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ENDOGENOUS ACETALDEHYDE IN RATS

EFFECTS OF EXOGENOUS ETHANOL, PYRAZOLE, CYANAMIDE AND DISULFIRAM

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Abstract—Male Long–Evans rats consumed the alcohol and aldehyde dehydrogenase inhibitors pyrazole, cyanamide or disulfiram, for 6 days. No endogenous blood acetaldehyde could be detected in controls and pyrazole treated rats, endogenous blood concentrations up to 2–5 nmoles/ml were, however, measured in the cyanamide and disulfiram-treated animals. Other rats received daily ethanol gastric intubations in addition to the consumption of the inhibitors. Little or no acetaldehyde was detected in the controls and pyrazole treated animals during acute ethanol intoxication or on the subsequent days. High blood levels (200–500 nmoles/ml) were observed in the rats consuming cyanamide and disulfiram, and concentrations up to 10–12 nmoles/ml were still found on the following day after all the ethanol had been eliminated. This acetaldehyde and the endogenous acetaldehyde could only be observed with the hemolyzation method in which blood hemolyzates were directly heated prior to headspace GC analysis; none was detected if blood proteins were first precipitated and removed with perchloric acid. It is suggested that aldehyde dehydrogenase inhibitors elevate endogenous concentrations of bound acetaldehyde and that exogenous ethanol increases this form of acetaldehyde.

In contrast to the situation during ethanol intoxication, little is known about endogenous acetaldehyde metabolism. The microbial and/or hepatic pathways from threonine [1], deoxyribose phosphate [2], β-alanine [3] and pyruvate [4] are best regarded as theoretically possible sources of acetaldehyde, since as yet there is no firm evidence for the *in vivo* existence of these intermediary reactions. On the other hand, it has been demonstrated that acetaldehyde is indeed formed and metabolized as an intermediate by microbial fermentation reactions in the gastrointestinal tract [5]. Most of the ethanol formed in the fermentation reactions is then absorbed into the circulation and oxidized to acetaldehyde and further to acetate on the first passage through the liver [5]. It is doubtful whether any endogenous acetaldehyde leaves the liver. In fact, based on recent advances in the methodology on blood acetaldehyde determination, it may be concluded that there is no solid evidence for detectable blood acetaldehyde concentrations during control situations [6, 7]. That endogenous acetaldehyde does exist, even if its origin is unknown, is, however, supported by findings of minute acetaldehyde levels in normal breath [8, 9].

An early study [10] examined endogenous acetaldehyde in rabbits treated with disulfiram, an inhibitor of acetaldehyde oxidation. No elevation of blood acetaldehyde was found during the disulfiram treatment, and it was concluded that the concentration of acetaldehyde never becomes important in normal metabolic processes. Shortly after this, conflicting results with disulfiram-induced endogenous human blood acetaldehyde levels were published [11]. Conflicting results, with no [12] or with some levels [13, 14] of endogenous acetaldehyde in rats treated with aldehyde dehydrogenase inhibitors, have also been reported. The aim of the present investigation was to examine the endogenous acetaldehyde levels in rats and the possible causes for previous discrepancies.

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MATERIALS AND METHODS

Rats. Male Long–Evans rats, 2–3 months of age, bred in our laboratory (originating from rats purchased from Simonsen, Gilroy, CA), were used in the experiments. The rats were given a standard laboratory diet (Astra–Ewos AB, Södertälje, Sweden) and tap water *ad libitum*. Some of the animals received additional drugs in the diet or water.

General procedures. Animals were randomly divided into four main groups: (1) control group, which received normal diet and tap water; (2) pyrazole group, which received normal diet, and tap water containing 0.15 (w/v) pyrazole (Fluka AG, Buchs, Switzerland), an alcohol dehydrogenase inhibitor; (3) cyanamide group, which received diet containing 0.2 g Ca-carbimide (Dipsan from Lederle, Montreal) per kg of diet, and tap water; (4) disulfiram group, received diet containing 2 g disulfiram (Antabus from A/S Dumex, Copenhagen) per kg of diet, and tap water. Each main group was further divided in two

Table 1. Food, water and inhibitor consumption*

Group (N)	Food consumption (g/kg day)	Water consumption (ml/kg day)	Inhibitor consumption (mg/kg day)	Body wt on day 1 (g)	Body wt on day 7 (g)
Control (8)	61 ± 4	109 ± 21		322 ± 24	340 ± 31
+ethanol (7)	53 ± 5†	96 ± 4		340 ± 23	345 ± 21
Pyrazole (8)	27 ± 6†	36 ± 6†	54 ± 9	328 ± 38	286 ± 38†
+ethanol (8)	27 ± 6†	41 ± 6†	58 ± 24	337 ± 25	295 ± 20†
Cyanamide (8)	55 ± 3†	96 ± 5	11 ± 1	332 ± 13	331 ± 24
+ethanol (8)	40 ± 8†	88 ± 7	8 ± 2	339 ± 22	322 ± 27
Disulfiram (8)	39 ± 6†	94 ± 20	78 ± 13	329 ± 35	312 ± 34
+ethanol (8)	34 ± 2†	121 ± 35	69 ± 4	322 ± 30	301 ± 28†

* Rats were treated as described in the Materials and Methods section. Means ± S.D. are given. Significance (compared with corresponding control) is indicated by †($P < 0.01$), based on Student's t-test.

subgroups, one receiving no additional drugs during the whole experimental period (Days 1–7), and the other receiving an oral dose of ethanol (2 g/kg as 10%, w/v, in tap water) in the mornings of Days 2–6.

Ethanol and acetaldehyde determinations. Blood samples were taken from the tip of the tail on Days 2, 4 and 6 from all rats before the subgroups receiving ethanol had been intubated and from the latter subgroups 1 hr after intubation. Samples of 0.1 ml were hemolyzed with 0.9 ml ice-cold distilled water, and ethanol and acetaldehyde were directly measured by headspace gas chromatography (Perkin–Elmer F 40, column: 15% polyethylene glycol on Celite 60/100) as previously described [15]. The identification of acetaldehyde was verified in preliminary experiments by using another gas chromatograph, Hewlett–Packard, and another column, packed with Carbopack containing 0.1% SP-1000 (Supelco Inc., Bellefonte, PA). Corrections for artefactual acetaldehyde formation [6, 15], based on control blood hemolyzed with water containing different amounts of ethanol, were applied (0–0.8 μM at ethanol concentrations of 0–4 mM in the headspace vessel). In the morning of Day 7 another tail blood sample was taken, which was treated as described for the previous days. The animals were then decapitated and additional blood samples were taken. Samples of 0.1 ml were hemolyzed with 0.5 ml ice-cold distilled water, or treated with 0.5 ml 0.6 M perchloric acid (at 4° or at room temperature). Blood precipitates were centrifuged at 4000 *g* for 15 min at 4°. Ethanol and acetaldehyde were measured from hemolyzates or perchloric acid supernatants by headspace gas chromatography as previously described [15].

In addition to the correction for artefactual acetaldehyde formation with the hemolyzation method (with ethanol present), all measurements were corrected for blank values (0–0.01 μM) obtained in zero samples containing only distilled water or perchloric acid. Because of this procedure, some group means were negative, and these means were listed as 0 on the tables. However, for the statistical evaluations also negative means ± S.D. were considered.

RESULTS

Food and water consumption, and growth, were followed to get a more comprehensive view of the effects of the alcohol and aldehyde dehydrogenase inhibitors used. The results are depicted in Table 1. Food consumption was somewhat reduced when either cyanamide or disulfiram was mixed with the food, and water intake was reduced when pyrazole was added to the water. The latter treatment also caused a marked decrease in food intake. The daily ethanol administration tended to further decrease the food consumption in all groups except for that treated with pyrazole. Body wts in general paralleled the changes in the food consumptions.

Blood acetaldehyde concentrations, determined 1 hr after the ethanol intubations, are listed in Table 2. Cyanamide consumption greatly elevated the blood acetaldehyde levels and the elevation was

Table 2. Blood acetaldehyde during ethanol oxidation*

Group (N)	Acetaldehyde (nmoles/ml tail blood) Day 2	Day 4	Day 6
Control (7)	5 ± 7	0 ± 2	2 ± 3
Pyrazole (8)	0 ± 3	0*	0 ± 4
Cyanamide (8)	365 ± 149	499 ± 157	351 ± 133
Disulfiram (8)	27 ± 11	206 ± 63	208 ± 51

* Rats were treated and analyses were made as described in the Materials and Methods section. Blood ethanol means ranged between 23 and 36 μmoles/ml in all groups, except for the pyrazole group, which displayed concentrations ranging between 35 and 44 μmoles/ml.

Table 3. Endogenous acetaldehyde I*

Group	Acetaldehyde (nmoles/ml tail blood) Day 2	Day 4	Day 6	Day 7
Control	1.3 ± 2.5	0.0 ± 0.8	1.0 ± 2.6	1.3 ± 2.4
+ethanol		1.9 ± 2.9	0.8 ± 3.1	0.6 ± 1.6
Pyrazole	0.1 ± 2.7	0.3 ± 1.6	1.4 ± 1.8	1.8 ± 2.6
+ethanol		0.5 ± 0.5	1.5 ± 1.7	1.7 ± 2.0
Cyanamide	0.7 ± 2.1	2.1 ± 1.6†	4.3 ± 4.9	2.9 ± 2.5
+ethanol		5.3 ± 2.2†	12.0 ± 2.8†	12.3 ± 2.9†
Disulfiram	1.6 ± 2.5	2.5 ± 1.2†	4.0 ± 3.2	4.1 ± 3.2
+ethanol		3.7 ± 1.5	10.1 ± 3.5†	10.4 ± 4.0†

* Rats were treated and analyses (hemolyzation method) were made as described in the Materials and Methods section. Blood ethanol concentrations were all below the detection limit (<0.1 μmoles/ml). Significance (compared with corresponding control) is indicated by †($P < 0.05$), based on Student's *t*-test. The number of animals is indicated in Table 1.

already apparent after 1 day. Disulfiram also elevated the acetaldehyde levels, with the effect gradually increasing during the 4 first days. No significant (from zero) acetaldehyde was, however, observed in the controls and pyrazole treated animals. Nevertheless, the possibility that the controls had some blood acetaldehyde on Day 2 cannot be excluded because of their large standard deviation. No differences between the different groups were observed in the magnitude of correction for artefactual acetaldehyde formation during the analytical procedures (6–8 nmoles/ml at ethanol concentrations of 30–40 μmoles/ml blood).

Table 3 lists acetaldehyde concentrations determined before the ethanol intubations, and on Day 7. No correction for artefactual acetaldehyde formation during incubation in the headspace vessels prior to analysis was needed because of the lack of ethanol in the samples. The values for the control and pyrazole groups (Table 3) are merely the result of variable blanks which were determined in zero samples containing only distilled water and then subtracted from the values determined for the blood hemolysates. The consumption of the aldehyde dehydrogenase inhibitors, cyanamide and disulfiram, produced measureable acetaldehyde levels without any ethanol treatment. This phenomenon was more apparent in the disulfiram group. In those groups receiving the daily ethanol intubations the "endogenous" acetaldehyde levels rose to 10–12 nmoles/ml blood.

Interestingly, these endogenous acetaldehyde levels could only be measured with the hemolyzation method (Table 4), according to which the blood hemolyzate was directly heated to 65° for 15 min prior to headspace analysis [15]. No acetaldehyde could be detected with the perchloric acid precipitation method [15] (Table 4), according to which blood proteins were first precipitated and acetaldehyde was then measured in the supernatant by headspace GC. No difference was observed as a result of whether cold (4°) or warm (20°) perchloric acid was used.

Table 4. Endogenous acetaldehyde II*

Group (N)	Acetaldehyde (nmoles/ml whole blood) Hemolyzation method	Precipitation method
Control (4)	0.0 ± 1.2	0.4 ± 1.1
+ethanol (4)	0*	0.3 ± 1.5
Pyrazole (5)	1.6 ± 1.9	0*
+ethanol (4)	1.8 ± 1.0†	0.3 ± 1.9
Cyanamide (6)	2.3 ± 2.7	0*
+ethanol (4)	10.0 ± 1.4†	0*
Disulfiram (7)	5.3 ± 3.9†	0.0 ± 0.7
+ethanol (6)	7.3 ± 1.4†	0.2 ± 1.3

* Rats were treated and analyses were made on blood after decapitation on Day 7 as described in the Materials and Methods section. The hemolyzation method involved direct headspace analysis of blood hemolysates (as in Table 3). With the precipitation method blood proteins were first precipitated after which headspace analysis was applied on the supernatants. Blood ethanol concentrations were all below the detection limit (<0.1 μmoles/ml). Significance (compared with corresponding control) is indicated by †($P < 0.05$), based on Student's *t*-test.

DISCUSSION

The present results suggest that endogenous acetaldehyde may be elevated as a result of treatment with aldehyde dehydrogenase inhibitors. An interesting question is why such acetaldehyde only could be detected in blood hemolyzates and not in precipitated blood supernatants, and whether these methodological differences may explain earlier conflicting results. It seems that the acetaldehyde measured in the present investigation represented some source of bound acetaldehyde which was released during the heating of the hemolysates prior to headspace GC. Apparently the perchloric acid precipitation was not effective to release the bound acetaldehyde. The occurrence of tightly bound acetaldehyde could explain why disulfiram treatment was previously not observed to elevate the endogenous acetaldehyde concentration [10], determined in rabbit blood after the proteins had been precipitated with sodium wolframate/sulphuric acid and removed [16]. The elevated endogenous acetaldehyde concentrations observed in human [11] and rat [13, 14] blood after treatment with several different aldehyde dehydrogenase inhibitors could be explained by the fact that blood proteins were precipitated, but not centrifuged away, before acetaldehyde trapping into semicarbazide [11, 14, 17] or direct heating followed by headspace GC [13].

The findings of disulfiram-induced endogenous acetaldehyde levels [13] were criticized in a study in which no acetaldehyde could be detected both with or without disulfiram treatment and in which the acetaldehyde was measured by direct headspace GC on whole blood [12]. That the control blood did not contain measureable (>1.7 μM) acetaldehyde concentrations, is in agreement with the results of the present study and with our general experience [7]. That no endogenous acetaldehyde was found with the disulfiram treatment, also agrees with the present data, since the study [12] used only 1 day of disulfiram treatment (compare results in Table 3). Thus, the previous results of rather high endogenous acetaldehyde levels (20–120 μM) after treatment with aldehyde dehydrogenase inhibitors [13, 14] seem difficult to understand. On the other hand, the explanation could be that acetaldehyde may occur in several different bound forms with different degrees of reversibility, and the recovery could depend on the analytical procedures used. This aspect should be further clarified in future studies.

Ethanol treatment clearly increased the "endogenous" acetaldehyde concentration in the present study (Tables 3 and 4), a finding which briefly has been reported earlier in cyanamide treated rats [18]. Whether the acetaldehyde elevation is an "exogenous" remainder of the high acetaldehyde concentration appearing after the administered ethanol, or the result of a long lasting disturbance to the metabolism of the endogenous acetaldehyde metabolism, is not clear. The former explanation seems more likely and opens the general question of whether ethanol treatment may lead to the creation of different kinds of bound acetaldehyde, which could then remain after the ethanol has been oxidized. Different kinds of such more [19], or less [20–23], reversible acetaldehyde adducts have been suggested to occur after ethanol treatment in human [20–23] and rat [19] blood. The previously reported reversible binding to rat blood hemoglobin [19] cannot explain the present results, because perchloric acid treatment did not release the bound acetaldehyde observed in the present study. Whether this binding is similar to that without the addition of exogenous ethanol, and the nature of the binding(s), remains to be settled in future studies.

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