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BLOOD AND LIVER ACETALDEHYDE CONCENTRATIONS DURING ETHANOL OXIDATION IN C57 AND DBA MICE

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Abstract—Hepatic and blood acetaldehyde concentrations during ethanol oxidation were determined in C57 and DBA mice. Liver acetaldehyde was determined with the perchloric acid-thiourea method (no artefactual acetaldehyde formation). Levels ranging from 5 to 118 nmole/g were observed. At ethanol concentrations below 50–60 μmole/g, liver acetaldehyde concentrations were higher in DBA compared with C57 mice. A positive correlation was found between the ethanol and acetaldehyde concentration, when ethanol concentration was below 25 (DBA) or 70 μmole/g (C57). At higher ethanol concentrations the correlations tended to become negative. Artefactual acetaldehyde formation during the analytical procedures was obtained with the use of hemolyzation, with or without thiourea, and semicarbazide methods for blood acetaldehyde determination. The magnitude of the artefactually formed acetaldehyde was of such order that no conclusions regarding the existence of true *in vivo* blood acetaldehyde concentrations could be drawn. Earlier reported mice blood acetaldehyde concentrations are suggested to be re-evaluated.

According to several observations [1–9], the DBA inbred mouse strain consume less ethanol during free choice compared with the C57 inbred mouse strain. Part of this strain difference has been attributed to higher circulating acetaldehyde concentrations during ethanol intoxication in DBA compared with C57 mice [10, 11]. Although higher blood acetaldehyde levels have been observed in DBA mice in some studies [6, 10–13], other observations of no differences in blood [14] or breath acetaldehyde levels [15] have also been made. In fact, one study reports higher blood acetaldehyde levels in C57 than in DBA at the time of regaining righting reflex [7]. In general, the average acetaldehyde concentration (25–340 μM, 1–2 hr after ethanol doses of 1.1–4.2 g//kg) reported in both C57 and DBA mice [6, 7, 10–14] have been higher than those (7–31 μM, 1–2 hr after an ethanol dose of 2–4 g/kg) observed in other mouse strains [16, 17]. In addition, average levels ranging between 21 and 155 μM have been observed in a genetically heterogenous mice strain, 0.25–2.5 hr after ethanol doses of 1.2–3.0 g/kg [18].

With a view to the general difficulties regarding blood acetaldehyde determination, especially concerning artefactual acetaldehyde formation during the analytical prodedures [19], it is possible that previously reported acetaldehyde concentrations need to be reevaluated. Thus, emphasis in checking for possible artefactual acetaldehyde formation was put in the present investigation, in which the DBA and C57 strains were compared with regard to their *in vivo* acetaldehyde metabolism.

Another approach in assessing possible C57–DBA strain differences in acetaldehyde metabolism would be determination of hepatic acetaldehyde concentrations. This should give a more comprehensive picture about hepatic acetaldehyde metabolism and the arterial acetaldehyde levels, reaching the target organs, than the venous blood acetaldehyde concentration, which is highly affected by peripheral acetaldehyde metabolism [20]. Thus, the present investigation was designed so that, in addition to blood acetaldehyde, hepatic acetaldehyde concentrations were also determined by the freeze-stop technique after the challenge of various ethanol doses.

MATERIALS AND METHODS

Animals. Male mice (age: 2–4 months, weight: 20–30 g) of the DBA and C57 inbred strains were used. These animals were obtained from the Institute for Behavioral Genetics, University of Colorado. Animals were provided water and diet (Wayne Sterilizable Lab Blox, Chicago, IL) *ad libitum*.

Liver freeze-stop determinations. Ethanol doses from 0.5 to 6.0 g/kg were injected intraperitoneally as 15% (w/v) solutions in water to 26 mice from both strains. Sixty minutes after ethanol injection, animals were cervically dislocated. The livers were excised and freeze-clamped within an average of 14 sec by means of aluminium clamps precooled in liquid nitrogen. The frozen livers were pulverized in a mortar and about 0.6 g of the liver powder was suspended in 5 ml of 0.6 M ice-cold perchloric acid containing

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40 mM thiourea, shaken, and the precipitate was centrifuged at 4000 *g* for 10 min at 4°. In preliminary experiments livers from untreated control mice were taken and processed in ethanol-containing perchloric acid with or without thiourea. Ethanol and acetaldehyde were determined from the supernatant fractions.

Blood determinations. Just before freeze-clamping, retro-orbital sinus blood samples of 50 μl were taken into a capillary pipette, sealed and shaken in a tube containing 0.95 ml water. To check for artefactual acetaldehyde formation, control blood samples from 14 mice of both strains were processed in water containing various amounts of ethanol.

In another series of experiments, ethanol (2 g/kg) was injected as a 20% (w/v) solution in water to 10 mice from both strains. Sixty minutes later, retro-orbital sinus blood samples were taken as described above. A second retro-orbital blood sample was taken and expelled into ice-cold isotonic semicarbazide solution described by Stowell [21]. The semicarbazide solutions were always fresh made to avoid such artefactual acetaldehyde formation, that has been observed in the determination of human blood acetaldehyde by using solutions 1–4 days old [22, 23]. After taking the retro-orbital blood samples, the animals were decapitated and additional blood samples of 50 μl were taken with micro pipettes and mixed in 0.95 ml cold water or isotonic semicarbazide solution. To check for artefactual acetaldehyde formation, control blood samples from 6 mice of both strains were similarly processed in water or semicarbazide solution, which contained 0.4–2 mM ethanol. An additional control blood sample was taken in water containing ethanol and 25 mM thiourea. Acetaldehyde was determined from the hemolysates. In the case of semicarbazide treatment, blood cells were first removed by centrifugation (2000 *g* for 10 min at 4°), plasma proteins were precipitated (with 0.2 vol of 3 M HCl) and centrifuged (4000 *g* for 10 min at 4°), and acetaldehyde was determined from the supernatants.

Analytical techniques. The samples to be analysed for acetaldehyde or ethanol were incubated in rubber-stoppered tubes for 15 min at 60–65°, at which time aliquots of headspace gas were injected into a Hewlett-Packard equipped with FID and a 6-ft column packed with Carbopack containing 0.1% SP-1000 (Supelco Inc., Bellefonte, PA). Headspace GC conditions, and ethanol and acetaldehyde determinations were as described previously [14]. GC detection limit was 0.05 μM. In the preliminary experiments, as in accordance with previous studies [24, 25] thiourea totally blocked artefactual acetaldehyde formation in liver supernatants during headspace incubation.

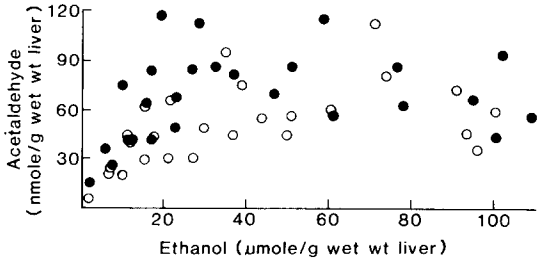

Fig. 1. Correlation between liver acetaldehyde and ethanol concentration. Mice were treated and analyses made as described in the Materials and Methods section. Individual values for C57 are marked with open circles and those for DBA with solid circles.

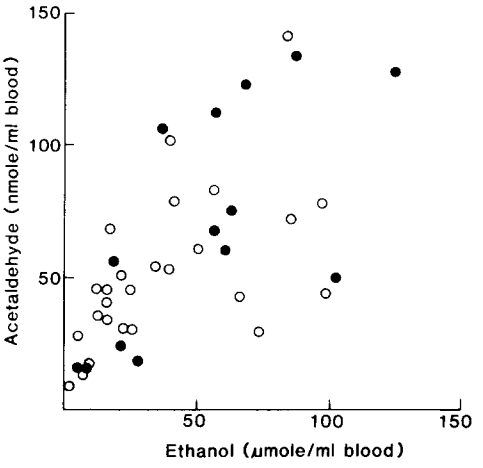

Fig. 2. Blood acetaldehyde concentration in DBA mice measured during ethanol intoxication and in control blood to which ethanol was added. Mice were treated and analyses made as described in the Materials and Methods section. Individual values for levels measured during intoxication are marked with open circles and those for artefactual formation with solid circles.

RESULTS

Hepatic acetaldehyde. Liver acetaldehyde as a function of the hepatic ethanol concentration is depicted in Fig. 1. A strain difference occurred with the DBA mice displaying higher hepatic acetaldehyde concentrations than C57 at ethanol concentrations below 50–60 mM. In the DBA mice acetaldehyde correlated positively ($r = 0.600$, $P < 0.05$) with hepatic ethanol concentration up to ethanol levels of 25 mM. From thereon, the acetaldehyde concentration tended to decrease with increasing ethanol concentration ($r = 0.505$, $P < 0.1$). In C57 mice hepatic acetaldehyde and ethanol concentrations also correlated positively at ethanol levels lower than 25 mM ($r = 0.709$, $P < 0.02$). In these mice, however, the positive correlation lasted up to higher ethanol concentrations, and the biphasic shape of the acetaldehyde–ethanol function, seemed not as clear, as with the DBA mice. Nevertheless, the possibility of a negative correlation at ethanol concentrations above 60–70 mM cannot be excluded.

Blood acetaldehyde. Blood acetaldehyde concentrations as functions of blood ethanol concentrations are depicted in Figs. 2 (DBA) and 3 (C57). The figures also depict artefactually formed acetaldehyde levels in control blood at corresponding ethanol concentrations. As demonstrated in Fig. 2, no differences were observed in DBA mice between blood acetaldehyde determined during ethanol oxi-

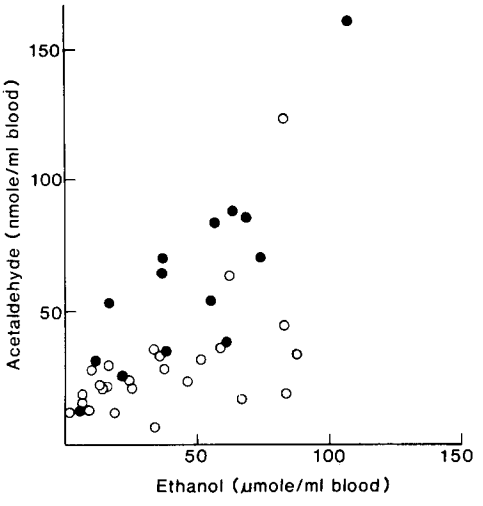

Fig. 3. Same as Fig. 2 but with C57 mice.

dation and blood acetaldehyde artefactually formed in control blood to which ethanol had been added. In C57 mice (Fig. 3) artefactually formed acetaldehyde concentrations were even higher than those measured during ethanol intoxication.

Table 1 lists the results from the experiments in which different blood sources and analytical procedures were used for determination of acetaldehyde in C57 and DBA mice. No acetaldehyde concentration differences were found between the strains. A tendency for higher acetaldehyde concentrations were observed in the DBA compared with C57 mice. However, artefactually formed acetaldehyde concentrations closely matched those measured during intoxication. Thiourea in the hemolyzing water had no effect on the artefactual acetaldehyde formation. It should be noted that the use of fresh semicarbazide did not abolish the artefactual acetaldehyde formation, as opposed to the case with human blood [22, 23]. In summary, these present results did not provide any evidence for the existence of measurable acetaldehyde levels *in vivo* during ethanol oxidation in any of the strains.

DISCUSSION

Liver acetaldehyde during ethanol oxidation. Hepatic acetaldehyde levels observed in the present investigation (5–118 nmole/g) fall within the normal hepatic acetaldehyde range for rats [26]. Moreover, the biphasic shape of the hepatic acetaldehyde–ethanol concentration function observed in rats [20, 26, 27], seems to exist also in mice. The present results are in agreement with a recent investigation, in which several mice strains were compared [17]. Possible explanations for the positive as well as negative correlations between hepatic ethanol and acetaldehyde concentrations in rats have been discussed earlier [26–28]. It remains, however, to be established if it is the rate of ethanol or acetaldehyde oxidations, which changes as a function of the hepatic ethanol concentration.

The present results from mice displayed higher hepatic acetaldehyde concentrations in DBA compared with C57 mice, at least at lower ethanol concentrations. Whether this is a reliable marker for a DBA–C57 strain difference is not conclusive from the present study. The difference seems to be supported by some earlier observations of higher blood acetaldehyde concentrations in the DBA compared with C57 mice [6, 10–13]. On the other hand, there are conflicting breath [15] and blood [7, 14] data, and moreover, as will be discussed further in the text, previously reported blood acetaldehyde levels need perhaps to be re-evaluated.

Determination of blood acetaldehyde. Based on the present strain differences with regard to the hepatic acetaldehyde concentrations, one could also expect higher blood acetaldehyde levels in the DBA compared with the C57 strain, at least at ethanol concentrations below 50 μM. Such a difference was observed (Table 1, and Fig. 2 compared with Fig. 3) if artefactual acetaldehyde formation was not recognized. The difference could, however, just as well have been an artefact as demonstrated by the high level of artefactually formed acetaldehyde during the analytical procedures. What further complicates the comparison of the acetaldehyde levels measured during intoxication with those measured in control blood with added ethanol, is the possibility of ethanol oxidation *in vivo* affecting the magnitude of artefactually formed acetaldehyde. This possibility is clearly demonstrated in Fig. 3, in which the artefactually formed acetaldehyde concentrations seem to exceed those found under comparable ethanol concentrations *in vivo*. Thus, in the present investigation there was no evidence for the existence of any measurable acetaldehyde in venous blood during ethanol oxidation.

Table 1. Blood acetaldehyde*

Blood source, treatment	Acetaldehyde (nmole/ml) During intoxication C57	DBA	Artefactual C57	DBA
Orbital blood, hemolyzation	38 ± 16	59 ± 21†	38 ± 16	59 ± 31
Orbital blood, semicarbazide	37 ± 25	56 ± 22	21 ± 5	67 ± 33†
Blood after decapitation, hemolyzation	37 ± 22	48 ± 17	34 ± 19	62 ± 24†
Blood after decapitation, semicarbazide	41 ± 25	51 ± 27	48 ± 28	49 ± 34

* Mice were treated and analyses made as described in the Materials and Methods section. Results are given as means ± S.D. (n = 10 for intoxication and 6 for artefactual means).

† Significant ($P < 0.05$) strain differences are calculated by means of the Student's *t*-distribution.

Also the fact that the measured blood acetaldehyde concentrations during ethanol oxidation were as high as those observed in the liver suggest the involvement of artefacts. In rat studies [20], acetaldehyde concentration gradients have been found between liver, arterial and venous blood so that very low, if any, venous acetaldehyde levels would be expected during conditions in which the hepatic acetaldehyde concentration is below 100 μM.

Previous mouse blood acetaldehyde determinations have used hemolyzation, with [14] or without [16, 18] thiourea, precipitation [6, 7, 10–12, 17, 29], or direct heating [12] procedures to treat the blood prior to normal or headspace gas chromatographic measurements. The problems with artefactual acetaldehyde formation during blood treatment procedures was, however, only mentioned in one of these investigations [16], in which it was stated that this phenomenon did not occur. With a view to the present results regarding the magnitude of the artefactual acetaldehyde production, it seems, however, that earlier mice blood acetaldehyde levels should be reevaluated. A similar reevaluation has been found to be necessary for human blood acetaldehyde determinations [23]. Thus, also previously reported DBA–C57 blood acetaldehyde differences [6, 10–13] may turn out to be artefacts. Future studies should be carried out to improve mouse blood acetaldehyde determinations and to examine the actual acetaldehyde concentration, if any, during ethanol oxidation.

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