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# SHORT COMMUNICATIONS

## Alcohol dehydrogenase activity in rat brain: evidence for the metabolism of succinic semialdehyde to gamma-hydroxybutyrate

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THE PRESENCE of alcohol dehydrogenase (AdH, alcohol NAD$^+$ oxidoreductase, EC1.1.1.1.) has been reported in rat brain[1] despite earlier negative reports [2,3]. Since the level of activity of the enzyme detected in the brain is low, about 0·05 per cent of that found in the liver, it is difficult to imagine that it can play a significant role in overall detoxification processes compared to the liver. It is possible though that the AdH in the brain is responsible for the metabolism of a normal metabolite possessing primary alcohol or aldehyde groups.

Gamma-hydroxybutyric acid (GHB) is present in rat brain[4] and, from tracer studies using $^3$H-GABA, appears to be readily formed from GABA.[5] The reverse reaction, that is the formation of GABA from GHB, does not occur directly, but involves the metabolism of GHB via the TCA cycle and glutamate.[6] The present report confirms the presence of AdH in rat brain and presents evidence that the enzyme can readily reduce succinic semi-aldehyde (SSA) to GHB *in vitro*.

*Materials and methods.* Pure succinic semialdehyde was prepared by the method of Taberner, Barnett and Kerkut.[7] Alcohol dehydrogenase from horse liver was obtained from the Sigma Chemical Co., London. All other reagents were analytical (A.R.) grade and were obtained from BDH, Poole, Dorset, England. Eighty Wistar albino rats of either sex were sacrificed and the brains quickly dissected out, rinsed in physiological saline at 0°, and homogenized in two volumes of saline at 2000 rev/min in an M.S.E. homogenizer. AdH was prepared from this extract, firstly by following exactly the method of Raskin and Sokoloff,[1] and secondly by the standard purification procedure described by Bonnischen and Brink.[8] Assays of AdH activity were carried out at room temperature (22°) in a total volume of 3 ml in cells of 1cm light path on a Unicam SP500 spectrophotometer. Assay conditions were as follows: (i) 0·1 M glycine sodium hydroxide buffer, pH 9·6, 0·5 mM NAD, 0·01 mM–1 mM GHB (sodium salt) and enzyme equivalent to 25 $\mu$g protein; (ii) 0·1 M sodium phosphate buffer, pH 6·6, 0·05 mM NADH, 0·01 mM–1 mM SSA and enzyme extract as above. Reaction rates were determined over a period of 6 min immediately following the addition of substrate. Enzyme activity was expressed as nmoles of NAD reduced (or NADH oxidized) per min per mg of protein.

*Results.* No AdH activity was detectable in the extract prepared by the method of Raskin and Sokoloff using the assay method described above. Similarly, no activity was detectable in a crude brain homogenate. AdH activity was, however, measurable in the partially purified brain extract (see Table 1) with ethanol, acetaldehyde or SSA as substrate. At pH 9·6 the conversion of alcohol to the corresponding aldehyde is favoured and a linear rate of reduction of NAD over a period of twenty minutes was obtained. The maximum rate was obtained with 2 mM ethanol and this was completely inhibited by 2 $\mu$M pyrazole. GHB, under the same conditions, did not produce any reduction of NAD. At pH 6·6, the addition of 0·2 mM acetaldehyde produced the maximum rate of oxidation of NADH. Under the same conditions the addition of 2 mM SSA produced an equivalent rate of oxidation of NADH. In order to ensure that the enzyme activity measured was not due to aldehyde reductase, incubations were performed in the presence of pyrazole and pentobarbitone and with NADPH as cofactor. Pyrazole (2 $\mu$M) completely inhibited the reaction in the presence of acetaldehyde of SSA. Pentobarbitone (0·5 mM) had no effect on the reduction of either substrate and neither substrate was reduced to any measurable extent in the presence of 0·05 mM NADPH.

There was not sufficient enzyme activity available to enable the kinetic parameters of SSA reduction to be determined using the partially purified brain extract. The Michaelis constant for the reaction of SSA with AdH was therefore determined using crystalline AdH from horse liver. The apparent $K_m$ for SSA was 0·87 mM (0·05 mM NADH). Under the same conditions the apparent $K_m$ for acetaldehyde was 0·075 mM.

*Discussion.* The present work confirms that no AdH activity is detectable in crude homogenates of rat brain.[1] However, in a partially purified preparation a low level of activity was detectable using traditional assay techniques (see Table 1). Raskin and Sokoloff[1] used an assay involving the coupled reduction of lactaldehyde to propanediol, which is more specific than the assay used here, and were able to measure

TABLE 1. ACTIVITY OF RAT BRAIN ALCOHOL DEHYDROGENASE (EC 1.1.1.1)

| Conditions | Reaction rate |
|---|---|
| 2 mM ethanol pH 9·6 | 4·0 ± 0·1 |
| 1 mM ethanol pH 9·6 | 2·7 ± 0·1 |
| 2 mM GHB pH 9·6 | 0 |
| 1 mM GHB pH 9·6 | 0 |
| 2 mM ethanol pH 9·6 pyrazole 2 μM | 0 |
| 2 mM succinic semialdehyde pH 6·6 | 6·3 ± 0·2 |
| 1 mM succinic semialdehyde pH 6·6 | 3·8 ± 0·2 |
| 2 mM succinic semialdehyde pH 6·6 pyrazole 2 μM | 0 |
| 0·2 mM acetaldehyde pH 6·6 | 5·9 ± 0·2 |
| 0·2 mM acetaldehyde pH 6·6 pyrazole 2 μM | 0 |

Assay conditions were as described in the text. The enzyme extract added was equivalent to 0·336 g wet wt brain tissue. Results are expressed as nMoles NADH oxidised or $NAD^+$ reduced/min/mg protein determined over a period of 6 min following the addition of the substrate and are the ± S.E.M. for four observations.

AdH activity in a 100,000 *g* supernatant fraction from rat brain. In this fraction no AdH activity was detectable by following the reduction of NAD or oxidation of NADH at 340nm. The specific activity of the AdH measured in the partially purified preparation agrees fairly closely with the value obtained by Raskin and Sokoloff[1] when the results are both expressed in terms of wet weight of tissue, i.e. 29 nmoles NADH oxidized/min/g wet wt.

The inhibition of the reduction of acetaldehyde and SSA by pyrazole and lack of effect of pentobarbitone suggests that AdH and not aldehyde reductase is responsible for the reaction, since the latter enzyme has been shown to be inhibited by pentobarbitone but unaffected by pyrazole.[9,10] In addition, Erwin and Dietrich have reported that SSA is not a substrate for brain aldehyde reductase.[9]

The inability of AdH from brain or liver to oxidize GHB confirms earlier results obtained with whole tissue homogenates,[11] and suggests that under the conditions prevailing *in vivo* the equilibrium would favour the formation of GHB from SSA. The latter is produced by the transamination of GABA with 2-ketoglutaric acid by GABA aminotransferase and so the appearance of $^3H$ in GHB following the injection of $^3H$-GABA[5] can be explained in terms of the activity of GABA aminotransferase and AdH in the brain. The further metabolism of GHB in the brain is not likely to be catalysed by AdH although tracer studies have suggested that GHB can be metabolized to glutamate via succinate.[6]

Further work is proceeding on the nature of the metabolic pathways involving GHB in rat brain.

*Department of Pharmacology,*
*University of Bristol Medical School*
*University Walk,*
*Bristol BS8 1TD, England.*

PETER V. TABERNER

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