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Inhibition of aldehyde reductase by acidic metabolites of the biogenic amines

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Reduced NADP-dependent aldehyde reductase (EC 1.1.1.2) is an enzyme of wide specificity capable of reducing aromatic aldehydes, long chain aliphatic aldehydes and certain aldoses [1, 2]. An important function of the aldehyde reductases from brain tissue may be in the metabolism of the aldehydes derived by deamination of the neurotransmitter biogenic amines [1–4]. These "biogenic aldehydes" may either be reduced to alcohols by aldehyde reductase or oxidized to acids by aldehyde dehydrogenase (EC 1.2.1.3) and several studies have shown that the preferred route of amine metabolism appears to be mainly dependent upon the kinetic parameters of the aldehyde metabolising enzymes [5–8]. Such studies have, however, not considered the possible inhibition of aldehyde reductase by acidic or alcohol metabolites of the biogenic amines although such inhibition has recently been reported to be significant [4]. This communication examines the nature and potency of the inhibition of brain aldehyde reductase by biogenic amine metabolites and suggests that such inhibition is unlikely to be a factor that regulates amine metabolism *in vivo*.

MATERIALS AND METHODS

All chemicals were of the highest grade commercially available and unless otherwise stated were obtained from British Drug Houses Ltd. (Poole, Dorset, U.K.). Coenzymes were obtained from Boehringer (Mannheim), Germany and were stored desiccated at 4°. 3-pyridine-carboxaldehyde (PC), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), DL-4-hydroxy-3-methoxymandelic acid (vanillylmandelic acid), 5-hydroxyindole-3-acetic acid, imidazole acetic acid and 3,4-dihydroxyphenylglycol were obtained from Sigma (London) Chemical Co., U.K. Pyridine-3-methanol was from R. N. Emanuel Ltd., Wembley, U.K.

Multiple forms of reduced NADP dependent aldehyde reductase occur in brain tissue [3, 4]. The major isoenzyme in sheep brain was purified to a sp. act. of 0·6 units/mg protein by the method previously described for the isolation of this enzyme from pig brain [1]. The purified product exhibited a single band of activity following polyacrylamide gel electrophoresis [1]. Initial rate kinetic studies with this enzyme produced linear Lineweaver–Burk reciprocal plots over a 100-fold range of aldehyde concentration [3].

Routine assays of brain aldehyde reductase were performed at 30° in 200 mM sodium phosphate buffer, pH 7·2, containing 0·8 mM PC and 0·1 mM NADPH. For kinetic studies, addition of enzyme was normally used to start the reaction. The reaction was monitored continuously by following the decrease in absorbance at 340 nm in a Gilford Model 240 Spectrophotometer coupled to a Servoscribe 8-in. chart recorder. The reaction rate was linear for at least 5 min and the initial rate was proportional to enzyme concentration. A unit of enzyme activity is defined as the amount that catalyses the oxidation of 1 μmole of NADPH/min at 30°.

The concentration of PC in solution was estimated [2] assuming a molar extinction coefficient of $3{\cdot}35 \times 10^3$. Stock solutions of NADPH in glass distilled water were made freshly each day and assayed as described previously [1]. The various inhibitors tested were dissolved in the assay buffer before use and their addition to the reaction mixture at the concentrations used in these experiments did not affect the pH of the assay medium. The experimental data were initially fitted to reciprocal plots by eye to determine linearity and kinetic constants were obtained using a modification of the computer program of Cleland [9] and the University of Leeds ICL 1906A computer. Data were fitted to rate equations describing linear non-competitive, uncompetitive and competitive inhibition to determine the equation giving best fit to the data and the most valid values of kinetic parameters.

RESULTS AND DISCUSSION

The apparent Michaelis constants for NADPH and PC were estimated as $3{\cdot}5 \times 10^{-6}$ M and $9{\cdot}0 \times 10^{-4}$ M respectively which are similar in magnitude to the values previously obtained for the enzymes isolated from pig brain and kidney [1, 2]. A variety of acid metabolites of the catecholamines and 5-hydroxytryptamine were shown to cause significant inhibition of sheep brain aldehyde reductase (Table 1). In contrast imidazole acetic acid, 3,4-dihydroxyphenylglycol (an alcohol metabolite of noradrenaline) and pyridine-3-methanol (the product of PC reduction) exerted little inhibitory effect on this enzyme. Of the compounds tested, the greatest inhibition was shown by HVA, a major metabolite of dopamine.

Abbreviations: HVA: 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid); DOPAC: 3,4-dihydroxyphenylacetic acid; PC: 3-pyridine-carboxaldehyde.

Table 1. Inhibition of sheep brain aldehyde reductase by acid and alcohol derivatives

Inhibitor	Rate (% of control)
Homovanillic acid	50
3,4-Dihydroxyphenylacetic acid	76
Vanillyl mandelic acid	85
5-Hydroxyindolyl-3-acetic acid	89
3,4-Dihydroxyphenylglycol	94
Pyridine-3-methanol	97
Imidazole acetic acid	101

Aldehyde reductase was assayed in the presence of 8×10^{-4} M pyridine-3-carboxaldehyde and 1×10^{-4} M NADPH at pH 7·2 and 30°. All inhibitors were tested at a concentration of 5×10^{-4} M except DL-vanillyl mandelic acid which was at a conc of 1×10^{-3} M. The reaction was started by addition of enzyme. The results are the mean of experiments from three separate preparations of enzyme.

Accordingly, the inhibition patterns exhibited by this compound were examined in more detail. The inhibition by HVA was observed to be reversible in nature by dilution experiments and pre-incubation of enzyme and HVA for 10 min at 30° did not affect the degree of inhibition obtained.

Reciprocal plots of data obtained for the inhibition of aldehyde reductase by HVA in the range 0–1 mM could be fitted most closely to an uncompetitive type of inhibition when either NADPH or aldehyde was the variable substrate and the co-substrate was held at non-saturating or saturating concentrations (Figs. 1 and 2). Secondary plots of intersection on the vertical axis against inhibitor concentration were linear and an apparent K_i value of $2{\cdot}2 \times 10^{-4}$ M was obtained when aldehyde was the variable substrate. It should be noted that at high concentrations of HVA (greater than 1 mM) deviation from uncompetitive inhibition was observed and the inhibition tended to become of mixed type. A similar pattern of inhibition was observed using 5-hydroxyindolyl-3-acetic acid. The studies reported here show that a variety of acid metabolites of the biogenic amines reversibly inhibit brain aldehyde reductase. This raises the possibility that the activity of aldehyde reductase *in vivo* may be regulated in part by these acidic compounds and in particular by HVA and DOPAC, the major metabolites of dopamine in mammalian brain.

Maximal levels of HVA in brain occur in the striatum and estimates of these levels vary between 0·12–0·48 μg/g tissue [10–12]. If it is assumed that there is a uniform distribution of HVA in striatum and that the intracellular space (non-inulin space) is approximately 0·56 ml/g tissue (calculated from [13]), then values in the range $1{\cdot}2 \times 10^{-6}$–$4{\cdot}7 \times 10^{-6}$ M are obtained for normal intracellular concentrations of HVA in striatum. These concentrations may be increased as much as 3-fold however after treatment with reserpine, amphetamines, neuroleptics and certain cholinomimetic drugs [10–12, 14]. By similar calculations the concentration of DOPAC in rat striatum may be estimated as approx 7×10^{-6} M [15], whereas concentrations of HVA and DOPAC in cerebrospinal fluid and plasma are much lower [16]. Despite the problems and approximations inherent in estimating intracellular metabolite concentrations in brain [13, 17] it would appear that the levels of HVA and DOPAC are considerably lower than the apparent K_i for inhibition of the enzyme ($2{\cdot}2 \times 10^{-4}$ M). Thus it seems unlikely that these metabolites exert a significant inhibitory effect *in vivo* on aldehyde reductase. However, until more is known of the properties

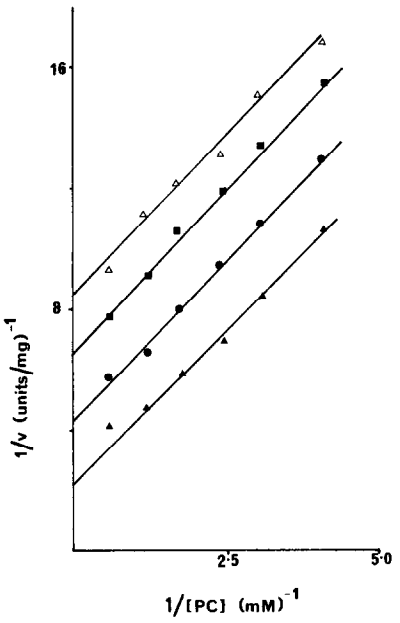

Fig. 1. Aldehyde reductase inhibition by homovanillic acid: aldehyde as variable substrate. Double reciprocal plot of the inhibition of aldehyde reductase by HVA with PC as variable substrate. NADPH was held constant at 5 μM and the concentrations of HVA used were: (▲) no added HVA, (●) $0{\cdot}2 \times 10^{-3}$ M HVA, (■) $0{\cdot}4 \times 10^{-3}$ M HVA, (△) $0{\cdot}6 \times 10^{-3}$ M HVA. The lines drawn are computer "best fits" to the rate equation describing uncompetitive inhibition.

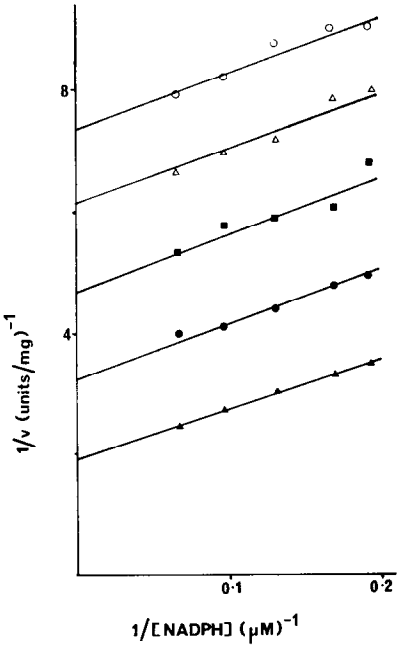

Fig. 2. Aldehyde reductase inhibition by homovanillic acid; NADPH as variable substrate. Double reciprocal plot of the inhibition of aldehyde reductase by HVA with NADPH as variable substrate. PC was held constant at 8×10^{-4} M and the concentrations of HVA used were: (▲) no added HVA, (●) $0{\cdot}25 \times 10^{-3}$ M HVA, (■) $0{\cdot}50 \times 10^{-3}$ M HVA, (△) $0{\cdot}75 \times 10^{-3}$ M HVA, (○) $1{\cdot}0 \times 10^{-3}$ M HVA. The lines drawn are computer "best fits" to the rate equation describing uncompetitive inhibition.

and the perisynaptic distribution of the multiple forms of brain aldehyde reductase, the possibility of such physiological regulation cannot be ruled out entirely.

The use of 'dead-end' inhibitors in enzyme kinetic studies may sometimes allow a distinction to be made between various kinetic mechanisms [18]. In this case, HVA was observed to show an uncompetitive type of inhibition when either NADPH or aldehyde was the variable substrate. Brain aldehyde reductase obeys a sequential kinetic mechanism ([19] and unpublished observations). The pattern of inhibition shown by HVA unfortunately does not allow a distinction to be made between an ordered or random addition of substrates to the enzyme but would not be inconsistent with the random order mechanism proposed for ox brain aldehyde reductase [19] if HVA bound reversibly to the enzyme central complex to form an inhibited species.

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