Brain Alcohol Dehydrogenase

Abstract. *Significant alcohol dehydrogenase activity has been demonstrated in the soluble fraction of rat brain and is very similar to the liver enzyme in kinetic properties and responses to inhibitors. A cerebral mechanism that oxidizes ethanol may play a significant role in local adjustments during exposure to ethanol and in the pathogenesis of the neural disorders associated with chronic alcohol ingestion or withdrawal.*

Previous attempts to demonstrate alcohol dehydrogenase (ADH) (alcohol: NAD oxidoreductase, E.C. 1.1.1.1.) activity in preparations from brain have been uniformly unsuccessful (*1*), and it has remained unclear whether brain tissue has the capacity to utilize ethanol. The assays for ADH that were generally used were based on the spectrophotometric measurement of ethanol-dependent formation or acetaldehyde-dependent disappearance of reduced nicotinamide-adenine dinucleotide (NADH). It is doubtful whether these assays have sufficient specificity and sensitivity in crude preparations of tissues that contain low levels of ADH activity relative to many other contaminating enzyme systems that catalyze the oxidation and reduction of the pyridine nucleotides. With other methods employed in this laboratory significant ADH activity has been found in the soluble portion of rat brain. Many of the properties of the brain enzyme appear to be similar, if not identical, to those of the liver enzyme.

The method used for assaying ADH is based on the reduction of lactaldehyde to propanediol, coupled to the ethanol-dependent generation of NADH (*2*). Alcohol dehydrogenase catalyzes the oxidation-reduction of a number of alcohols and aldehydes, lactaldehyde among them (*3*). There is evidence that when NADH is generated by oxidation of ethanol, lactaldehyde reacts preferentially with the NADH still bound to the enzyme (*2*). The net reaction is as follows:

$$\text{Ethanol} + \text{lactaldehyde} \overset{\text{ADH, NAD}^+}{\rightleftharpoons} \text{1,2-propanediol} + \text{acetaldehyde}$$

One mole of propanediol is formed per mole of ethanol oxidized to acetaldehyde (*2*); measurement of ethanol-dependent propanediol formation is, therefore, a measure of ADH activity. Since the NAD^+ functions catalytically, the assay does not depend on a net change in the content of NADH and is, therefore, less sensitive to the effects of contaminating pyridine nucleotide oxidoreductase systems.

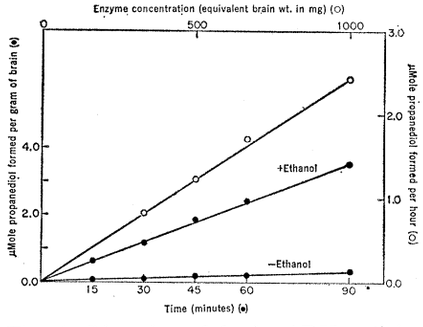

Fig. 1. Linearity of brain ADH activity with respect to time and concentration of enzyme. The reaction mixture contained 250 m*M* ethanol, 0.7 m*M* NAD^+, 10 m*M* L-lactaldehyde, 90 m*M* potassium phosphate buffer (*p*H 7.6), 0.2 ml of the 100,000*g* supernatant fraction of brain (for the time course studies) or the indicated amount of enzyme (for the enzyme concentration studies), and water to achieve a final volume of 1.0 ml. Mixtures were incubated at 37°C for 1 hour in the enzyme concentration studies or for the times indicated in the time course studies. Reactions were terminated by the addition of 0.3 ml of 45 percent trichloroacetic acid. Propanediol was measured in the protein-free supernatant fraction.

Adult male Sprague-Dawley rats were decapitated, and their brains were rapidly removed and homogenized by means of a motor-driven, glass-Teflon homogenizer in 2 ml of ice-cold 0.1*M* potassium phosphate buffer (*p*H 7.6) per gram of tissue. The homogenate was centrifuged at 100,000*g* for 60 minutes, and the supernatant fraction was used for enzyme assays. Liver enzyme was prepared similarly, except that the livers were homogenized in 5 ml of phosphate buffer per gram of tissue and the 100,000*g* supernatant fraction was further diluted 100-fold with phosphate buffer.

Contents of the reaction mixtures and incubation conditions are described in the legends to the tables and figures. These were established by preliminary experiments which demonstrated that activity of brain ADH, like the liver enzyme (*4*), is essentially confined to the soluble fraction of the cell and that the *p*H optimum is 7.6 for the activity of both enzymes. Addition of nicotinamide and glutathione to the reaction system was without effect. Lactaldehyde was synthesized by a modification of the method of Huff and Rudney (*5*). Propanediol was measured colorimetrically by the ninhydrin–sulfuric acid method (*6*).

The results shown in Fig. 1 demonstrate the presence of ADH activity in brain and illustrate some of the properties of the assay system. The ethanol-dependent formation of propanediol is proportional to enzyme concentration and linear with respect to time for at least 90 minutes. Production of propanediol in the absence of added ethanol was also linear with respect to time, but never exceeded one-tenth the rate of the ethanol-dependent reaction. Activity was absent in boiled enzyme, NAD^+-free, and lactaldehyde-free controls.

Additional and more direct evidence of ADH activity in brain was obtained by confirmation of enzyme-dependent oxidation of ethanol to acetaldehyde under the conditions of the assay. Ethanol-1-^{14}C was incubated with the brain extract in sealed Thunberg tubes under conditions similar to those described in the legend to Fig. 1; acetaldehyde-^{14}C, volatile (boiling point, 21°C) under conditions of the experiment, was trapped as the 2,4-dinitrophenylhydrazone and was identified

Table 1. Comparison of the apparent K_m values obtained with the brain and liver ADH activities for the various components of the assay reaction. Assay conditions were the same as those described in Fig. 1. The enzyme addition was 0.2 ml of the 100,000*g* supernatant fractions obtained from brain or liver as described in the text. Incubation time at 37°C was 1 hour. The apparent K_m values were estimated from double reciprocal plots of reaction velocity versus substrate concentration.

Substrate	Apparent K_m (moles/liter)	
	Brain	Liver
Ethanol	2.3×10^{-2}	1.1×10^{-2}
NAD^+	1.8×10^{-5}	1.7×10^{-5}
L-Lactaldehyde	3.3×10^{-3}	5.0×10^{-3}
D-Lactaldehyde	3.9×10^{-2}	9.5×10^{-2}

Table 2. Comparative effects of inhibitors on alcohol dehydrogenase activity of brain and liver. Assay conditions are the same as those described in Table 1; V_i/V is the ratio of reaction velocity in the presence of inhibitor to the velocity in the absence of inhibitor.

Agent	Concentration (mole/liter)	Fractional residual activity (V_i/V)	
		Liver	Brain
Pyrazole	1×10^{-5}	0.72	0.76
	2×10^{-5}	.57	.50
	5×10^{-5}	.38	.39
Sodium sulfide	1.7×10^{-3}	.67	.72
	3.3×10^{-3}	.46	.49

by paper chromatographic procedures (Fig. 2).

Activity of brain ADH was similar in a number of respects to the liver activity. Their apparent K_m values for ethanol, NAD+, and lactaldehyde were comparable, and both exhibited clear and quantitatively similar stereospecificity in regard to the D- and L-isomers of lactaldehyde (Table 1). Two different types of inhibitor of ADH, pyrazole and sulfide (*9*), inhibited the velocities of the brain and liver reactions to almost identical degrees (Table 2). Native activity in liver, however, exceeded that of brain by several orders of magnitude; the maximal rate of ethanol oxidation in liver was 9 mmole/g of liver per hour in contrast with 2.4 μmole/g per hour in brain.

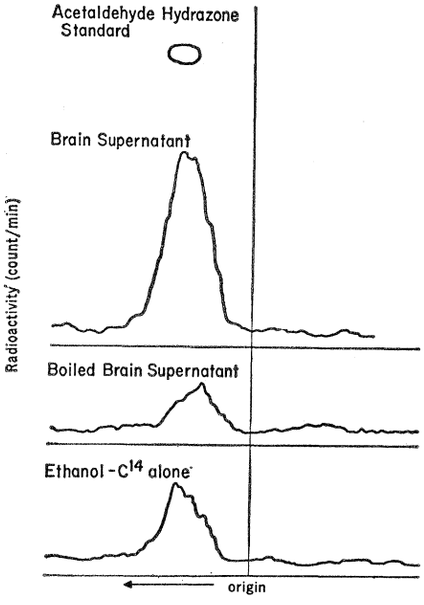

Fig. 2. Identification of acetaldehyde as the product of the reaction used for the assay of ADH in brain. Reaction mixtures were the same as those described in Fig. 1, except that the ethanol content was 200 m*M* ethanol-1-^{14}C (specific activity, 0.5 mc/mmole). The enzyme content was 0.2 ml of the 100,000*g* supernatant portion of brain. Incubation time at 37°C was 2 hours. Reactions were carried out in sealed Thunberg tubes, the sidearms of which contained 0.5 ml of 0.01*M* 2,4-dinitrophenylhydrazine hydrochloride. At the end of the incubation contents of the sidearms were extracted into chloroform, and the extracts were then evaporated to dryness to remove unreacted ethanol-^{14}C. The residue was redissolved in 0.3 ml of chloroform, and 5-μl samples were chromatographed on Whatman No. 1 filter paper impregnated with phenoxyethanol in parallel with an authentic standard of the 2,4-dinitrophenylhydrazone of acetaldehyde. The chromatogram was developed in phenoxyethanol-saturated heptane by the descending chromatographic method of Lynn *et al.* (*7*). Strip-scanning revealed a single radioactive peak that migrated the same distance from the origin as the yellow spot of an authentic preparation of the 2,4-dinitrophenylhydrazone of acetaldehyde (top two panels) (*8*). A similar but much smaller peak, 22 percent of the first, was detected in boiled enzyme controls, but was probably derived from acetaldehyde-^{14}C contamination of the ethanol-^{14}C, since a peak of comparable magnitude was obtained by incubating ethanol-^{14}C alone in water (lower two panels).

The relatively low level of activity of brain ADH raises the question of its physiological significance. It should be noted that metabolic capacities of tissues estimated on the basis of in vitro enzyme assays need not reflect their actual activities in vivo. For example, in the intact adult rat ethanol is metabolized at a rate of 1.0 to 1.5 μmole/hour (*10*) despite a hepatic capacity to oxidize 9 mmole of ethanol per hour per gram of tissue. The liver alone, however, does account for at least 90 percent of the total body metabolism of ethanol (*11*), and the brain probably plays a minor role in the body's disposition of an ethanol load. A cerebral mechanism for the oxidation of ethanol may be important for local adjustments during exposure to ethanol; or, since alcohol dehydrogenases of low substrate specificity are found in abundance in the tissues of many organisms that never come into contact with ethanol (*12*), Theorell has suggested that ADH functions in the metabolism of some as yet unidentified physiological substrate (*9*). Sustained tissue burdens of ethanol may affect the steady-state levels of this physiological substrate; such alterations may occur in brain and may be relevant to understanding the neural disorders associated with prolonged alcohol ingestion or withdrawal.

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Neuronal Geometry: Determination with a Technique of Intracellular Dye Injection

Abstract. *In a study of the specificity of neuronal connections in lobster abdominal ganglia, the dye Procion Yellow M4RS was electrophoretically injected into identified cell bodies. This dye spreads into fine branches of cells, survives fixation and routine histological procedures, and permits the reconstruction of cell shapes through examination of serial sections of ganglia. Certain cells were found to have an internal bilateral symmetry. Repeated injection of the same cells in ganglia from different animals showed that cells have characteristic shapes and that the neuropil is highly structured. This method of dye injection should have general applicability in studies where a knowledge of the geometry of specific cells is important.*

In a consideration of how a structure as complicated as a nervous system is built, one of the most basic questions is how accurately are the elements of the system, the neurons, connected together. Is there a well-defined circuit diagram for each nervous system? Are the same cells always connected in the same way?

A great aid in answering such questions may arise from recent studies in a variety of invertebrates and in a primitive chordate (*1, 2*) where it has been possible to identify and tabulate individual cells, find the same cells in different individuals of the same species, and establish maps of the positions of cell bodies of known function.