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Comparative Study of the Tissue Distribution of NADH and NADPH-Dependent Chloral Hydrate Reducing Enzymes in the Rat

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Chloral hydrate (CH), an intermediate metabolite of trichloroethylene, is reduced to trichloroethanol (TCE) by alcohol dehydrogenase(Friedman and Cooper, 1960) and aldehyde reductase (Tabakoff et al., 1974; Ikeda et al., 1981). Alcohol dehydrogenase requires reduced nicotinamide adenine dinucleotide (NADH), and aldehyde reductase requires reduced nicotinamide adenine dinucleotide phosphate (NADPH). No reports have appeared concerning comparative studies of the tissue distribution of CH-reducing enzymes. In this report, NADH and NADPH-dependent CH-reducing activities were investigated in various organs of the rat.

MATERIALS AND METHODS

Male Wistar rats weighing about 230 g were used. The rats were fasted for 24 h, killed by decapitation, and the different organs removed by dissection. Stomach, intestine and colon were cut out, cleaned and washed in ice-cold distilled water. Almost all of the organs were stored at -80°C. Prior to analysis, organs were homogenized in 10 mM potassium phosphate buffer, pH 7.4, containing 1 mM EDTA and 1 mM 2-mercaptoethanol. The homogenates were centrifuged at 105,000 x g for 90 min at 4°C. Supernatant were used to test for CH-reducing activity with NADPH and NADH (Ikeda et al., 1981). Spectrophotometric assays were performed by measuring absorbance at 340 nm at 25°C. One unit of enzyme was defined as an amount of activity that caused a 0.01/min change in absorbance. Protein was determined by Bio-Rad protein assay (Bradford, 1976).

RESULTS AND DISCUSSION

CH-reducing activity of the rat was compared in various organs. CH-reducing activity is theoretically equal to the total amount of CH-reducing activity of NADH-linked alcohol dehydrogenase and NADPH-linked aldehyde reductase. However, there is a report that alcohol dehydrogenase is capable of reducing CH in the presence of an oxidazable alcohol because it is converted into an enzyme-NADH complex which can then reduce the compound (Shultz and Weiner, 1979).

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Table 1. Chloral hydrate reducing activity in various organs of the rat

Organ	Activity (units/g)		Specific activity (units/mg of protein)	
	NADH	NADPH	NADH	NADPH
Brain	9.8 ± 0.6	16.9 ± 2.0	0.16 ± 0.02	0.28 ± 0.03
Liver	538.2 ± 34.6	359.2 ± 22.1	2.37 ± 0.14	1.59 ± 0.12
Lung	56.6 ± 3.3	81.2 ± 4.3	0.41 ± 0.09	0.64 ± 0.06
Heart	42.1 ± 2.8	66.2 ± 1.4	0.33 ± 0.04	0.53 ± 0.01
Kidney	46.9 ± 2.7	236.8 ± 9.0	0.29 ± 0.01	1.44 ± 0.03
Stomach	25.2 ± 2.3	71.3 ± 3.3	0.32 ± 0.02	0.85 ± 0.08
Intestine	26.5 ± 2.6	45.2 ± 2.0	0.33 ± 0.03	0.56 ± 0.03
Colon	52.3 ± 5.8	39.8 ± 3.4	0.77 ± 0.12	0.58 ± 0.05
Testis	19.1 ± 1.3	60.2 ± 3.8	0.25 ± 0.02	0.78 ± 0.07
Spleen	27.3 ± 3.1	48.7 ± 4.2	0.16 ± 0.03	0.25 ± 0.02
Pancreas	30.6 ± 3.8	44.4 ± 7.7	0.24 ± 0.05	0.27 ± 0.09
Adrenals	*	322.1 ± 27.6	*	3.1 ± 0.56
Thymus	8.1 ± 0.2	10.9 ± 1.9	0.06 ± 0.04	0.1 ± 0.08
Muscle	32.1 ± 3.6	33.9 ± 3.2	0.15 ± 0.12	0.39 ± 0.03

*Activity present were below the limit of quantitation. Results are mean ± SE of five rats.

In this study, liver, kidney and adrenals had higher CH-reducing activity than other organs (Table 1). However, from the viewpoint of CH metabolizing capacity, CH may be mainly metabolized in liver and kidney, because adrenals have the lowest weight of all the organs tested. Liver had greater activity with NADH as a cofactor than NADPH. Organs other than liver, on the other hand, had high CH-reducing activity with NADPH. These results agreed with previous reports in part (Tabakoff et al., 1974; Ervin et al., 1972). It is of particular interest to note that adrenals had high NADPH-linked CH-reduction activity in spite of having no NADH-linked CH-reduction. The adrenal gland is known to secrete corticosteroids. Ikeda et al. (1981) discovered a NADPH-dependent CH-reducing enzyme which use C24-3-ketosteroids as their optimal substrate in liver of the rat. However, it is not clear why the adrenals have high CH-reducing activity. The nature and character of CH-reducing enzymes in the adrenals must be investigated.

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