Short Communications

Inhibition by Acetaldehyde of the Pyruvate Dehydrogenase Complex from Ox Brain and Ox Kidney

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Low concentrations of acetaldehyde, similar to those that can occur in the brain of ethanol-treated animals, effectively inhibit the pyruvate dehydrogenase complex purified from ox brain or from ox kidney, although the precise mechanism of this inhibition remains to be defined.

The effects of ethanol ingestion on neurological function have been recognized for centuries, but the detailed mechanisms by which these effects are achieved remain a problem of both theoretical and practical interest. In this communication we report that concentrations of acetaldehyde similar to those that accumulate in the brains of ethanol-treated rats effectively inhibit the brain pyruvate dehydrogenase complex and point out the possible physiological significance of this effect.

The pyruvate dehydrogenase complex was purified from ox brain and ox kidney and assayed spectrophotometrically as described previously (Blass & Lewis, 1973), except that in some experiments a cooling bath was used to maintain the temperature at 15°C, i.e. well below the boiling point for acetaldehyde. These enzyme preparations also contained the α -oxoglutarate dehydrogenase complex but were devoid

of detectable activity of alcohol dehydrogenase (EC 1.1.1.1). Aldehydes were redistilled before use.

Acetaldehyde effectively inhibited the pyruvate dehydrogenase complex from either brain or kidney, with a K_i of 0.13 ± 0.03 mM (S.D., for six preparations, three from brain and three from kidney). Removal of the acetaldehyde by dilution completely reversed the inhibition. Cysteine partly counteracted the inhibitory effect of acetaldehyde, but a number of other thiols did not (Table 1). Not only acetaldehyde, but, in fact, all the aldehydes we tested were inhibitory (Table 2). By contrast, 20 mM-ethanol or 20 mM-acetate caused barely detectable inhibition. Kinetic studies suggested that the inhibition by acetaldehyde was non-competitive with pyruvate, thiamin pyrophosphate, CoA and NAD⁺, but did not allow definite conclusions about the nature of the inhibition. Concentrations of acetaldehyde that completely inhibited the

Table 1. *Effect of thiols on the inhibition of ox pyruvate dehydrogenase complex by acetaldehyde*

The activity of a preparation from kidney was measured spectrophotometrically at 15°C in the presence of 1 mM-acetaldehyde and 5 mM concentrations of the thiol compounds or amino acids. Without acetaldehyde the addition of the thiol compounds or amino acids did not significantly affect activity. Results were similar with two other preparations from kidney and two from brain.

Addition(s)	Specific activity	
	(μ mol/min per mg of protein)	(%)
None	0.160	(100)
Acetaldehyde	0.018	11
Cysteine + acetaldehyde	0.076	48
Dithiothreitol + acetaldehyde	0.020	13
GSH + acetaldehyde	0.022	14
2-Mercaptoethanol + acetaldehyde	0.015	9
Leucine + acetaldehyde	0.012	8
Glutamic acid + acetaldehyde	0.020	13

Table 2. *Inhibition of ox pyruvate dehydrogenase complex by various aldehydes*

The concentration of each of the aldehydes was 5 mM. Activity was determined spectrophotometrically at room temperature with 3 mM-cysteine present. Results are shown with a preparation from brain, and were similar with another preparation from brain and three from kidney.

Addition	Specific activity	
	($\mu\text{mol/min per mg of protein}$)	(%)
None	0.124	(100)
Acetaldehyde	0.006	5
Propionaldehyde	0.019	15
Butyraldehyde	0.015	12
Benzaldehyde	0.002	2

pyruvate dehydrogenase complex had little if any effect on the α -oxoglutarate dehydrogenase complex: thus α -oxoglutarate dehydrogenase activity in the presence of 5 mM-acetaldehyde was $83 \pm 16\%$ of the activity without acetaldehyde (s.d., for four preparations, three from kidney and one from brain). The oxidation of $[1-^{14}\text{C}]$ pyruvate to $^{14}\text{CO}_2$ by the purified pyruvate dehydrogenase complex, measured in the presence of $\text{K}_3(\text{FeCN})_6$ but without added CoA or NAD^+ (Blass *et al.*, 1970), was also not inhibited by acetaldehyde or by other aldehydes. In agreement with previous results, we noted no significant differences between preparations of the pyruvate dehydrogenase complex from brain and from kidney.

Two lines of evidence suggest that the inhibition of the pyruvate dehydrogenase complex may be of physiological significance in the brain, in certain circumstances. First, the concentrations of acetaldehyde required to inhibit the enzyme are comparable with those reported to occur in the brains of ethanol-treated animals (Kiessling, 1962; Mendelson, 1970a,b) and are much below those occurring in the tissues of individuals who have ingested ethanol after treatment with the drug disulfiram [Antabuse; bis(diethylcarbamoyl) disulphide]. Secondly, the

utilization of glucose (Flock *et al.*, 1970) and of pyruvate (Kiessling, 1962; Majchrowicz, 1965) is known to be impaired in the brains of ethanol-treated rats. Further, impairment of the activity of the pyruvate dehydrogenase complex has been associated with neurological disorders in children with inherited defects affecting these enzymes (Blass *et al.*, 1970, 1972), and has also been proposed as a cause of brain damage in thiamin deficiency. It is noteworthy in this regard that the pathological effects of ethanol ingestion have not been clearly distinguished from those of thiamin deficiency in human chronic alcoholics, the expected result if both conditions affected the same molecular site (Davison, 1963).

The mechanisms by which acetaldehyde affects the mammalian pyruvate dehydrogenase complex may not prove to be identical with its well-characterized effects on the yeast enzyme complex. Aldehydes appear to inhibit the yeast enzyme complex by combining both with the active site and with a control site of its thiamin-dependent first enzyme, but this effect requires concentrations of acetaldehyde 100 times those that inhibit the mammalian enzyme complex (Juni, 1961; Huebner *et al.*, 1970). More detailed studies of the mechanisms of inhibition of the mammalian enzyme would allow firmer conclusions on this point.

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