REFERENCES

1. J. A. Coderre, S. M. Beverley, R. T. Schimke and D. V. Santi, *Proc. natn. Acad. Sci. U.S.A.* **80**, 2132 (1983).
2. M. M. Bradford, *Analyt. biochem.* **72**, 248 (1976).
3. D. A. Carson and K. P. Chang, *Biochem. biophys. Res. Commun.* **100**, 1377 (1981).
4. D. J. Nelson, S. W. LaFon, J. V. Tuttle, W. H. Miller, R. L. Miller, T. A. Krenitsky, G. B. Elion, R. L. Berens and J. J. Marr, *J. biol. Chem.* **254**, 11544 (1979).
5. P. Rainey and D. V. Santi, *Proc. natn. Acad. Sci. U.S.A.* **80**, 288 (1983).
6. R. L. Cybulski, D. W. Fry and I. D. Goldman, *J. biol. Chem.* **256**, 4455 (1981).
7. J. D. Berman, P. Rainey and D. V. Santi, *J. exp. Med.* **158**, 252 (1983).
8. D. J. Nelson, S. W. LaFon, T. E. Jones, T. Spector, R. L. Berens and J. J. Marr, *Biochem biophys. Res. Commun.* **108**, 349 (1982).
9. J. J. Marr and R. L. Berens, *Molec. biochem. Parasit.* **7**, 339 (1983).

Biochemical Pharmacology, Vol. 33, No. 8, pp. 1377–1378, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

The formation of the acidic and alcoholic metabolites of MD 780236

(*Received* 1 *September* 1983; *accepted* 18 *November* 1983)

The compound 3-{4[(3-chlorophenyl)-methoxy]phenyl}-5-(methylamino)methyl)-2-oxazolidinone methane sulphonate (MD 780236) has been shown to act as both a substrate and an inhibitor of monoamine oxidase-B and mainly as a substrate for monoamine oxidase-A [1–3]. The immediate product of the monoamine oxidase-catalysed oxidation of MD 780236 will be the corresponding aldehyde [2] and studies of the metabolic fate of this compound have shown the corresponding acid and alcohol metabolites to be formed *in vivo* and *in vitro* [2]. These results suggest that, like the aldehyde produced by the oxidation of the biogenic amines, the immediate oxidation product of MD 780236 is a substrate for aldehyde reductase and dehydrogenase actvities and that the relative rates of formation of the two products will depend on the kinetic properties of these two enzymes [4, 5].

The formation of these metabolites may be important to the monoamine oxidase inhibitory potency of MD 780236 *in vivo* since the alcohol metabolite has been shown to be a potent inhibitor of the B-form of monoamine oxidase whereas the acidic metabolite is considerably less potent [1, 6]. This paper reports the action of aldehyde-metabolizing enzymes on MD 240233, the aldehyde derived from the oxidation of MD 780236.

Materials and methods

The high-K_m aldehyde reductase was purified from ox brain by the method of Rivett *et al.* [7] and the low-K_m enzyme was purified from the same source by a modification of the method of Daly and Mantle [8]. Both enzyme preparations were homogeneous by the criteria of polyacrylamide electrophoresis in the presence and absence of sodium dodecyl sulphate. Aldehyde dehydrogenase was purified from ox liver by a modification [9] of the method of Deitrich *et al.* [10]. Crystalline horse liver alcohol dehydrogenase and coenzymes were obtained from the Boehringer Corporation Ltd., London.

MD 780236 and MD 240233, its hydrated aldehyde derivative, were synthesized by the Department of Chemistry, Centre de Recherche Delalande, Paris. Stock solutions of MD 780236 were prepared in water whereas those of MD 240233 were dissolved in 10% dimethyl sulphoxide. At the concentrations used in the enzyme assays dimethyl sulphoxide had no effect on the activities of aldehyde dehydrogenase or reductase.

All enzyme assays were carried out spectrophotometrically at 30° by following the increase or decrease in absorbance at 340 nm. Aldehyde dehydrogenase activity was determined in a reaction mixture containing, in a total volume of 2.0 ml, 200 μM NAD^+, the appropriate buffer, aldehyde and the enzyme preparation. Alcohol dehydrogenase was assayed in a reaction mixture containing, in a total volume of 2.0 ml, 100 mM potassium phosphate buffer, pH 7.2 250 μM NAD^+, aldehyde and enzyme. The activity of aldehyde reductase was determined in a mixture containing, in a total volume of 3.2 ml, 100 mM potassium phosphate buffer, pH 7.2, 125 μM NADPH and the appropriate concentrations of aldehyde and enzyme. Protein concentration was determined by the method of Markwell *et al.* [11].

Results and discussion

Two major forms of aldehyde reductase have been detected in the livers and brains from several animal species. The more active form has been termed the high-K_m aldehyde reductase [12] and it has been suggested that this enzyme may be identical with L-hexonate dehydrogenase [13] whereas the other form, which has been termed the low-K_m aldehyde reductase [12], may be identical with aldose reductase [13]. Studies with the high-K_m aldehyde reductase from ox brain indicated that it had no significant activity towards MD 240233 at concentrations of up to 50 μM. At this concentration MD 240233 had no inhibitory effect on the reduction of 500 μM pyridine-3-aldehyde by this enzyme. In contrast the low-K_m aldehyde reductase was active towards this substrate with a K_m value of 2.8 ± 0.3 μM and a maximum velocity of 102 nmol · min^{-1} mg^{-1} (Fig. 1). This maximum velocity was 28 ± 3% of that determined with pyridine-3-aldehyde, a good substrate for this enzyme [14]. The activity towards a mixture of MD 240233 and pyridine-3-aldehyde, each at a concentration of 10 times its K_m value, was 50.4 ± 3.2% of the sum of the activities obtained when the two substrates were assayed separately indicating that the same enzyme was responsible for the metabolism of these two aldehydes [15].

Another enzyme that might catalyse the reduction of aldehydes is alcohol dehydrogenase. However, no significant activity of this enzyme towards MD 240233 at concentrations of up to 100 μM could be detected. The compound was, however, an inhibitor of the reduction of acetaldehyde, a concentration of 25 μM MD 240233 resulting in 90% inhibition of the activity towards 900 μM acetaldehyde.

Dimethyl sulphoxide, in which the MD 240233 was dissolved, also proved to be an inhibitor of alcohol dehydrogenase at higher concentrations. In the studies reported above dimethyl sulphoxide was present at a constant concentration of 0.1% in all determinations. However, if this concentration was increased to 1.0% the activity towards 900 μM acetaldehyde was inhibited by more than 90%.

The partly purified preparation of aldehyde dehydrogenase showed only slight activity towards MD 240233 when assayed at pH 7.2. As shown in Fig. 1, the K_m value was found to be 4.3 ± 0.2 μM under these conditions. Studies on the effects of pH on the activity of the enzyme towards this and other aldehydes, shown in Fig. 2, revealed that the optimum pH was considerably higher and that the enzyme was more active in pyrophosphate than in phosphate buffer. In 100 mM pyrophosphate buffer, pH 8.5, the K_m value was found to be 7.0 ± 1.0 μM. The maximum velocity at this pH value was about 10% of that determined with 3 mM acetaldehyde. In contrast the optimum pH of the reaction catalysed by the low-K_m aldehyde reductase was found to be below pH 7.0 (data not shown).

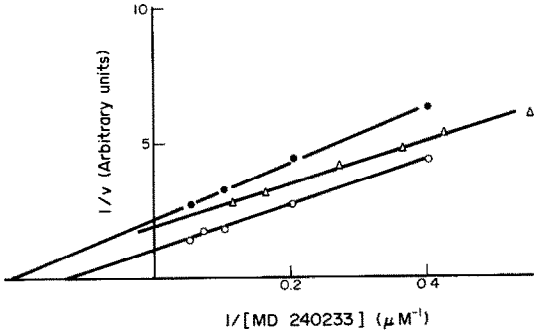

Fig. 1. Double-reciprocal plots of the oxidation and reduction of MD 240233. The reduction in the presence of the low-K_m aldehyde reductase and NADPH was determined at pH 7.2 (△) and the oxidation in the presence of aldehyde dehydrogenase and NAD^+ was determined in 50 mM phosphate buffer, containing 25 mM pyrophosphate, pH 7.2 (●) or in 50 mM pyrophosphate buffer pH 8.5 (○). Other details are as described in the text.

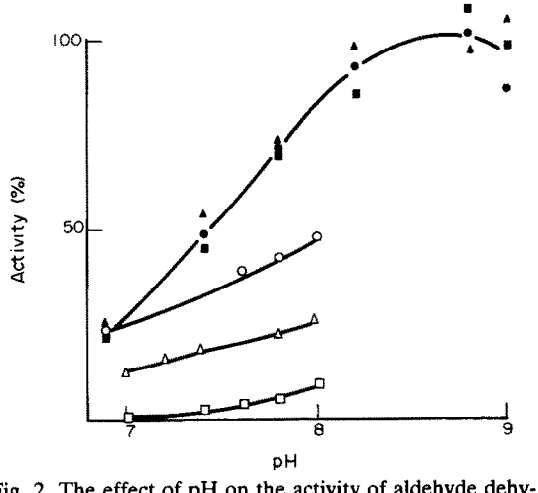

Fig. 2. The effect of pH on the activity of aldehyde dehydrogenase. Initial rate measurements were made in 100 mM phosphate buffer (open symbols) or 100 mM pyrophosphate buffer (closed symbols) with 50 μM MD 240233 (□, ■), 100 μM acetaldehyde (△, ▲) or 800 μM pyridine-3-aldehyde (●, ○). Activities are expressed as a percentage of that determined at pH 8.6 in pyrophosphate buffer in each case.

The compound MD 240233 was in its hydrated aldehyde (gem-diol) form and it is possible that the kinetic constants for the unhydrated form may be different. In the case of aldehyde dehydrogenase, however, it is still unclear whether the enzyme shows a preference for one of these forms [15].

The relatively high pH optimum for the oxidation of MD 240233 may explain the observation that the production of the acid metabolite in brain homogenates was considerably increased when the pH of the medium was raised from 7.4 to 8.8 [2] and the relatively low-K_m value and high activity of aldehyde reductase is consistent with the importance of the alcohol metabolite *in vivo* [3]. Since the low-K_m aldehyde reductase has been shown to play the dominant role in catalysing the reduction of the aldehydes derived from the biogenic amines [4, 5], competition by MD 240233 might be expected to reduce the flux of those aldehydes through this pathway. Since these aldehydes are believed to have specific pharmacological actions of their own (see [5] for review) this might contribute to the effects of MD 240233 *in vivo*. The possible significance of the inhibitory effect on alcohol dehydrogenase remains to be evaluated.

Department of Biochemistry KEITH F. TIPTON
Trinity College JOHN M. MCCRODDEN
Dublin 2 GARY T. M. HENEHAN
Republic of Ireland

Centre de Recherche Delalande THIERRY BOUCHER
F 92500 Rueil-Malmaison
France

Research and Development Laboratories CHRISTOPHER J. FOWLER
Astra Läkemedel AB
S-15185 Södertälje
Sweden

REFERENCES

1. K. F. Tipton, C. J. Fowler, J. M. McCrodden and M. Strolin Benedetti, *Biochem. J.* **209**, 235 (1983).
2. M. Strolin Benedetti and J. Dow, *J. Pharm. Pharmac.* **35**, 238 (1983).
3. M. Strolin Benedetti, P. Dostert, T. Boucher and C. Guffroy, in *Monoamine Oxidase: Basic and Clinical Frontiers* (Eds. K. Kamijo, E. Usdin and T. Nagatsu) pp. 209–220. Exerpta Medica, Amsterdam (1982).
4. A. J. Turner, J. A. Illingworth and K. F. Tipton, *Biochem. J.* **144**, 353 (1974).
5. K. F. Tipton, M. D. Houslay and A. J. Turner, *Essays Neurochem. Neuropharmac.* **1**, 103 (1977).
6. C. J. Fowler, K. F. Tipton, M. Strolin Benedetti and J. Dow, *Acta Physiol. Scand. Suppl.* **508**, 39 (1982).
7. A. J. Rivett, I. L. Smith and K. F. Tipton, *Biochem. J.* **197**, 473 (1981).
8. A. Daly and T. J. Mantle, *Biochem. J.* **205**, 373 (1982).
9. M. D. Houslay and K. F. Tipton, *Biochem. J.* **135**, 173 (1973).
10. R. A. Deitrich, L. Hellerman and J. Wein, *J. biol. Chem.* **237**, 560 (1962).
11. M. A. K. Markwell, S. M. Haas, L. L. Bieber and N. G. Tolbert, *Analyt. Biochem.* **27**, 206 (1978).
12. A. J. Turner and K. F. Tipton, *Biochem. J.* **130**, 765 (1972).
13. T. G. Flynn, *Biochem. Pharmac.* **31**, 2705 (1982).
14. C. M. Ryle and K. F. Tipton, *Biochem. J.* **197**, 715 (1981).
15. M. Dixon and E. C. Webb, *Enzymes*, pp. 72–75. Longmans, London (1979).
16. H. Weiner, S. Freytag, J. M. Fox and J. H. J. Hu, in *Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase and Aldo/Keto Reductase* (Eds. H. Weiner and B. Wermuth) pp. 91–102. Alan R. Liss, New York.