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THE ENZYMES CATALYSING SUCCINIC SEMIALDEHYDE REDUCTION IN RAT BRAIN

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Abstract—Pure preparations of the high-K_m aldehyde reductase from rat brain catalyse the NADPH-dependent reduction of succinic semialdehyde to γ-hydroxybutyrate. The high-speed supernatant fraction from rat brain contains a second enzyme, succinic semialdehyde reductase, that will catalyse this reaction using either NADPH or NADH but, unlike the high-K_m aldehyde reductase, is insensitive to inhibition by barbiturates. Alcohol dehydrogenase is also able to catalyse the reversible NAD^+-dependent oxidation of γ-hydroxybutyrate. The kinetic parameters of these enzymes have been compared and indicate that only the succinic semialdehyde reductase is likely to play a significant role in the metabolism of this compound *in vitro*.

Succinic semialdehyde (SSA) formed by transamination of the neurotransmitter γ-aminobutyric acid, is largely metabolised in brain by oxidation to succinate in a reaction catalysed by SSA dehydrogenase (see e.g. [1]). It can, however, also be reduced to γ-hydroxybutyrate (GHB) and this compound has been shown to be a naturally-occuring metabolite in brain [2, 3]. Systemically administered GHB has been shown to have a number of pharmacological effects [4]. These include an anaesthetic action [5, 6] and anesthetic doses of GHB have been shown to cause an increase in the concentrations of dopamine in brain [7, 8]. Despite these intriguing actions little is known about the factors that may effect GHB levels in the brain and the nature and properties of the enzymes involved in its formation are still poorly understood.

Two major enzymes have been identified that are capable of catalysing the reduction of the aldehydes derived from the biogenic amines to the corresponding alcohols in mammalian brain [9, 10]. Since these differ in their K_m values for a number of aldehydes they have been termed the high- and low-K_m aldehyde reductases. The high-K_m enzyme appears to be specific for NADPH as the coenzyme whereas the low K_m enzyme can also use NADH, although less efficiently. Other differences between them include substrate specificity and inhibitor sensitivity, the high K_m enzyme being, for example, considerably more sensitive to inhibition by barbiturates [9–12]. Evidence for the involvement of either, or both, of these two enzymes in the production of GHB is controversial.

Tabakoff and von Wartburg [13] reported that the high-K_m aldehyde reductase was not involved in SSA reduction in rat brain, but Anderson *et al.* [14] demonstrated the presence of an NADPH-dependent and barbiturate-sensitive SSA-reducing enzyme in the cytoplasm. These workers also suggested that there were two cystosolic enzymes that catalysed SSA reduction. Two such enzymes have recently been purified from human brain [15, 16] one of which appeared to be typical of the high-K_m aldehyde reductase, but the other which did not resemble the low-K_m enzyme, was highly specific for SSA. An $NADPH^+$-dependent enzyme capable of interconverting GHB and SSA has also been purified from hamster liver and brain and shown to be sensitive to inhibition by barbiturates [17].

It has been suggested that alcohol dehydrogenase may play a role in SSA reduction [18], but a proposal that lactase dehydrogenase might be involved [19] is unlikely since inhibition of this enzyme does not affect SSA reduction [13, 14]. It has been shown that both NADPH- and NADH-dependent SSA reduction are largely confined to the cytoplasm [14, 17] and in this study the enzymes responsible for SSA reduction in rat brain cytosol have been investigated.

MATERIALS AND METHODS

Succinic semialdehyde and *p*-nitrobenzaldehyde were obtained from Sigma Chemical Company (London) Ltd., Poole, Dorset, UK. γ-hydroxybutyrate (sodium salt) was obtained from Aldrich Chemical Company Ltd., Gillingham, Dorset, UK and D-glucuronate, D,L-glyceraldehyde and acetaldehyde were from British Drug Houses, Poole, Dorset, UK. Sodium barbitone was from May & Baker, Dagenham, Essex, UK and pyrazole from Eastman Chemicals, New York, USA. Coenzymes of the highest purity available were obtained from The Boehringer Corporation (London) Ltd., Lewes, East Sussex, UK. Lactaldehyde was synthesised by the method of Zagalak *et al.* [20].

p-Nitrobenzaldehyde (mp. 105–107°) was recrystallised from water and γ-hydroxybutyrate was recrystallised from ethanol.

The dinitrophenylhydrazone of SSA was made by addition of a saturated solution of 2,4-dinitrophenylhydrazine in 2N HCl to a 1 M solution of SSA.

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Abbreviations—SSA: succinic semialdehyde; GHB: γ-hydroxybutyrate.

After leaving in the cold for a few hours the yellow, 2,4-dinitrophenylhydrazone precipitate was filtered off and recrystallised from ethanol, its melting-point was determined as 202–204°.

The concentration of SSA was determined using purified rat brain succinic semialdehyde dehydrogenase assuming total conversion to succinate. The assay mixture consisted of SSA and 325 μM NAD^+ in 0.1 M sodium pyrophosphate buffer containing 10 mM 2-mercaptoethanol, pH 8.6. Reactions were started by the addition of enzyme and the change in absorbance at 340 nm was measured spectrophotometrically for three different concentrations of SSA.

Thin-layer chromatography of succinic semialdehyde was carried out using silica plates developed with chloroform/methanol (10:1 vol:vol). The plates were pre-run before use. SSA was detected by spraying the developed plates with 2,4-dinitrophenylhydrazine (0.4%, wt:vol. in 2 N HCl).

Enzyme preparations. Rats (Wistar strain) were killed by decapitation and a 20% (wt:vol.) homogenate in 0.1 M sodium phosphate buffer, pH 7.4, was made using a Dounce homogenizer. This was then centrifuged at 48,000 *g* for 1 hr and the resulting supernatant was used for enzyme assays. All operations were carried out at a temperature of 0–4°.

The high-K_m aldehyde reductase from rat brain was purified by a modification (Rivett, Smith and Tipton, manuscript in preparation) of the method of Felsted *et al.* [21] to give a preparation that was homogeneous as judged by polyacrylamide gel electrophoresis, both in the presence and absence of sodium dodecylsulphate. The enzyme from rat liver was prepared in a similar way.

Assay methods. Enzyme activities were determined spectrophometrically by following the changes in absorbance at 340 nm at 30°. For assays of aldehyde reducing activity the reaction mixture contained, in a total volume of 3 ml, 100 μM NADPH or NADH, 0.1 M sodium phosphate buffer, pH 7.0, the aldehyde substrate and the enzyme-containing preparation. Unless otherwise stated the concentrations of the aldehyde substrates were: 200 μM succinic semialdehyde, 330 μM *p*-nitrobenzaldehyde, 3 mM D,L-glyceraldehyde, and 10 mM D-glucuronate. The reverse reaction was determined in a reaction mixture containing 1 mM $NADP^+$, 0.1 M glycine–NaOH buffer, pH 9.5, either 100 mM propane-1, 2-diol or 100 mM γ-hydroxybutyrate and the enzyme-containing preparation in a total volume of 3 ml. In all cases any blank rates, determined in the absence of substrate, were subtracted.

The activity of alcohol dehydrogenase was determined by the coupled assay procedure described by Raskin and Sokoloff [22]. Linear relationships between initial velocity and enzyme concentration were established in each case.

Kinetic treatments. When *p*-nitrobenzaldehyde and D,L-glyceraldehyde were used as substrates for enzymes preparations containing both the high- and low-K_m aldehyde reductase activities, the kinetic parameters for both these enzymes were determined from double-reciprocal plots using a computer program to fit the data to the equation for the sum of two hyperbolas:

$$v = \frac{v_1 s}{K_{m1} + s} \quad \frac{v_2 s}{K_{m2} + s}$$

Where V_1, K_{m1} and V_2, K_{m2} are the maximum velocities and K_m values for the high and low K_m enzymes respectively. Estimation of the kinetic constants by extrapolation of the two apparently linear portions of a biphasic double-reciprocal plot does not give accurate estimates of these parameters [23, 24].

For mixed-substrate experiments initial velocities were determined using K_m concentrations of each aldehyde substrate and then with a mixture of the two substrates at the same concentrations. If a single enzyme is involved in catalysing the reactions involving both the substrates, competition between them will result in the activity determined with the mixture being 67 per cent of the sum of the activities determined when each substrate was assayed alone [25].

RESULTS

Some kinetic parameters of the high-K_m aldehyde reductase purified from rat brain are shown in Table 1. Similar values were obtained for the rat liver enzyme and in both cases the K_m value for SSA was found to be about 130 μM. No significant activity could be detected when NADPH was replaced by 100 μM NADH as the cofactor and the activity towards these substrates was inhibited by 0.1 mM sodium barbitone to the extent of about 75 per cent. When the reverse reaction was assayed slow rates of GHB and propane-1,2-diol oxidation could be detected. The rates of oxidation of these two substrates were similar and corresponded to some 5–10 per cent of the SSA-reducing activity determined in the presence of 450 μM SSA.

Mixed substrate experiments showed that the purified enzyme preparation contained only a single enzyme that was responsible for the NADPH-dependent reduction of these substrates (Table 2).

The presence of both the low- and high-K_m aldehyde reductase in rat brain supernatant was indicated by non-linear double-reciprocal plots of initial rate

Table 1. Kinetic constants for the purified rat brain high-K_m aldehyde reductase

Substrate	K_m	Relative V
p-Nitrobenzaldehyde	80 μM	(100)
D-Glucuronate	4.5 mM	87
D,L-Glyceraldehyde	1.5 mM	41
Succinic semialdehyde	130 μM	83
NADPH	3–5 μM	—

The relative maximum velocities are expressed as percentages of that given when *p*-nitrobenzaldehyde was used as the substrate. Assays were carried out in 0.1 M sodium phosphate buffer, pH 7.0, as described in the text. Kinetic parameters for the aldehyde substrates were determined in the presence of 100 μM NADPH, and K_m values in the range shown for NADPH were determined in the presence of 10 mM D-glucuronate, 450 μM SSA or 333 μM *p*-nitrobenzaldehyde.

Table 2. Mixed substrate experiment with purified rat brain high-K_m aldehyde reductase

Substrates Added					
D-Glucuronate (mM)	SSA (μM)	*p*-Nitrobenzaldehyde (μM)	v (nmoles/min)	$\frac{(V_{obs}) \times 100}{v_1 + v_2}$	%
4.5	—	—	7.06	—	
—	130	—	6.54	—	
—	—	80	7.8	—	
4.5	130	—	9.56	70	
—	130	80	9.65	67	
4.5	—	80	9.8	66	

Initial rates of NADPH oxidation were determined as described in text. v is the average value from two determinations. V_{obs} is v measured with two substrates present. v_1 and v_2 are the rates with the two substrates separately.

data obtained with either *p*-nitrobenzaldehyde or D, L-glyceradehyde as the substrate. Figure 1 shows the double-reciprocal plot determined when a wide range of *p*-nitrobenzaldehyde concentrations were used, and a qualitatively similar curve was obtained with D,L-glyceraldehyde as the substrate. Since similar plots were linear when the purified enzyme was used, such curves can be ascribed to the presence of the two aldehyde reductases in the supernatant fraction. The kinetic constants determined by computer fitting of these data are given in Table 3.

When the supernatant from rat brain was used as the enzyme source the double-reciprocal plot, obtained from initial-rate measurements with SSA as the substrate was non-linear (Fig. 2) although the curvature was not nearly so marked as that shown in Fig. 1. The double-reciprocal plot obtained with this substrate, in the same concentration range, showed no deviation from linearity when the purified high-K_m aldehyde reductase was used. The relatively

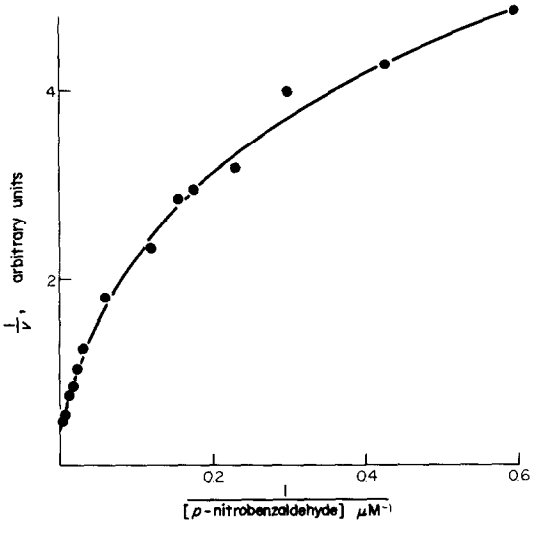

Fig. 1. Double-reciprocal plot for the reduction of *p*-nitrobenzaldehyde by rat brain supernatant. Assays were carried out in 0.1 M sodium phosphate buffer, pH 7.0, in the presence of 100 μM NADPH. Other details are as described in the text. The points are experimental values and the line is that determined by computer as the best fit for the presence of two enzymes.

small degree of curvature shown in Fig. 2 renders computer analysis of the data in terms of two enzyme components inaccurate, although an estimate of K_{m1} by this procedure gave a value similar to that obtained from studies with the purified high-K_m enzyme.

Low levels of NADH-dependent aldehyde-reducing activity could be detected in rat brain supernatant using *p*-nitrobenzaldehyde, D,L-glyceraldehyde or SSA as the substrate, but no such activity could be detected when D-glucuronate was used (Table 4). With D-glucuronate as the substrate initial-rate measurements of NADPH oxidation gave a linear double-reciprocal plot, as shown in Fig. 3, that gave a similar K_m value to that calculated from studies with the purified preparation. These results suggest that, in the concentration range used, only the high-K_m aldehyde reductase has significant activity towards D-glucuronate. Assuming this to be the case the relative activity of the two SSA-reducing enzymes can be estimated at different SSA concentrations by comparing the activities of the brain supernatant and the purified enzyme using SSA and D-glucuronate as the substrates. From this comparison it is possible to calculate the contribution of the high-K_m aldehyde reductase to the total SSA-reducing activity of the supernatant fraction, and thus the contribution of any other activities. Application of the approach to the results shown in Fig. 2 gave the curves shown in Fig. 4. The double-reciprocal plot, calculated for a range of SSA concentrations, for the component distinct from the high-K_m reductase, can be seen to be linear. The K_m value for SSA was approximately 15–20 μM and the maximum velocity was about 6 per cent of that of the high-K_m aldehyde reductase in the preparation.

The purified high-K_m aldehyde reductase from rat brain was found to be non-competitively inhibited by sodium barbitone with a K_i value of 30–40 μM. When the brain supernatant was used with 5 mM D-glucuronate and 100 μM NADPH the same K_i value could be determined from Dixon [26] plots, supporting the conclusion that this substrate is reduced only by the high-K_m aldehyde reductase. With either SSA or *p*-nitrobenzaldehyde, at concentrations of 20 μM, inhibition by sodium barbitone was found to be considerably less than that obtained with the purified enzyme (Table 5).

The rate of $NADP^+$-dependent oxidation of GHB

Table 3. Kinetic constants for the NADPH-dependent reduction of aldehydes by rat brain supernatant

Substrate	K_{m1}	K_{m2}	V_2/V_1
p-Nitrobenzaldehyde	80 μM	0.5 μM	0.09
D,L-Glyceraldehyde	1.4 mM	26 μM	0.17
D-Glucuronate	4.5 mM	—	—
Succinic Semialdehyde	130 μM	15–20 μM	0.06

Details of the assay methods are as described in the text. The values for *p*-nitrobenzaldehyde and D,L-glyceraldehyde were obtained from computer-fits to curves such as that shown in Fig. 1. Those for D-glucuronate were determined from the data shown in Fig. 3, and the method described in the text and illustrated in Fig. 4 was used to calculate the values for succinic semialdehyde. K_{m1} and V_1 are the kinetic parameters of the enzyme with the higher K_m value and K_{m2} and V_2 those of the enzyme with the lower value in each case.

by the brain supernatant (assayed as described in Materials and Methods) was about 10 per cent of the maximum rate of SSA reduction. From a comparison of the rates of the reverse reaction relative to those of the forward reaction, with SSA and D-glucuronate as the substrates, for the supernatant and purified preparations it was found that the high-K_m aldehyde reductase could account for only about 75 per cent of the total GHB dehydrogenase activity of the brain supernatant.

Using NADH as the coenzyme the K_m value of the brain supernatant for SSA was found to be 100–200 μM and that for *p*-nitrobenzaldehyde was 130–190 μM. Mixed-substrate experiments using 100 μM NADH with 120 μM SSA and 200 μM *p*-nitrobenzaldehyde gave an initial rate for the mixture of approximately 95 per cent of that calculated for the sum of the rates given when the two substrates were assayed separately. Because of the low NADH-dependent activities inhibition studies were difficult to perform with great accuracy. In the presence of 0.1 mM sodium barbitone the activity with SSA as the substrate was inhibited by less than 10 per cent and the activity towards *p*-nitrobenzaldehyde was inhibited by about 20 per cent. No inhibition by pyrazole (2 mM) oxaloacetate (5 μM) or ethanol (200 mM) could be detected when either of the substrates was used. The effects of pH on the activity of the NADH-dependent SSA reductase activity in the brain supernatant are compared with the effects on the NADPH-dependent activity of the purified high-K_m aldehyde reductase in Fig. 5. It was not possible to determine the activity in the supernatant at pH values much below 6.0 because protein precipitation occurred.

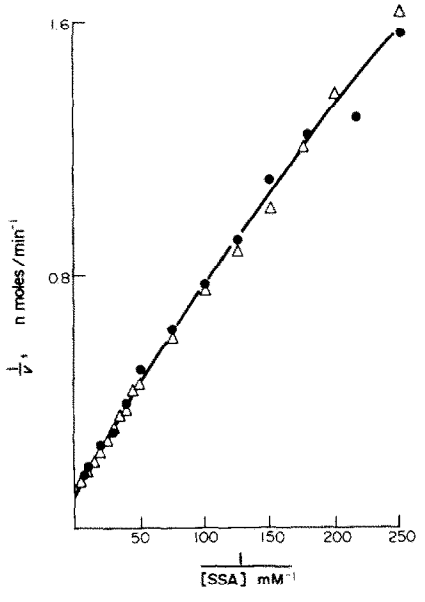

Fig. 2. Double-reciprocal plot for the reduction of succinic semialdehyde by rat brain supernatant. Assays were carried out in 0.1 M sodium phosphate buffer, pH 7.0, in the presence of 100 μM NADPH. Other details are as described in the text. △ and ● represent data obtained from two separate experiments.

Because of its very low activities and the presence of interfering enzymes, alcohol dehydrogenase cannot be easily detected in brain by direct spectrophotometric methods [27]. Using the coupled-assay of Raskin and Sokoloff [22] the rate of NAD^+-dependent oxidation of GHB was found to be similar to that of ethanol. A high-speed supernatant fraction of rat liver showed a higher NADH-dependent activity in SSA reduction than that in the presence of NADPH when 450 μM SSA was used. Apparently linear double-reciprocal plots were obtained using both coenzymes and K_m values of 150 μM and 1.8 mM were calculated for the NADPH- and NADH-dependent activities respectively. The NADPH-dependent activity was inhibited by sodium barbitone to a similar extent to that found with the purified high-K_m aldehyde reductase whereas the NADH-dependent activity was inhibited by pyrazole.

DISCUSSION

The specificity data for the purified high-K_m aldehyde reductase, shown in Table 1, are similar to those reported for the enzyme from other sources [9, 12, 28, 30] and the mixed-substrate experiments, shown in Table 2, indicate that the same enzyme is responsible for the oxidation of D-glucuronate, *p*-nitrobenzaldehyde and SSA. There has been some disagreement as to whether the high K_m aldehyde

Table 4. Releative specific activity for the reduction of aldehydes by rat brain supernatant

	Rat brain supernatant		Purified high-K_m aldehyde reductase	
	NADPH	NADH	NADPH	NADH
D-Glucuronate				
(10 mM)	100	0	100	0
SSA (30 μM)	31	—	26	—
(300 μM)	104	9	98	0
(2 mM)	90	—	84	—
p-Nitrobenzaldehyde				
(10 μM)	30	—	18	—
(300 μM)	142	11	130	0

Assays are carried out in 0.1 M sodium phosphate buffer, pH 7.0, as described in the text and NADPH and NADH were used at concentrations of 100 μM. Relative specific activity was taken as the percentage of the specific activity obtained with D-glucuronate and NADPH for supernatant or purified enzyme.

reductase is active towards SSA. Whittle and Turner [31] reported that it was a substrate for the enzyme from ox brain, but no such activity could be detected by Tabakoff and von Wartburg [13]. These latter workers also found that the total NADPH-dependent SSA-reductase activity was only some 10 per cent of that obtained with *p*-nitrobenzaldehyde (assayed at substrate concentrations of 500 μM), a value much lower than that obtained in this work (Table 4). It may be that the reason for these discrepancies is the presence of impurities in the preparation of SSA used. The preparation used by Tabakoff and von Wartburg [13] was synthesised by the oxidation of γ-butyrolactone [32]. Thin layer-chromatography of this preparation using the methods described earlier, yielded a number of spots that stained with 2,4-dinitrophenylhydrazine whereas that obtained from Sigma Chemical Company, which is prepared by hydrolysis of formyl succinate [33] gave only a single spot. We found that the material prepared by the oxidation of γ-butyrolactone gave much lower initial rates with aldehyde reductase than that obtained from Sigma, and from experiments with purified liver aldehyde reductase using this preparation Felsted, *et al.* [34] obtained a K_m value of 10.9 mM, which is considerably higher than the value of 130 μM determined in this work. SSA synthesised from glutamate [33] was also found to contain several impurities as judged by thin layer chromatography, and Bruce *et al.* [36] have also reported the presence of impurities in SSA prepared by this method. The melting points of the 2,4-dinitrophenylhydrazones derived from SSA prepared from glutamate or γ-butyrolactone have also been reported to be slightly lower at 200–202° [32, 35] than that reported here for the material supplied by Sigma.

The curved double-reciprocal plot obtained for the NADPH-dependent reduction of *p*-nitrobenzaldehyde catalysed by the brain supernatant (Fig. 1) is similar to that found with pig brain [9] and the relative activities of the two reductase are similar to those determined in that [9] and other [10] sources. The higher K_m values for *p*-nitrobenzaldehyde and D,L-glyceraldehyde determined from analysis of the curves agreed well with those found for the purified high-K_m aldehyde reductase (Tables 1 and 3). The

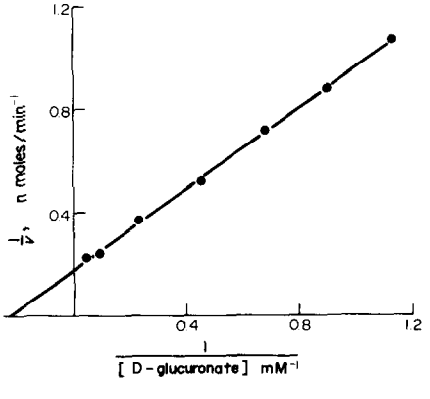

Fig. 3. Double-reciprocal plot for the reduction of D-glucuronate by rat brain supernatant. The assay conditions were as described in the legend to Fig. 1.

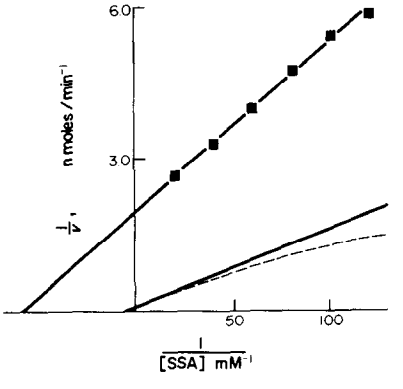

Fig. 4. Determination of the kinetic parameters for succinic semialdehyde reduction by rat brain supernatant. The broken line shows the experimentally determined curve (see Fig. 2). The solid line represents the activity of the high-K_m aldehyde reductase calculated as described in the text. From the difference between this and the experimental curve the line represented by (■——■) was drawn as the contribution of the SSA reductase.

Table 5. Inhibition of aldehyde-reducing activity of rat brain supernatant and purified high-K_m aldehyde by sodium barbitone

	Sodium barbitone (mM)	% Activity 5 mM D-Glucuronate	20 μM SSA	20 μM *p*-Nitrobenzaldehyde
Supernatant	0	100	100	100
	0.05	38	61	75
	0.1	25	41	59
	0.2	—	32	46
Purified high-K_m aldehyde reductase	0	100	100	100
	0.05	40	41	44
	0.1	24	26	27

Assays were carried out in 0.1 M sodium phosphate buffer, pH 7.0 with 100 μM NADPH as cofactor. Activity = 100% for each substrate when no inhibitor is present. Other details are as described in the text.

lower K_m values are likely to be less accurate because of the difficulties involved in spectrophotometric assays at such low substrate concentrations. D-Glucuronate is apparently only reduced by the high-K_m reductase at the concentrations used here, in agreement with the results of Tulsiani and Touster [11] who could detect no reduction of this compound by the low-K_m reductase from mouse liver.

The slight curvature of the double-reciprocal plot with SSA as the substrate (Fig. 2) suggests that this substrate is reduced by more than one enzyme in agreement with the results of Anderson *et al.* [14]. Comparison of the aldehyde-reducing activities of the brain supernatant with those of the purified high-K_m aldehyde reductase provided further evidence for the involvement of a second enzyme that was capable of reducing SSA with NADPH as the coenzyme and allowed approximate values for its kinetic constants to be calculated. Comparison of the NADH-dependent activity and the effects of sodium barbitone indicated that this enzyme could also use that coenzyme and that it was relatively insensitive to inhibition by barbiturates.

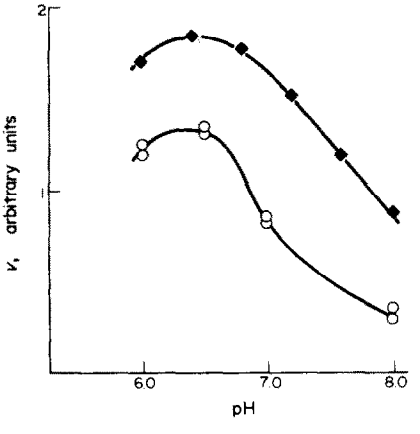

Fig. 5. The effects of pH on the activities of succinic semialdehyde-reducing enzymes from rat brain. All assays were carried out in 0.1 M sodium phosphate buffers. The SSA concentration was 450 μM and the coenzyme concentration was 100 μM. (◆) denotes the activity of the purified high-K_m aldehyde reductase assayed with NADPH, and ○ is that of SSA reductase assayed with NADH.

Although the low-K_m aldehyde reductase may have a predominantly mitochondrial location [14] (see also [13]), several observations support the results presented here indicating that a proportion of the low-K_m reductase is present in the high-speed supernatant fractions obtained after relatively mild homogenisation procedures [9, 11, 37]. The mixed-substrate experiments suggested that the NADH-dependent activity did not correspond to that of the low-K_m aldehyde reductase but was due to a separate SSA reductase. Higher K_m values were found for both these reductases when NADPH was replaced by NADH. A similar result has been reported for the ox brain aldehyde reductase with *m*-nitrobenzaldehyde as the substrate [38].

The K_m value for SSA and sensitivity to inhibition by sodium barbitone indicate that the NADPH-dependent SSA-reducing activity in rat liver supernatant is due to the high-K_m aldehyde reductase. Since the activity of this enzyme is greater in liver than in brain it would be difficult to detect the presence of the specific SSA-reductase activity unless it too was present in significantly higher concentrations. The sensitivity of the NADH-dependent activity to inhibition by pyrazole suggests that it is mainly due to alcohol dehydrogenase, and the K_m value determined for SSA (1.8 mM) is similar to the value reported for this compound as a substrate for horse liver alcohol dehydrogenase [18]. Since brain and liver alcohol dehydrogenases are probably identical [39] it is reasonable to conclude that this enzyme is also capable of reducing SSA in brain, and studies using the coupled assay system of Raskin and Sokoloff [22] indicate that the reverse reaction could take place with GHB as the substrate. The observation that pyrazole had no significant effect on the NADH-dependent reduction of SSA or *p*-nitrobenzaldehyde catalysed by the brain supernatant, however, indicates that, because of the high K_m value and low activity of rat brain alcohol dehydrogenase, it did not play any significant role under the conditions used here. The insensitivity of this activity to inhibition by oxalate supports earlier conclusions that lactate dehydrogenase is not involved in SSA reduction [13, 14].

The SSA reductase detected in rat brain appears to be quite similar to that from human brain [15, 16]

in its insensitivity to inhibition by barbiturates, its K_m value for SSA, its relatively low pH optimum and its lower activity with NADH as the substrate. Recently, however, the enzyme from human brain has been shown also to have significant activity towards *p*-nitrobenzaldehyde [46]. The non-specific SSA reducing enzyme found in human brain [15, 16] may correspond to the high-K_m aldehyde reductase although the K_m value determined for that tissue (56 μM) was lower than that determined in this work. The properties of the NADP$^+$-dependent GHB reductase purified from hamster liver and brain by Kaufmann *et al.* [17] also has similar properties to the rat brain high-K_m aldehyde reductase. These workers also detected the presence of a small amount of an NADPH-dependent SSA-reducing activity with a higher molecular weight, which may be the same as the SSA reductase described here, but they did not characterize it. SSA also has been reported to be a substrate for L-hexanoate dehydrogenase [40] but this enzyme may be identical with the high-K_m aldehyde reductase [41].

Although it appears that rat brain contains three enzymes that are capable of reducing SSA, the relatively high K_m values of alcohol dehydrogenase and the high-K_m aldehyde reductase suggest that these two enzymes will not play important roles in the metabolism of SSA *in vivo*. This conclusion is supported by the observations that barbiturates have no effect on the levels of GHB [42] or on its formation from γ-aminobutyric acid [43] and that pyrazole, which inhibits ethanol metabolism *in vivo* [44] has no effect on the formation of GHB [43] or on its further metabolism [45].

Since this paper was completed Rumigny *et al.* [47] have reported the separation of SSA-reducing enzymes from rat brain. The properties of the specific SSA-reductase detected by these workers were similar in several ways to those reported here, although these workers did not investigate its kinetic with NADH as the cofactor and the pH optima for enzyme activity that they reported with NADPH were rather different from those determined here.

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