Biochemical Pharmacology, Vol. 35, No. 9, pp. 1597–1600, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

SHORT COMMUNICATIONS

Inhibition of diamine oxidase and *S*-adenosylmethionine decarboxylase by diminacene aceturate (berenil)

(*Received* 5 *August* 1985; *accepted* 14 *October* 1985)

One approach to determining the role of polyamines has been to use specific inhibitors of their biosynthesis and degradation. Most of these prevent the decarboxylation by *S*-adenosylmethionine decarboxylase and hence the conversion of putrescine into spermidine and spermine [1], and others inhibit the putrescine formation by ornithine decarboxylase [2, 3]. Methylglyoxal bis(guanylhydrazone) (MGBG) and related compounds are potent competitive inhibitors of *S*-adenosylmethionine decarboxylase [4] and non-competitive inhibitors of diamine-oxidase—the first enzyme of putrescine degradation [5]—in mammalian tissues. These compounds have a major disadvantage in that they stabilize the *S*-adenosylmethionine decarboxylase by prolonging its half-life [6]. Diminacene aceturate (Fig. 1) is a well-known antiprotozoal drug whose biochemical mechanism is not clear nowadays. In the present work, we show that this compound is a potent inhibitor of *S*-adenosylmethionine decarboxylase and diamine oxidase from rat tissues.

DIMINACENE ACETURATE

Fig. 1. Structure of the drug used in the study.

Materials and methods

An important source of diamine oxidase is the small intestine of the rat [7]. To determine diamine oxidase activity, male Wistar strain rats were euthanatized by cervical fracture and their small intestines were immediately extracted, washed, minced and homogenized at 4° in a volume (ml) of phosphate Na^+/K^+ buffer, 0.1 M, pH 7.8, twice its weight (g). These homogenates were centrifuged at 15,000 rpm for 30 min at 4°. A most convenient source of putrescine-activated *S*-adenosylmethionine decarboxylase is rat liver [8]. To determine this enzyme activity, the rats were injected 24 hr prior to their death with a dose of 80 mg/kg body weight in order to increase its activity [8]. The livers of these rats were extracted and homogenized in 2 vol. (ml) of ice-cold Na^+ phosphate buffer, 2.5 mM, pH 7.5, containing 2.5 mM dithiothreitol, 2.5 mM putrescine and 0.1 mM EDTA. The homogenate was then centrifuged at 105,000 *g* for 30 min; this material could be used as an enzymatic source after an exhaustive dialysis for 24 hr against 50 vol. of the same buffer in order to remove MGBG. Diamine oxidase activity was essentially assayed as described by Snyder and Hendley [9], with some variations. The reaction mixture contained: 2.7 ml of phosphate Na^+/K^+ buffer 0.1 M, pH 7.8, 85 μl of 25 mM unlabelled putrescine, 6 μl of (1.4-^{14}C) putrescine (0.10 μCi), and enzymatic extracts to complete a final volume of 3 ml. This reaction mixture was incubated in culture tubes at 37° in a shaking bath for 30 min. δ-1-Pyrroline formed by spontaneous cyclation from γ-aminobutyraldehyde was extracted twice with a toluene-based scintillation fluid (4 g PPO, 0.1 g POPOP, 700 ml toluene and 300 ml ethanol). After shaking vigorously for 5 min, the tubes were frozen at −20°, and the upper phase was decanted and extracted again. The blank tube contained the same assay mixture with the enzymatic extracts inactivated by heat. One unit of diamine oxidase activity is defined as the capacity of the enzymatic extracts to oxidize 1 μmol of putrescine per hour at 37°. *S*-adenosylmethionine decarboxylase was assayed as described by Pegg and Pössö [8]. In both cases specific activity is expressed as units per milligram of protein. Diamine oxidase and *S*-adenosylmethionine decarboxylase activities were lineal with time for at least 90 min even in the presence of the inhibitor.

To determine the inhibition constants (K_i) from diminacene aceturate, Dixon and Linewaver–Burk plots were used [10].

Protein was measured colorimetrically by the Lowry method [11], using crystalline bovine serum albumin as standard.

Diminacene aceturate, unlabelled putrescine and unlabelled *S*-adenosylmethionine were purchased from Sigma. (1,4-^{14}C) putrescine, *S*-adenosyl(carboxy-^{14}C)methionine were of the highest purity commercially available.

Results and discussion

In the present work, the inhibitory effects of diminacene aceturate (berenil) has been tested on diamine oxidase and *S*-adenosylmethionine decarboxylase activities from rat small intestine and rat liver respectively. Dose–response curves for the inhibition of these enzymes at threefold K_m substrate concentration of each one showed that to reduce by 50% the enzyme activities a concentration of 6 μM and 0.9 μM was needed for *S*-adenosylmethionine decarboxylase and diamine oxidase, respectively (Fig. 2). Diminacene aceturate is, however, a strong uncompetitive inhibitor of diamine oxidase with a K_i value of 0.21 μM (Fig. 3). Furthermore, this compound is a potent competitive

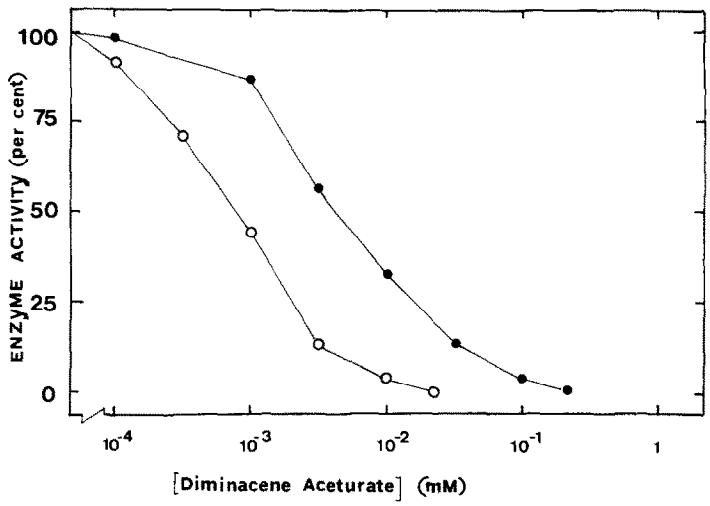

Fig. 2. Dose–response curves for inhibition of *S*-adenosylmethionine decarboxylase (●) and diamine oxidase (○) by diminacene aceturate. *S*-Adenosylmethionine and putrescine concentrations were three-fold K_m values for their respective enzyme. Each point is the average of three determinations.

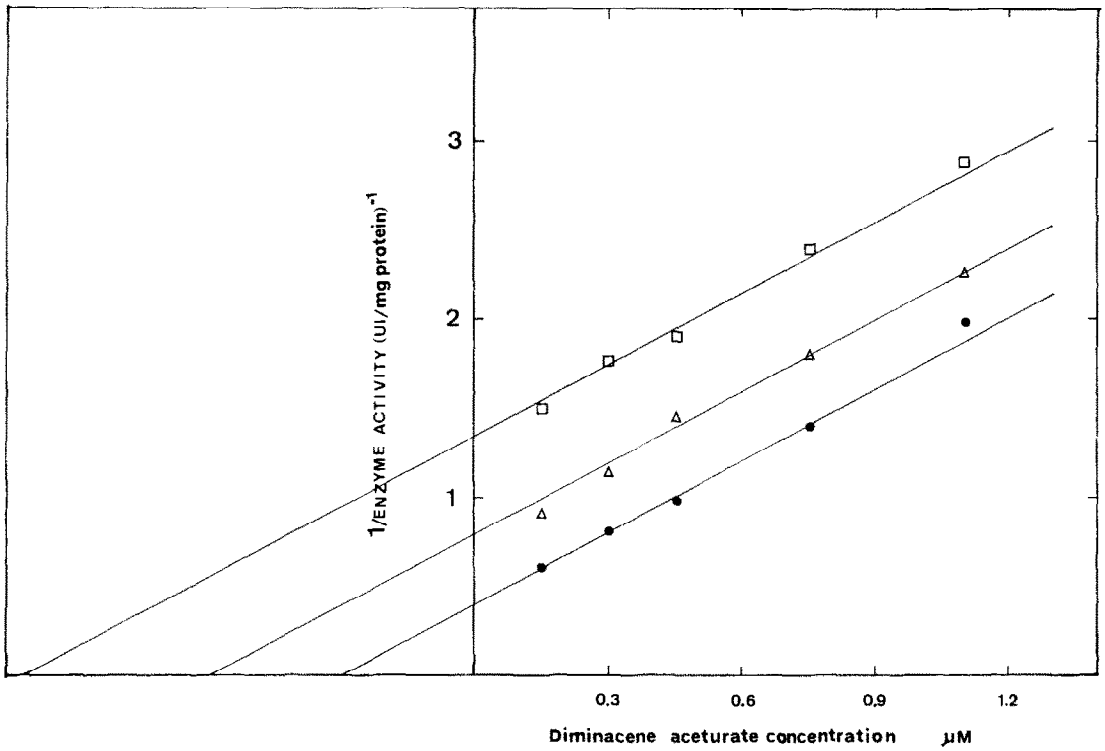

Fig. 3. Dixon plot for the inhibition of diamine oxidase by diminacene aceturate. Key: □, 125 μM putrescine; △, 250 μM putrescine; ●, 500 μM putrescine. Each point is the average of two determinations.

inhibitor of *S*-adenosylmethionine decarboxylase with a K_i of 1.8 μM (Fig. 4). While the K_i from diamine oxidase is quite similar to those found for MGBG in pig kidney [5], the K_i value from *S*-adenosylmethionine decarboxylase is slightly greater than those found for MGBG in rat liver and other mammalian tissues [12, 13]. The role of polyamines, putrescine, spermidine and spermine is almost always associated with rapidly growing tissues and proliferative processes [14, 15]. There is an ever-increasing number of reports of indirect correlations between synthesis and accumulation polyamines and tissue or cell growth. However, the physiological functions of these compounds are not clear nowadays. It seems that the only approach to solve the importance of polyamines for living animal cells and other eukaryotic organism is to find a specific inhibitor that blocks the synthesis of polyamines under conditions

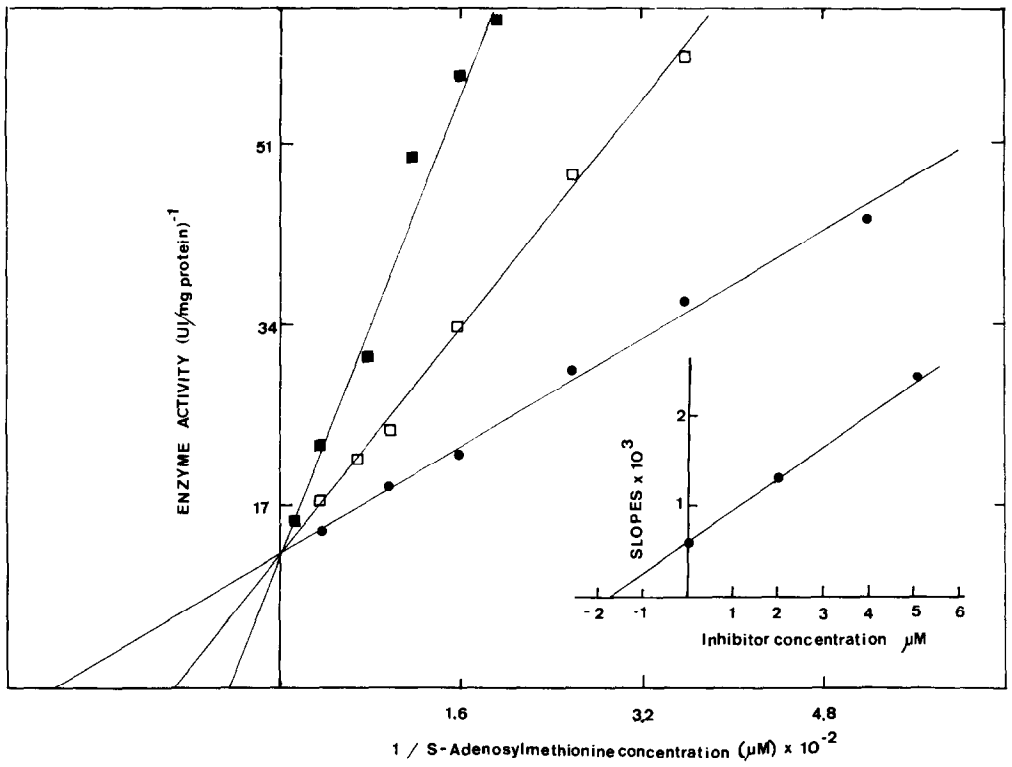

Fig. 4. Double reciprocal plot of initial velocity vs *S*-adenosylmethionine as a variable substrate at two inhibitor concentrations. Key: ●, no inhibitor, □, 2 μM diminacene aceturate; ■, 5 μM diminacene aceturate. Inset shows the replot of the slopes vs inhibitor concentration. Each point is the average of two determinations.

in which they normally accumulate. MGBG and related compounds, have been used closely as specific inhibitors of *S*-adenosylmethionine decarboxylase and diamine oxidase in proliferative and tumoral processes [16] but their high toxicity made them non advisable drug for therapeutic use; however, the berenil DL_{50} is clearly higher than the others [17]. These facts, and the wide use of diminacene aceturate (berenil) as an antiprotozoal drug, may be the first steps to understanding its pharmacological application.

In summary we found that: to reduce the diamine oxidase and *S*-adenosylmethionine decarboxylase activities by 50%, a concentration of 0.9 μM and 6 μM of inhibitor was needed; inhibition constans (K_i) of diminacene aceturate against both enzymes were estimated in 0.21 μM and 1.8 μM for diamine oxidase and *S*-adenosylmethionine decarboxylase respectively.

Acknowledgements—We wish to express our appreciation to Ms Sonia Travé-Bernabeu and to Mr Mateo González-Rouco for their excellent help in the preparation of this manuscript.

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Biochemical Pharmacology, Vol. 35, No. 9, pp. 1600–1603, 1986.
Printed in Great Britain.

0006-2953/86 $3.00 + 0.00
Pergamon Press Ltd.

Reevaluation of the products of tryptamine catalyzed by rabbit liver *N*-methyltransferases

(*Received* 22 *June* 1985; *accepted* 25 *September* 1985)

A non-specific *N*-methyltransferase [1] from rabbit liver has been described which, by reason of the wide range of xenobiotics that are acceptable substrates, suggests a possible role for this enzyme in the detoxication of exogenous amines. This catalytic activity had been referred to previously as indolethylamine *N*-methyltransferase and arylamine *N*-methyltransferase [1–6].

Interestingly, one set of data offered for the methylation of one of its substrates, tryptamine [1], implied that methylation occurred at the ring nitrogen rather than at the primary amino group of this compound. The evidence was based mainly on the observation that methylation took place without a concomitant decrease in the free amino group of tryptamine [1] when amines were measured by fluorescence after reaction with fluorescamine [7]. The unusual nature of these findings, and the present availability of two separate enzymes, amine *N*-methyltransferases A and B, of distinct but overlapping specificity [1, 8], prompted our reinvestigation of the methylation of tryptamine by both crude and homogenous rabbit liver *N*-methyltransferases. By means of a new, high-performance liquid-radiochromatographic procedure we have been able to resolve side chain and ring *N*-methylated derivatives of tryptamine. Reexamination was also expected to and did reveal the presence of enzymes responsible for the known formation of α-*N*-methyltryptamine.

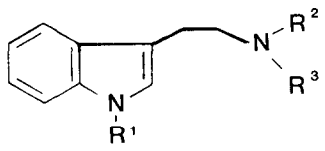
Materials and methods

Chemicals. Tryptamine hydrochloride (see Scheme 1) and α-*N*-methyltryptamine (NMT) were purchased from the Aldrich Chemical Co. (Milwaukee, WI). α,α-*N*,*N*-Dimethyltryptamine (DMT) and *S*-adenosyl-L-methionine (AdoMet) were purchased from the Sigma Chemical Co. (St. Louis, MO). N^1-Methyltryptamine (RMT) was prepared from α-*N*-methyltryptophan (Aldrich Chemical Co.) using the method of Leete [9]. *S*-Adenosyl-L-[*methyl*-^{3}H]-methionine was obtained from ICN Radiochemicals (Irvine, CA; 50 Ci/mole) or from New England Nuclear (Boston, MA; 75 Ci/mmole).

Preparation of tissue homogenates. Male New Zealand rabbits (2.0 to 2.3 kg) were killed by ear vein injection of a mixture of sodium pentobarbitone solution (4 ml; 50 mg/ml) and heparin sodium solution (0.25 ml; 10,000 units/ml). Liver and lungs were removed; lungs were inflated with room air and immediately perfused with 200 ml of saline. Perfused lungs and liver were each homogenized in three parts of 1.15% (w/v) KCl solution, and the resulting homogenate was centrifuged at 25,000 *g* for 45 min. The supernatant liquid was dialyzed against three changes, 3 liters each, of 1 mM sodium phosphate at pH 7.9 over a total of 36 hr. The final preparation was stored at −20°.

Enzyme activity in the dialyzed lung and liver preparation was determined by using AdoMet and L-tryptamine as substrates. A mixture of the dialyzed supernatant fluid (63 μl, 7 mg/ml protein), *S*-adenosyl-L-[*methyl*-^{3}H]-methionine (66 μM, 50 μCi/ml in 1 mM HCl), and 0.2 M sodium phosphate at pH 7.9 (33 μl) were incubated at 37° for 2 min. Reaction was initiated by addition of tryptamine (13 μl of a 1 mg/ml solution in 0.2 M sodium phosphate at pH 6.9) to the mixture. Incubations were carried out at 37° for 60 min and terminated by addition of an equal volume of 0.125 M potassium borate at pH 10. A control incubation was performed in the absence of tryptamine, as well as one in which enzyme heated to 100° for 5 min was substituted for an active preparation. The reaction mixtures were extracted with ethyl acetate (2 × 250 μl), the organic phase was separated and washed with 0.13 M potassium borate at pH 10, and the solvent was evaporated at room temperature under a stream of nitrogen.

Amine *N*-methyltransferases A and B were prepared by



Tryptamine — a) $R^1 = R^2 = R^3 = H$
(Ring) N^1-Methyltryptamine (RMT): — b) $R^1 = CH_3$, $R^2 = R^3 = H$
α-*N*-Methyltryptamine (NMT): — c) $R^1 = R^3 = H$, $R^2 = CH_3$
α,α-*N*,*N*-Dimethyltryptamine (DMT): — d) $R^1 = H$, $R^2 = R^3 = CH_3$

Scheme 1. Structure of methylated tryptamines.