-5′-deoxyadenosine, 40 μM decarboxylated *S*-adenosylmethionine and 0.0125–0.5 mM spermidine and 97 μg enzyme protein.

Department of Biochemistry
Mie University School of Medicine
Tsu, Mie 514, Japan

HIROSHIGE HIBASAMI
MINORU TANAKA
JUN NAGAI

REFERENCES

1. D. R. Morris and J. Harada, in *Polyamines in Biomedical Research* (Ed. J. M. Gaugas), p. 1. John Wiley & Sons, Chichester (1980).
2. A. E. Pegg and H. G. Williams-Ashman, *Archs Biochem. Biophys.* **137**, 156 (1970).
3. A. Raina and J. Jänne, *Med. Biol.* **53**, 121 (1975).
4. A. Raina, R.-L. Pajula and T. Eloranta, *FEBS Lett.* **67**, 252 (1976).
5. H. Hibasami and A. E. Pegg, *Biochem. J.* **169**, 709 (1978).
6. J. K. Coward, N. C. Motola and J. D. Moyer, *J. med. Chem.* **20**, 500 (1977).
7. H. Hibasami and A. E. Pegg, *Biochem. biophys. Res. Commun.* **81**, 1398 (1978).
8. R.-L. Pajula and A. Raina, *FEBS Lett.* **99**, 343 (1979).
9. K. Samejima and Y. Nakazawa, *Archs Biochem. Biophys.* **201**, 241 (1980).
10. H. Hibasami, M. Tanaka, J. Nagai and T. Ikeda, *FEBS Lett.* **116**, 99 (1980).
11. H. Hibasami, R. T. Borchardt, S. Y. Chen, J. K. Coward and A. E. Pegg, *Biochem. J.* **187**, 419 (1980).
12. K. C. Tang, A. E. Pegg and J. K. Coward, *Biochem. biophys. Res. Commun.* **96**, 1371 (1980).
13. W. Trager, M. Robert-Géro and E. Lederer, *FEBS Lett.* **85**, 264 (1978).
14. A. Raies, F. Lawrence, M. Robert-Géro, M. Loche and R. Cramer, *FEBS Lett.* **72**, 48 (1976).
15. C. Terrioux, M. Crépin, F. Gros, M. Robert-Géro and E. Lederer, *Biochem. biophys. Res. Commun.* **83**, 673 (1978).
16. H. Pösö, P. Hannonen and J. Jänne, *Acta chem. scand.* **B30**, 807 (1976).

Biochemical Pharmacology, Vol. 31, No. 8, pp. 1650–1652, 1982.
Printed in Great Britain.

0006-2952/82/081650–03 $03.00/0

Effect of chronic clofibrate feeding on the activities of enzymes involved in glycerolipid synthesis and in peroxisomal metabolism in rat liver

(*Received* 14 *September* 1981; *accepted* 11 *November* 1981)

Dihydroxyacetone phosphate is an alternative precursor to glycerol phosphate for the biosynthesis of glycerolipids in mammalian cells [1–3]; although the physiological importance of these two routes of metabolism is not established. The initial esterification of fatty acids to phosphatidate could involve three different acyltransferases: (a) a glycerol phosphate acyltransferase (EC 2.3.1.15) located in the endoplasmic reticulum which can also use dihydroxyacetone phosphate as an acyl acceptor [4, 5]; (b) a specific glycerol phosphate acyltransferase located in the mitochondrial outer membrane [6–9]; and (c) a specific dihydroxyacetone phosphate acyltransferase that is found in peroxisomes [10–12].

The latter activity has only recently been described and its possible importance in producing diacylglycerolipids is unknown.

The present work was performed to investigate how the peroxisomal acyltransferase changes in activity in relation to other enzymes of phosphatidate metabolism when the peroxisomal content of the liver is modified. This was achieved by feeding rats with a metabolite of the hypolipidaemic drug, clofibrate, which is known to produce peroxisomal proliferation in the liver.

Sodium-4-chlorophenoxyisobutyrate was a gift from Imperial Chemical Industries Ltd. (Macclesfield, U.K.), the source of other materials, the rats, and the conditions of enzymic analysis in liver homogenates is described elsewhere [13]. For some incubations the liver homogenate was preincubated with *N*-ethylmaleimide [13] to inhibit the glycerol phosphate acyltransferase of the endoplasmic reticulum that can also esterify dihydroxyacetone phosphate [4, 5]. The *N*-ethylmaleimide-insensitive glycerol phosphate acyltransferase is mitochondrial [11, 14–16], whereas the insensitive dihydroxyacetone phosphate acyltransferase is mainly peroxisomal [10–12]. This latter activity was measured by using a concentration of 400 μM palmitoyl-CoA rather than 523 μM which was used previously [13].

The standard 41B diet [13] was crushed and a solution of sodium chlorophenoxyisobutyrate was mixed with the powder to form a stiff paste with a final concentrate of 5 g of drug per kg of dry diet. The mixture was reconstituted