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BIOGENIC ALDEHYDE METABOLISM IN RAT BRAIN

DIFFERENTIAL SENSITIVITY OF ALDEHYDE REDUCTASE ISOENZYMES TO SODIUM VALPROATE

SUSAN R. WHITTLE and ANTHONY J. TURNER *

Department of Biochemistry, University of Leeds, 9 Hyde Terrace, Leeds LS2 9LS (U.K.)

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Summary

The effects of inhibitors of aldehyde reductase (alcohol:$NADP^+$ oxidoreductase, EC 1.1.1.2) on the formation of 3-methoxy-4-hydroxyphenethylene glycol from normetanephrine have been studied in rat brain homogenates. The reaction pathway was shown to be unaffected by several inhibitors of the major (high K_m) form of aldehyde reductase such as sodium valproate. Two isoenzymes of aldehyde reductase have been separated and characterized from rat brain. The minor (low K_m) isoenzyme is shown to be relatively insensitive to sodium valproate and exhibits a similar inhibitor-sensitivity profile to that obtained for methoxyhydroxyphenethylene glycol formation. The low K_m isoenzyme is therefore implicated in catecholamine metabolism. The metabolism of succinic semialdehyde and xylose by rat brain cytosol has also been examined. Aldose metabolism may also be attributed to the action of the low K_m reductase, but the existence of a separate succinic semialdehyde reductase is postulated. The possible roles of aldehyde reductases in brain metabolism and the relationship between these enzymes and aldose reductase (alditol: $NADP^+$ 1-oxidoreductase, EC 1.1.1.21) are discussed.

Introduction

NADPH-dependent aldehyde reductases (alcohol:$NADP^+$ oxidoreductase, EC 1.1.1.2) are widely distributed throughout the animal kingdom [1]. These

* To whom correspondence should be addressed.

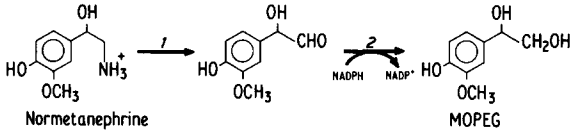

Fig. 1. Conversion of normetanephrine to its major metabolite in brain, 3-methoxy-4-hydroxyphenethylene glycol (MOPEG). The enzymes involved in this pathway are monoamine oxidase and aldehyde reductase. In rat brain less than 10% of the aldehyde is converted to its corresponding acidic metabolite 3-methoxy-4-hydroxymandelic acid under the conditions used [3].

enzymes are monomeric oxidoreductases that show a broad specificity towards aromatic and aliphatic aldehydes [2,3] and are characteristically sensitive to inhibition by phenobarbitone [4]. Because of the range of substrates reduced by aldehyde reductase, its physiological role is unclear. It may well peform distinct functions in different tissues. For example, the enzyme has been implicated in the metabolism of aldoses and carbonyl-containing drugs in liver [5-7], whereas in cardiac muscle it may be involved in the formation of long-chain aliphatic alcohols [8]. In brain tissue the most likely candidates as natural substrates include aldoses and the aldehydes formed by deamination of catecholamines, indoleamines and γ-aminobutyric acid [3].

Multiple forms of aldehyde reductase have been shown to occur in mammalian brain. The major isoenzyme (referred to in the literature as AR1 or high K_m form) is cytosolic in location, NADPH-dependent and exhibits a relatively high K_m towards the model substrate *p*-nitrobenzaldehyde [9,10]. The minor, low K_m reductase (AR2) can use both NADPH and NADH as cofactors and is less sensitive to inhibition by phenobarbitone [11,12]. Human brain has been reported to contain additional aldehyde-reducing enzymes [12].

The major aldehyde reductase in brain tissue is inhibited by sodium valproate (2-propyl pentanoate) a branched-chain fatty acid that is particularly effective in the treatment of epilepsy [13]. It has been suggested that inhibition of aldehyde reductase by anti-convulsants may be an important physiological site of action of these drugs [4,14]. Both the aldehyde reductase isoenzymes in brain can use biogenic aldehydes as substrates in vitro [11,15] and have been implicated in the metabolism of the catecholamine normetanephrine (3-*O*-methyl noradrenaline) to its major metabolite 3-methoxy-4-hydroxyphenethyleneglycol (Fig. 1) [16]. Sodium valproate would seem to be a useful tool for examining the role of aldehyde reductases in the metabolism of physiologically relevant aldehydes in brain.

The reduction of aldoses such as xylose to their corresponding polyols has been ascribed to the enzyme aldose reductase (alditol:$NADP^+$ 1-oxidoreductase, EC 1.1.1.21). This enzyme occurs in nervous tissue [17] and has been implicated in some of the secondary effects observed in diabetes, such as neuropathy and cataract formation [18,19]. The relationship of aldose reductase to aldehyde reductases is unclear. Boghosian and McGuinness [20] have presented evidence that aldose reductase from pig brain is identical with the low K_m aldehyde reductase previously described by Turner and Tipton [11]. In contrast, Tulsiani and Touster [21] have claimed that the two isoenzymes of aldehyde reductase in mouse liver are distinct from aldose reductase.

The reduction of succinic semialdehyde to γ-hydroxybutyrate is a minor but significant route of degradation of the inhibitory neurotransmitter γ-aminobutyric acid. The formation of γ-hydroxybutyrate in brain may be relevant to seizure mechanisms since it has been shown that it can cause epileptic-like activity in animals [22,23]. Although the major isoenzyme of aldehyde reductase can reduce succinic semialdehyde in vitro [24], there is evidence for the presence in brain of a specific high-affinity succinic semialdehyde reductase [25,26]. The relative contributions of these two enzymes to succinic semialdehyde reduction in vivo remains to be clarified.

The present work examines the metabolism in rat brain of normetanephrine, succinic semialdehyde and xylose in relation to the isoenzymes of aldehyde reductase. The two isoenzymes are shown to differ dramatically in their sensitivity to inhibition by sodium valproate, allowing the low K_m minor isoenzyme to be identified as the principal reductase involved in catecholamine metabolism.

Experimental

Materials

All chemicals were of the highest grade commercially available and unless stated otherwise were obtained from British Drug Houses Ltd., Poole, Dorset, U.K. Coenzymes and triethanolamine hydrochloride were from the Boehringer Corp., Lewes, Sussex, U.K. and pyrazole and diphenylhydantoin from Aldrich Chemical Co., Gillingham, U.K. Succinic semialdehyde, indol-3-ylacetaldehyde bisulphite compound, pyridine-3-aldehyde, quercetin, diphenylacetic acid, methoxyhydroxyphenethylene glycol and normetanephrine were purchased from Sigma (London) Ltd. Phenylacetylurea (phenacemide) and 2-phenylbutyric acid were from Aldrich Chemical Co., Milwaukee, WI, U.S.A., and quercitrin was from I.C.N. Pharmaceuticals Inc., Plainview, NJ, U.S.A. Microgranular DEAE-cellulose (DE-52) was obtained from Whatman Ltd., Maidstone, Kent, U.K. 3,4-Dihydroxyphenyl[1-^{14}C]ethylamine hydrochloride ([^{14}C]-dopamine hydrochloride, 53 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, U.K. Sodium valproate and carbamazepine were gifts from Reckitt and Colman Pharmaceutical Division, Hull, U.K.

Preparation of substrates. *p*-Nitrobenzaldehyde was recrystallized from water before use. Indole-acetaldehyde was prepared free of $NaHSO_3$ immediately before use by the method of Erwin et al. [27]. A solution (0.01 M) of the $NaHSO_3$ compound dissolved in 0.03 M sodium pyrophosphate buffer, pH 9.6, was extracted three times with equal volumes of diethyl ether. The combined ether extracts were washed three times with an equal volume of water, reduced under N_2 to approximately half the original volume, and finally shaken with an equal volume of 0.1 M sodium phosphate buffer, pH 6.5. The ether phase was then completely removed under N_2 and the concentration of aldehyde in the final solution was determined enzymically with purified aldehyde reductase.

Methods

Assay of aldehyde reductase. The standard assay mixture contained 0.1 M sodium phosphate buffer, pH 6.5/0.1 mM NADPH/0.5 mM *p*-nitrobenzal-

dehyde in a total volume of 3 ml. The reaction was followed by measuring the decrease in absorbance at 340 nm in a Gilford 240 spectrophotometer at 30°C. Under the above conditions, the reaction rate was linear for at least 5 min and was proportional to enzyme concentration in the range used. 1 unit of enzyme activity catalyses the oxidation of 1 μmol NADPH/min at 30°C.

Assay of monoamine oxidase. The activity of monoamine oxidase (amine: oxygen oxidoreductase (deaminating) (flavin-containing), EC 1.4.3.4) was assessed radiometrically, using 5 mM [^{14}C]dopamine as substrate, by the method of Southgate and Collins [28] taking note of the precautions discussed by Tipton and Youdim [29].

Assay of methoxyhydroxyphenethylene glycol. The production of methoxyhydroxyphenethylene glycol from normetanephrine by rat brain homogenates was measured by a modification of the method of Anderson et al. [16]. Rat brains were homogenized in 4 vol. 0.1 M sodium phosphate buffer (pH 7.2) and a sample (equivalent to 25 mg tissue, wet weight) was incubated for 60 min, at 37°C, in the same buffer containing 0.2 mM NADPH and 1 mM normetanephrine in a total volume of 1 ml. When inhibitors were included in the reaction mixture the sample was pre-incubated for 10 min with the inhibitor, before starting the reaction with normetanephrine. Reactions were terminated by the addition of 3 M HCl (1 ml) and denatured protein was removed by centrifugation. A sample (1 ml) of the supernatant was then added to 4.7 ml 0.15 M HCl and fluorescence was developed by a modification of the method of Meek and Neff [30]. To the solution was added 0.3 ml perchloric acid (60%, v/v) and the mixture was divided into 3 aliquots of 2 ml, to provide a blank incubation and duplicate experimental samples. The latter were heated at 100°C for 12 min, whereas the control sample was kept at room temperature. Re-distilled diaminomethane (0.3 ml) was added to all tubes, which were then heated at 100°C for 5 min. After cooling to room temperature the fluorescent intensity of each sample was measured in an Aminco SPF 125 spectrofluorimeter (excitation at 375 nm, emission at 465 nm, uncorrected). Each batch of assays included an internal standard containing 5 nmol methoxyhydroxyphenethylene glycol to allow correction for recovery, which was in the range 90 ± 2%. A standard curve was produced by adding various amounts of methoxyhydroxyphenethylene glycol to 0.15 M HCl and developing fluorescence as above. The standard curve was linear up to 10 nmol methoxyhydroxyphenethylene glycol.

Separation of aldehyde reductase isoenzymes. All steps were carried out at 4°C and all buffers contained 0.5 mM 2-mercaptoethanol. Brains were stored frozen at −70°C and thawed when required.

(i) Rat brain (150—200 g) was homogenized with 2 vol. 100 mM triethanolamine-HCl buffer, pH 7.5 and the homogenate was subjected to centrifugation at 23 000 × g_{av} for 30 min. The residue was discarded and the pH of the supernatant was lowered to 5.4 with 50% (v/v) acetic acid. After 5 min at 4°C the denatured protein was removed by centrifugation and the pH of the supernatant readjusted to pH 7.5, with 1 M NaOH.

(ii) To each 100 ml supernatant was added 22.6 g $(NH_4)_2SO_4$ and after stirring for 30 min, the precipitated protein was removed by centrifugation. A further 18.7 g was then added to each 100 ml supernatant and, after centrifugation, the precipitate was dissolved in a minimum volume of 15 mM tri-

ethanolamine-HCl buffer, pH 7.5, and dialysed overnight against 5 l of this buffer.

(iii) The sample was applied to a column (3 × 8 cm) of DEAE-cellulose equilibrated and eluted with 15 mM triethanolamine-HCl buffer pH 7.5. Fractions (5-ml) were collected until the E_{280} had fallen below 0.2. The material unadsorbed to DEAE-cellulose contained the major, high K_m isoenzyme, AR1. The column was then washed with at least 1 l of equilibration buffer to ensure its complete removal. The second isoenzyme, AR2, was then eluted by washing the column with equilibration buffer containing 1 M KCl. Fractions containing the two isoenzymes were pooled separately, concentrated by precipitation with $(NH_4)_2SO_4$ (60 g/100 ml) and dialysed overnight against 0.1 M sodium phosphate buffer, pH 6.5 containing 0.5 mM 2-mercaptoethanol. The two isoenzymes were stored at 4°C and lost no significant activity over a period of 7 days. Freezing and thawing resulted in a loss of activity of the low K_m isoenzyme.

Polyacrylamide gel electrophoresis. Electrophoresis in 7.5% polyacrylamide gels was performed by the method of Zweig and Whitaker [31]. The buffer system used was 10 mM Tris-glycine, pH 8.5. Samples were applied to the gels in 30% (v/v) glycerol as was Bromothymol blue (0.01%) which was used as tracking dye. Electrophoresis was performed at 4°C with a constant current of 2 mA/gel until the tracking dye had neared the bottom of the gel. The gels were washed with 0.1 M glycine/NaOH buffer, pH 9.5, before staining for enzyme activity by the method of Turner and Tipton [11]. Gels were incubated at room temperature in the dark, with fresh glycine buffer containing the following components: $NADP^+$ (0.4 mg/ml)/phenazine methosulphate (0.04 mg/ml)/Nitro-blue tetrazolium (0.4 mg/ml)/propane-1,2-diol (1 M). Control gels were incubated in the absence of propane-1,2-diol.

Results

Methoxyhydroxyphenethylene glycol formation in rat brain. Rat brain homogenates incubated in the presence of NADPH and normetanephrine produced approx. 12 nmol methoxyhydroxyphenethylene glycol/min per g brain (wet weight). The formation of methoxyhydroxyphenethylene glycol was linear with time up to 70 min under the conditions used. When NADH was substituted as cofactor, methoxyhydroxyphenethylene glycol formation was still seen but only at 20% of the rate observed with NADPH. The effects of a variety of aldehyde reductase inhibitors on methoxyhydroxyphenethylene glycol formation are shown in Table I. Surprisingly, sodium valproate and its structural analogues did not affect methoxyhydroxyphenethylene glycol formation at concentrations that caused substantial inhibition (more than 95%) of the activity of the major, high K_m aldehyde reductase. Carbamazepine also had no inhibitory effects. One possible explanation for these results might be that aldehyde reductase is not rate-limiting in the overall pathway from normetanephrine to methoxyhydroxyphenethylene glycol. However, all other aldehyde reductase inhibitors caused significant inhibition of methoxyhydroxyphenethylene glycol synthesis (Table I). No significant effect on monoamine oxidase activity was observed with any of the compounds that inhibited methoxy-

TABLE I

EFFECTS OF ALDEHYDE REDUCTASE INHIBITORS ON THE FORMATION OF METHOXYHYDROXYPHENETHYLENE GLYCOL FROM NORMETANEPHRINE IN RAT BRAIN HOMOGENATES

Samples of rat brain homogenate (equivalent to 25 mg tissue) were incubated for 60 min at 37°C in the presence of 0.2 mM NADPH and 1 mM normetanephrine. The methoxyhydroxyphenethylene glycol formed was estimated as described in Methods. The results quoted are the mean ± S.E. for four incubations, with the exception of the uninhibited reaction which represents the mean of 12 incubations. All additives were at a concentration of 1 mM. MOPEG, methoxyhydroxyphenethylene glycol.

Additive	MOPEG formed (nmol/min per g tissue)	Inhibition (%)
None	12.82 ± 0.72	—
Phenobarbitone	7.83 ± 0.83 *	39
Diphenylhydantoin	3.02 ± 0.20 **	76
Quercetin	0.51 ± 0.26 **	96
Quercitrin	8.56 ± 0.63 *	33
Carbamazepine	13.63 ± 1.72	0
Sodium valproate	12.49 ± 0.50	0
2-Phenylbutyric acid	13.80 ± 1.9	0
2-Ethylbutyric acid	15.90 ± 1.53	0

* Significantly different from control incubation ($P < 0.01$).
** Significantly different from control incubation ($P < 0.001$).

hydroxyphenethylene glycol formation with the exception of diphenylhydantoin (21% inhibition at 1.0 mM, mean of three determinations). These data suggest that methoxyhydroxyphenethylene glycol formation from normetanephrine may involve a valproate-insensitive form of aldehyde reductase. In order to confirm this possibility it was necessary to separate and compare the isoenzymes of aldehyde reductase in rat brain. Resolution of two such isoenzymes has previously been reported by Ris and Von Wartburg [12]. However, we were unable to resolve two distinct isoenzymes satisfactorily by this method. The reason for this is unclear, but it may relate to the difficulties involved in equilibrating DEAE-cellulose in 5 mM phosphate buffer. An alternative and reproducible procedure was eventually adopted (see Methods) which used triethanolamine-HCl buffer and succeeded in clearly resolving two peaks of activity. The first of these, the high K_m isoenzyme, was unadsorbed to the DEAE-cellulose under the conditions used and contained over 95% of the total activity as assessed with NADPH and *p*-nitrobenzaldehyde as substrates. The second peak (low K_m form) contained the remainder of the applied activity. This latter isozyme was unstable at low concentrations of protein and it was, therefore, important to elute it stepwise with a pulse of KCl. When a linear gradient was used for elution, the recovery of activity was much lower. Neither isoenzyme showed evidence of any contaminating aldehyde reductase activity, as judged by the linearity of Lineweaver-Burk plots over a wide range of substrate concentrations (cf. Turner and Tipton [11]) and by polyacrylamide gel electrophoresis. The two enzymes showed different mobilities on 7.5% acrylamide gels with R_F values of 0.28 (high K_m form) and 0.38 (low K_m form), respectively.

Substrate specificity. In order to try to clarify the roles of the two isoenzymes, their substrate specificities were examined. Table II illustrates the wide variety of aldehydes reduced by the two isoenzymes. The chief distinction

TABLE II

SUBSTRATE SPECIFICITIES OF ALDEHYDE REDUCTASES FROM RAT BRAIN

Relative maximal velocity (V) is expressed as a percentage of the rate with p-nitrobenzaldehyde. For determination of the apparent K_m values for coenzymes, p-nitrobenzaldehyde was held constant at 0.5 mM.

Substrate	AR1		AR2	
	Apparent K_m (M)	V	Apparent K_m (M)	V
p-Nitrobenzaldehyde	$1.61 \cdot 10^{-4}$	100	$1.79 \cdot 10^{-5}$	100
Succinic semialdehyde	$1.55 \cdot 10^{-4}$	91	$1.64 \cdot 10^{-4}$	34
Pyridine-3-aldehyde	$4.3 \cdot 10^{-4}$	133	$2.7 \cdot 10^{-5}$	101
Indoleacetaldehyde	$6.1 \cdot 10^{-4}$	26	$2.0 \cdot 10^{-4}$	46
Glucuronic acid	$5.25 \cdot 10^{-3}$	79	—	—
Xylose	1.51	69	$8.4 \cdot 10^{-2}$	32
NADPH	$2.2 \cdot 10^{-6}$	100	$2.8 \cdot 10^{-6}$	100
NADH	$1.2 \cdot 10^{-4}$	6	$7.3 \cdot 10^{-5}$	59

between the two enzymes was the ability of the high K_m, but not the low K_m, isoenzyme to reduce 20 mM glucuronate. In general, the differences in K_m values for aldehydes for the two isoenzymes were not as substantial as have been reported in other species [11]. There was a particularly marked difference, though, in the affinities of the two enzymes for xylose. Both isoenzymes could use NADPH or NADH as cofactors (Table II), although NADPH was preferred in both cases. The maximum velocity with NADH was 6% of that with

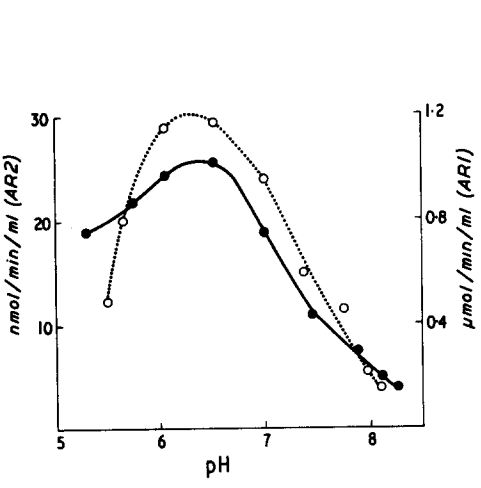

Fig. 2. pH-activity curves for rat brain aldehyde reductases. The activities of the high K_m reductase AR1 (●——●) and the low K_m reductase AR2 (○——○) were measured at various pH values in 0.1 M phosphate buffer, in the presence of 0.1 mM NADPH and 0.5 mM p-nitrobenzaldehyde. The pH of the reaction mixture was checked both before and after the assay.

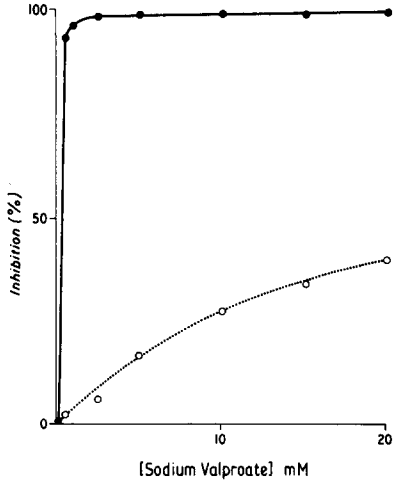

Fig. 3. Inhibition of rat brain aldehyde reductases by sodium valproate. Assays were performed using 0.1 mM NADPH and 0.5 mM p-nitrobenzaldehyde. The percentage inhibition of the high K_m reductase AR1 (●——●) and the low K_m reductase AR2 (○——○) is expressed as a function of the concentration of sodium valproate in the reaction mixture.

NADPH for the high K_m reductase but 60% in the case of the low K_m reductase.

pH optima. Both enzymes showed similar pH optima of approx. 6.3 (Fig. 2), which is in contrast to the isoenzymes from pig brain [11].

Inhibition by sodium valproate. The two isoenzymes differed substantially in their relative inhibition by sodium valproate (Fig. 3). The low K_m isoenzyme was almost insensitive to concentrations of sodium valproate of 1 mM or less. The mode of inhibition of these two isoenzymes was also apparently different. The low K_m isoenzyme showed inhibition of mixed type with a K_i value of $9.5 \cdot 10^{-2}$ M estimated from an intercept re-plot. The high K_m isoenzyme, however, showed a more complex pattern of inhibition similar to that observed with the isoenzyme from ox brain [13]. At low concentrations of valproate (less than 0.1 mM) inhibition was uncompetitive ($K_i = 3.4 \cdot 10^{-5}$ M), whereas at higher concentrations the pattern of inhibition became of mixed type.

Effects of other inhibitors. Table III illustrates the effects of a range of putative inhibitors on the two isoenzymes. In general, the low K_m isoenzyme was found to be less sensitive to inhibition by most anti-convulsant drugs and structural analogues of sodium valproate. Both enzymes were affected similarly by the flavonoids quercetin and quercitrin, and neither was significantly inhibited by 1 mM pyrazole, a potent inhibitor of alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1).

Although it is often difficult to correlate enzyme inhibition data in vitro with physiological effects of these inhibitors in vivo or in tissue extracts, it is clear from Tables I and III that inhibition of the low K_m isoenzyme closely parallels inhibition of methoxyhydroxyphenethylene glycol formation. Thus we conclude, on the basis of the effects of sodium valproate, that the low K_m, but not the high K_m, isoenzyme is principally involved in catecholamine metabolism. Similar conclusions have been arrived at on theoretical grounds, by computer simulation studies of the pathways of catecholamine metabolism in pig brain [32].

TABLE III

EFFECTS OF ANTICONVULSANT DRUGS AND ANALOGUES OF SODIUM VALPROATE ON THE ISOENZYMES OF ALDEHYDE REDUCTASE

Enzyme assays were performed using 0.5 mM *p*-nitrobenzaldehyde and 0.1 mM NADPH as substrates. All the results are the means of two determinations on separate batches of enzyme. All inhibitors were at a concentration of 1 mM unless stated otherwise.

Compound	Inhibition (%)	
	AR1	AR2
Phenobarbitone	85.2	31.6
Diphenylhydantoin (0.5 mM)	96.2	49.2
Quercetin (0.01 mM)	93.9	89.2
Quercitrin (0.01 mM)	37.4	43.5
Carbamazepine	37.7	11.7
Sodium valproate	93.0	2.0
2-Phenylbutyric acid	90.1	12.5
2-Ethylhexanoic acid	92.9	8.8
Diphenylacetic acid	86.2	42.5

Succinic semialdehyde metabolism. Both isoenzymes are capable of reducing succinic semialdehyde in vitro and show similar K_m values towards this substrate. In human brain, it has been suggested that a separate and specific reductase for succinic semialdehyde ($K_m = 2.4 \cdot 10^{-5}$ M) may exist [25]. In order to investigate this possibility in rat brain a cytosolic fraction was prepared by centrifugation at $38\,000 \times g_{av}$, for 60 min, of a 10% (w/v) homogenate in 0.1 M sodium phosphate buffer, pH 6.5/0.5 mM 2-mercaptoethanol. The extract was dialysed against the same buffer solution and then concentrated by ultrafiltration. When the variation of initial rate against succinic semialdehyde concentration was examined over a broad range of substrate concentrations, a non-linear Lineweaver-Burk double-reciprocal plot was consistently obtained (Fig. 4a). A similar phenomenon has been reported by Anderson et al. [26]. The occurrence of non-linear double-reciprocal plots has previously been used as evidence for the possible presence in tissue extracts of two or more enzymes with widely-differing affinities for their substrates [11,33]. The partially purified preparations of the aldehyde reductase isoenzymes isolated here exhibited very similar Michaelis constants towards succinic semialdehyde and individually gave linear double-reciprocal plots over the range tested. The non-linear of the double-reciprocal plot observed in Fig. 4a suggests the possible presence in rat brain of an additional succinic semialdehyde reductase, which may be of considerable importance in γ-hydroxybutyrate formation in vivo. Purification and characterization of this enzyme from rat brain will be required to confirm this hypothesis.

Xylose metabolism. Brain tissue has been reported to contain significant cytosolic aldose reductase activity using D-xylose as model substrate [17]. However pig brain and ox brain have been reported to contain aldose reductases with K_m values towards xylose substantially lower than those reported here [17,20] (Table II). In order to examine whether a separate 'high-affinity' aldose reductase occurs in rat brain, the kinetics of xylose reduction was examined. Again, a non-linear double-reciprocal plot was obtained (Fig. 4b) but

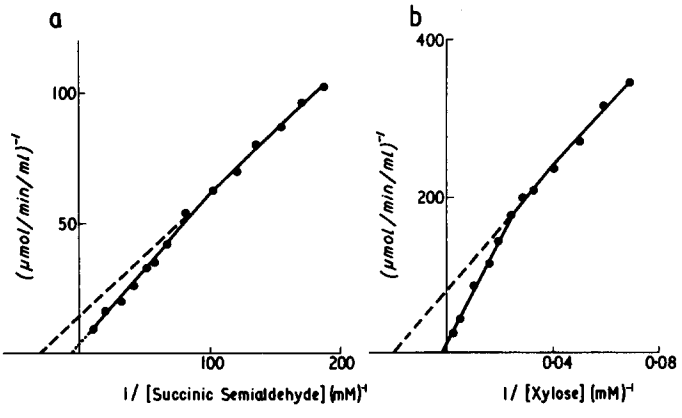

Fig. 4. Reduction of succinic semialdehyde and xylose by rat brain cytosol. A cytosol fraction was prepared from rat brain and assayed in the presence of 0.1 mM NADPH and various concentrations of either succinic semialdehyde or xylose. (a) Double-reciprocal plot of the reduction of succinic semialdehyde by rat brain cytosol. (b) Double-reciprocal plot of the reduction of xylose by rat brain cytosol.

no high-affinity component distinct from the two resolved isoenzymes was observed. Furthermore, no detectable reduction of 1 mM xylose by rat brain cytosol or mitochondrial fractions was found. These data would suggest that the majority of aldose reduction in rat brain is catalysed by the low K_m aldehyde reductase and that there may be no distinct aldose reductase in this tissue. Such a conclusion would be in agreement with the observations by Ris and Von Wartburg [12], that there are only two aldehyde reductases in rat brain yet four in human brain. The significance of such differences between species is unclear.

Discussion

Many of the discrepancies in the literature concerning K_m values for aldehyde substrates with aldehyde reductases have been ascribed to the use of preparations containing mixtures of isoenzyme species [11]. The evidence for this has come from the non-linearity of double-reciprocal plots and the occurrence of multiple activity bands following gel electrophoresis [11]. The demonstration here that the minor, low K_m isoenzyme is relatively insensitive to inhibition by the anti-convulsant drug sodium valproate provides an additional discriminator between these two reductases. Since the high K_m isoenzyme constitutes by far the major activity in rat brain, even a small contamination of a preparation of the low K_m reductase can markedly affect kinetic data. The sensitivity of preparations of the low K_m reductase to 1 mM sodium valproate is, therefore, a useful diagnostic tool to check for residual contamination with the major isoenzyme.

The occurrence of multiple forms of aldehyde reductase in various species and tissues has been well documented [11,12]. However, it has proved difficult to pinpoint the physiological roles of these enzymes. These studies go some way towards resolving these difficulties. The two enzymes isolated here correspond in many respect to the high K_m and low K_m isoenzymes previously purified from pig brain [11]. However, there are several differences in properties that merit attention. The rat isoenzymes show similar pH optima to each other and smaller differences in K_m values towards aldehydes compared with the pig. In addition the ability of the high K_m aldehyde reductase in rat brain to use NADH as cofactor, albeit poorly, is apparently unique. The demonstration of the differential sensitivity of the two isoenzymes to sodium valproate has permitted an examination of their relative contributions to catecholamine metabolism. The data presented here would support the previous theoretical conclusions [15,32] that the minor, low K_m reductase is primarily responsible for the formation of the glycol metabolite of noradrenaline. It would be of interest to ascertain whether or not this isoenzyme is specifically localized to aminergic neurons or is generally distributed throughout the brain.

On kinetic grounds, the low K_m isoenzyme would also seem to be responsible for the reduction of aldoses in rat brain and may, therefore, be implicated in some of the secondary effects seen in diabetes. The neurological effects of diabetes may be due, in part, to competition for this reductase between sugars and catecholamine-derived aldehydes. The rat brain reductase exhibits a considerably higher K_m value for xylose compared with some other species [17,20,

34]. Thus, the rat may not be a suitable model for studies on the development of diabetic neuropathy.

It is at present unclear whether either of the isoenzymes studied here have any role to play in the reduction of succinic semialdehyde to γ-hydroxybutyrate [24]. In view of the suggested presence in brain of a 'high-affinity' reductase for this substrate (Fig. 3) [25,26], it may be that they have little functional activity in this respect. Clarification of this situation might reveal whether inhibition of γ-hydroxybutyrate formation is relevant to the molecular action of sodium valproate as has been suggested elsewhere [13].

We are left with the perplexing problem of the physiological role of the high K_m isoenzyme in brain. It constitutes by far the major reductase activity in rat brain yet is probably not involved under normal conditions in the metabolism of aldoses, catecholamines or γ-aminobutyric acid. It may play a scavenging function in the removal of potentially toxic aldehydes or be involved in some, as yet unidentified, metabolic sequence. In view of the sensitivity of this enzyme to sodium valproate and other anti-convulsants of diverse structures, it becomes important to ascertain the endogenous substrates for this enzyme. One possibility is that it may be involved in the reduction of long-chain aliphatic aldehydes to alcohols, an important step in the biosynthesis of *O*-alkyl-lipids [35,36]. Such lipids are known to occur in significant concentrations in brain tissue [37,38]. Thus, inhibition of aldehyde reductase by anti-convulsants may lead to an alteration in the lipid composition of nerve-cell membranes. This possibility is currently under investigation. The hypothesis that sodium valproate and other anti-convulsants act by modifying the levels of catecholamine-derived aldehydes in brain [39] is not supported by the data presented here.

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