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Tissue Distribution of Acetaldehyde in Rats following Acetaldehyde Inhalation and Intragastric Ethanol Administration

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It is conceivable that ethanol will be blended with gasoline and used as a fuel in the future because of the depletion of petroleum resources (Hashimoto et al. 1984). Ethanol is metabolized to acetaldehyde, which is more toxic than the parent compound (Ortiz et al. 1974). However, there are few studies on the effects of acetaldehyde on the liver, kidney and other organs. While acetaldehyde levels in blood and liver following ethanol ingestion have been reported in rats (Eriksson et al. 1977), little is known about tissue distributions of acetaldehyde following inhalation exposure. The present communication comparatively describes the tissue distributions of acetaldehyde following acetaldehyde inhalation and intragastric ethanol administration.

MATERIAL AND METHODS

Three male Sprague-Dawley rats, weighing 230 to 280 g each, were starved overnight. Rats were exposed to acetaldehyde gas at a flow rate of 1 liter/minute for 1 hour by passing air through 400 ml of a 2.5% aqueous acetaldehyde solution in a sealed box (53 x 42 x 32 cm), as reported previously (Sakata et al. 1983). Acetaldehyde levels in the air in the box were kept between 1 and 20 mM. In addition, a 15% aqueous ethanol solution was administered intragastrically at a dose of 3 g ethanol/kg body weight. Blood was obtained serially from a jugular vein.

To prevent tissue contamination by blood acetaldehyde, rats were killed by exsanguination from the carotid artery. Determinations of acetaldehyde and ethanol levels in various tissue required 0.25 g of each, which were frozen immediately in liquid nitrogen. In the acetaldehyde inhalation test, tissue acetaldehyde contents were determined immediately after discontinuation of inhalation, because of the rapid disappearance of acetaldehyde from the blood. The frozen tissue samples were pulverized in mortars cooled with liquid nitrogen. Tissue powders were suspended in 1.23 M iced

perchloric acid containing 20 mM thiourea (Wako Pure Chem. Ind., Osaka, Japan), to prevent non-enzymatic formation of acetaldehyde from ethanol, and 1.34 mM n-propanol (Wako Pure Chem. Ind.) as an internal standard for gas chromatography. 0.15 ml of blood was added to 1.95 ml of ice-cold 1.06 M perchloric acid solution in an ice-cold test tube, which was tightly capped with a rubber stopper. The content was immediately mixed thoroughly by using a vibration shaker for 60 seconds. Both tissue extracts and blood were centrifuged at 2500 x g for 5 minutes at 4°C. 1.0-ml aliquots of protein-free supernatants were placed in cooled 15-ml glass bottles sealed with butyl-rubber septa. The vials were warmed at 56°C for 20 minitues. One ml of the head-space gas was injected into a Hitachi (Tokyo, Japan) Type 163 gas chromatography unit (column size 2.0 m x 3 mm internal diameter). The column media consisted of 25% polyethylene glycol 1000 and 60-80 mesh Shima light (Nanikawa & Kotoku 1969). The operating conditions were: injection and hydrogen flame ionization detector temperature 110°C; column temperature 85°C; and flow rate of carrier nitrogen gas 40 ml/minitue. Standard solution of acetaldehyde and ethanol in 1.23 M perchloric acid were prepared fresh for each study.

RESULT AND DISCUSSION

Standard curves of acetaldehyde concentrations were linear in the ranges of 0.4-2.8 nmol/ml for blood and nmol/g for liver. Those of ethanol levels also were linear in the ranges of 1.5-189.8 μmol/ml for blood and μmol/g for liver (data not shown). Accuracy of the procedure, as determined by a recovery study, was 90.8% and 93.8% for the blood and liver ethanol levels and 81.6% and 122.6% for the blood and liver acetaldehyde levels, respectively. The lowest detectable levels of ethanol were 1.5 μmol/ml of blood and g of liver, and of acetaldehyde 0.4 nmol/ml of blood and g of liver (data not shown).

Disappearance of acetaldehyde from blood following discontinuation of acetaldehyde inhalation was rapid (Fig. 1). Its level was reduced to less than 2% of the inital value within 20 minutes. The mean of disappearance rate of 3 rats was calculated to be 13.4 $hour^{-1}$, giving a half-life of 3.1 minutes.

Acetaldehyde was found in all tissues after both acetaldehyde inhalation and intragastric ethanol administration (Table 1). Following acetaldehyde inhalation, periperal blood acetaldehyde levels were highest; other tissue levels were similar except for the liver which had a much lower level. The data suggested that either blood acetaldehyde barely entered hepatocytes or liver acetaldehyde metabolism was very rapid. Arotic blood acetaldehyde levels were found to be 55% higher than peripheral venous blood

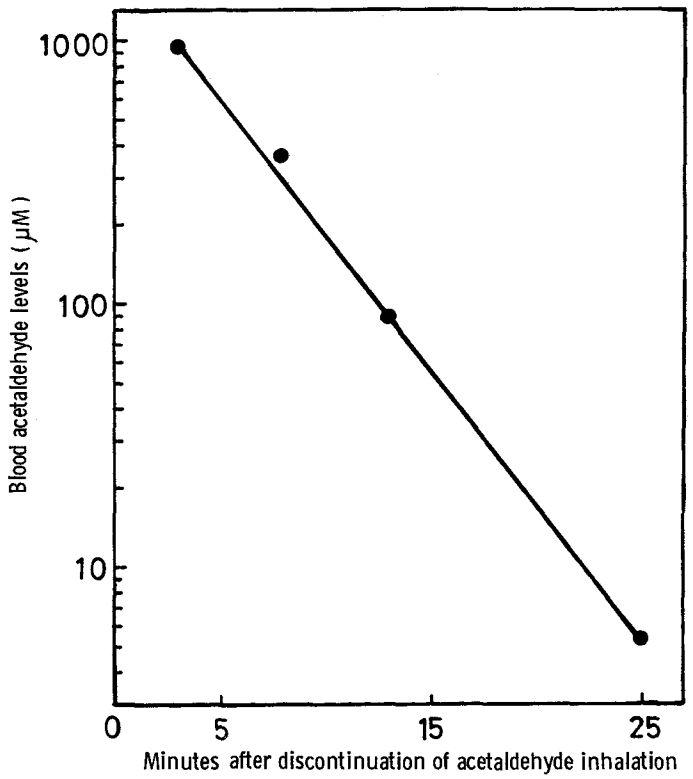

Fig. 1. Blood acetaldehyde levels in rats after discontinuation of acetaldehyde inhalation. Data from one representative rat are shown in this figure. Rats were exposed to acetaldehyde gas for 1 hour as described under MATERIALS AND METHODS.

levels 15 minutes after discontinuation of acetaldehyde inhalation. Acetaldehyde levels after intragastric ethanol administration were higher in the liver and blood than in other tissues, although blood ethanol levels were slightly higher than liver levels (blood 60.2 μmol/ml, liver 44.6 μmol/g). Marchner & Tottmar (1976) have already reported that aortic blood acetaldehyde levels after intraperitoneal ethanol administration were 30-40% lower than that in hepatic venous blood. In conclusion, acetaldehyde exposure by inhalation serves as a viable model for studying acetaldehyde toxicity. Secondly, rapid careful exsanguination is necessary to avoid falsely high tissue levels from blood contamination.

Table 1. The tissue distribution of acetaldehyde following acetaldehyde inhalation and intragastric ethanol administration

Tissue	Acetaldehyde inhalation (nmol/g)	Ethanol administration (nmol/g)
Blood*	1210	4.2
Liver	55	9.4
Kidney	213	2.1
Spleen	183	2.1
Heart muscle	277	2.3
Skeleton-muscle	345	1.7

*Blood levels were expressed as nmol/ml. Rats were exposed to acetaldehyde gas for 1 hour as described under MATERIALS AND METHODS. The acetaldehyde levels were determined immediately after discontinuation of inhalation and 3 hours after the intragastric administration of ethanol (3 g/kg body weight). One representative rat.

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