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BREATH AND BLOOD ACETALDEHYDE CONCENTRATIONS AND THEIR CORRELATION DURING NORMAL AND CALCIUM CARBIMIDE-MODIFIED ETHANOL OXIDATION IN MAN

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Abstract—With the use of new and improved analytical techniques, concentrations of acetaldehyde in antecubital venous blood and breath of human volunteers were measured after (a) pretreatment of subjects with ethanol and the aldehyde dehydrogenase inhibitor, calcium carbimide and (b) treatment with ethanol only. Breath acetaldehyde concentrations were converted to equivalent pulmonary blood concentrations using an experimentally determined blood: breath partition ratio for acetaldehyde of 190. Under all experimental conditions, blood acetaldehyde concentrations calculated from breath analysis were seen to closely reflect those measured by direct blood analysis. Treatment of subjects with calcium carbimide resulted in elevated blood and breath acetaldehyde concentrations which were rapidly lowered by the intravenous infusion of 4-methyl pyrazole. Peak blood acetaldehyde concentrations ranged from 25 to 188 μM after calcium carbimide and ethanol treatment, but were only 6–11 μM after ethanol treatment alone (1.2 g/kg).

Acetaldehyde is considered as a potential toxic metabolite of ethanol which could exert significant pharmacological effects during ethanol intoxication in man [1]. However, studies on the metabolism of acetaldehyde in man have been hampered by methodological problems associated with the measurement of this compound in human blood [2, 3]. Some attempts have been made to study human metabolism of acetaldehyde by its measurement in breath samples [4–6], the assumption being made that alveolar breath acetaldehyde is in equilibrium with acetaldehyde in pulmonary blood, so that changes in breath acetaldehyde concentrations may be taken to reflect those in blood. Although such a correlation has been demonstrated for ethanol [7, 8], this relationship has not yet been shown to exist for acetaldehyde.

In view of the ease of collection and analysis of human breath samples [6, 9], and the possibility that breath analysis could replace blood analysis in future studies of human acetaldehyde metabolism, this study was carried out to determine whether breath acetaldehyde concentrations reflect those in blood. The techniques used in this study were also designed to give additional information relating to biochemical mechanisms involved in acetaldehyde production and disposition mechanisms involved in acetaldehyde production and disposition during ethanol metabolism in humans.

MATERIALS AND METHODS

Reagents. The buffered (pH 7.0) isotonic semicarbazide solution used for the treatment of blood samples prior to acetaldehyde analysis contained: 16 mM NaCl, 29.9 mM NaH_2PO_4, 70.1 mM Na_2HPO_4, 6 mM semicarbazide hydrochloride and 1000 i.u. heparin per 100 ml. The same solution, without heparin and NaCl, was used to trap breath acetaldehyde.

Calcium carbimide was purchased from Lederle, Montreal, Canada and 4-methyl pyrazole was obtained from Orion, Espoo, Finland.

Human subjects. Informed consent was obtained from all subjects, who were healthy males with ages ranging from 25 to 40 yr. Subjects given ethanol doses of 0.2 g/kg body weight had fasted for at least 9 hr prior to ethanol ingestion, while those given 1.2 g/kg had consumed a standard light breakfast prior to ethanol consumption.

Ethanol, calcium carbimide and 4-methyl pyrazole administration. Four subjects were treated orally with the aldehyde dehydrogenase inhibitor calcium carbimide [10] (0.25 mg/kg) and with ethanol (0.2 g/kg) between 2.3 and 3.3 hr later. The ethanol was consumed within 1 min. Beginning between 26 and 29 min after ethanol consumption, the alcohol dehydrogenase inhibitor 4-methyl pyrazole [11] (5 mg/kg) in buffered saline was infused into an antecubital vein over a 2–3 min period. A control study with saline being infused instead of 4-methyl pyrazole was done on a separate day.

The calcium carbimide treatment was designed to produce readily measurable blood actaldehyde concentrations without using a large dose of ethanol and the 4-methyl pyrazole was used in an attempt to decrease blood acetaldehyde so that a corresponding elimination of breath acetaldehyde would be seen if the two were correlated. It has been shown previously that 4-methyl pyrazole treatment drastically

reduces blood acetaldehyde in rats metabolizing ethanol [12].

Where ethanol alone was administered, it was given to subjects as an oral dose of 1.2 g/kg and was consumed during five 5-min drinking periods separated by 5-min intervals. In the first period, 0.4 g/kg was consumed, while in subsequent periods, 0.2 g/kg was taken.

Blood sampling and analysis. Blood samples taken via an indwelling cannula inserted in an antecubital vein were immediately mixed with 0.5–3 vol. of the fresh isotonic semicarbazide solution described above and stored for up to 6 hr at 0–4°. Diluted plasma samples were obtained by centrifugation and analysed for acetaldehyde and ethanol after treatment with 0.2 vol. of 3.0 M perchloric acid and centrifugation to obtain clear, protein-free supernatants. Supernatant samples (0.5 ml) were assayed for ethanol and acetaldehyde by head-space gas chromatography [13]. Quantitative recoveries of ethanol and acetaldehyde added to fresh human blood samples were obtained in the concentration ranges measured in this study. Dilution of blood with 0.5–9 vol. of the semicarbazide reagent had no effect on acetaldehyde or ethanol recoveries. Sensitivity limits of the method for acetaldehyde and ethanol were approximately 0.5 μM and 0.5 mM, respectively, when blood was diluted two-fold with the semicarbazide reagent.

Artifactual acetaldehyde formation from ethanol during perchloric acid treatment of blood cells, a major problem associated with many previous methods for acetaldehyde analysis in blood [2], was negligible when plasma analysis was employed [14]. Semicarbazide reacts with acetaldehyde to form its semicarbazone derivative [15] and was used to stop the decay of acetaldehyde which occurs in blood on storage [16]. Acetaldehyde is readily regenerated from its semicarbazone by acid hydrolysis [9], this being carried out in the present method by perchloric acid treatment.

When calculating blood ethanol concentrations from analyses of protein-free perchloric acid supernatants, it was assumed that ethanol is distributed only throughout the water phase of blood and for correction purposes it was assumed that blood cells contain 70 per cent (v/v) water, and plasma, 94 per cent [17]. No such correction was carried out for acetaldehyde.

Breath acetaldehyde sampling and analysis. Two 10 ml volumes of ice-cold semicarbazide solution described in Reagents were placed in two gas bubbler tubes connected in series. Subjects passed 4.0 l of breath through the tubes in a single expiration after holding their breath for 2–3 sec. Breath flow rate was approximately 300 ml/sec and its volume was recorded by a spirometer connected to the outlet of the second bubbler tube.

After combining and mixing the contents of the bubbler tubes, samples (0.4 ml) of the resulting solution were acidified with 0.1 ml of sulphuric acid (5 M) and analysed for ethanol and acetaldehyde as above. Recoveries of acetaldehyde and ethanol added to the buffered semicarbazide solution were 100 per cent with respect to aqueous standards, and sensitivity limits for acetaldehyde and ethanol were approximately 0.2 nmoles/100 ml breath and 40 nmoles/100 ml breath, respectively. Breath ethanol and acetaldehyde concentrations were corrected for trapping efficiencies which were determined to be 98 per cent and 88 per cent for ethanol and acetaldehyde, respectively.

Conversion of breath ethanol and acetaldehyde concentrations to blood concentrations. All measured breath acetaldehyde and ethanol concentrations were converted to their equivalent blood concentrations using blood: breath partition ratios of 2100 [18] and 190 [9] for ethanol and acetaldehyde, respectively, assuming that breath samples were in equilibrium with pulmonary blood with respect to these two compounds.

RESULTS

Correlation between blood and breath acetaldehyde after calcium carbimide and ethanol treatment. Figures 1a and b show that concentrations of acetaldehyde in breath reflect those in peripheral venous blood in similar manner to that which occurs for ethanol, at least within the acetaldehyde concentration range observed in these experiments.

There was a considerable inter-individual variation in blood acetaldehyde levels produced after calcium carbimide and ethanol ingestion; peak concentrations determined from blood and breath analysis ranging from 25 to 188 μM and from 43 to 161 μM, respectively. Therefore, the standard errors of the mean concentrations presented in Figs. 1a and b

Table 1. Linear regression equations and correlation coefficients for blood acetaldehyde concentrations determined by direct and indirect (breath) analysis after calcium carbimide and ethanol treatment

Number of pairs of samples	4-Methyl pyrazole treatment	Sampling period (min after ethanol ingestion)	Regression equation*	Correlation coefficient
12	+	0–25	$Y = 0.71X + 36$	0.75 ($P < 0.002$)
16	−	0–30	$Y = 0.78X + 29$	0.98 ($P < 0.001$)
18	+	25–60	$Y = 0.98X - 0.9$	0.99 ($P < 0.001$)
16	−	30–60	$Y = 0.62X + 16$	0.96 ($P < 0.001$)

* Regression equations are in the form $Y = mX + b$, where Y = blood acetaldehyde concentration calculated from breath analysis, X = blood acetaldehyde concentration determined by direct analysis, m = slope of the regression line and b = intercept of this line on the X-axis. The 25–60 and 30–60 min sampling periods were chosen to coincide with the time during which blood ethanol levels could be accurately predicted from breath ethanol levels.

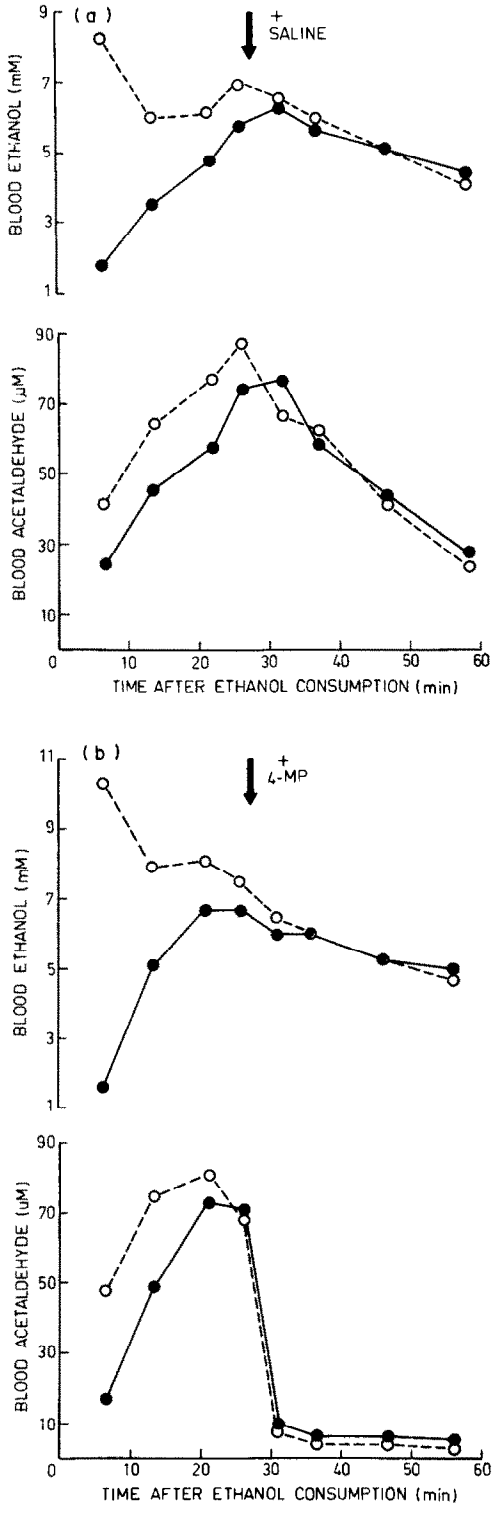

Fig. 1. Mean blood ethanol and acetaldehyde concentrations of subjects treated orally with calcium carbimide (0.25 mg/kg) and ethanol (0.2 g/kg). Acetaldehyde and ethanol concentrations were either determined directly from blood (●——●) or indirectly from breath analysis (O-----O) as described in Materials and Methods. The arrows indicate the mean time at which intravenous infusion of saline (panel a) or 4-methyl pyrazole (5 mg/kg) (panel b) was begun. All points represent the means of single determinations from four subjects.

were large (up to 50 per cent of the mean values) and have been omitted from the figures for the sake of clarity. However, for each individual, blood concentrations calculated from breath analysis were seen to closely reflect those determined by direct blood analysis at various time intervals. Therefore, for blood–breath comparison, the mean results presented in Figs. 1a and b are representative of the data from each individual. Table 1 illustrates this point, showing the excellent correlation between blood and breath acetaldehyde in both the ascending and descending phases of the acetaldehyde vs time curves. It may be noted that breath analysis tended to give overestimates of antecubital venous blood acetaldehyde levels (indicated by the positive Y-intercept values in Table 1), but these were marked only in the time during which the venous blood ethanol levels could not be predicted with accuracy from breath levels, i.e. 0–25 min after ethanol ingestion when 4-methyl pyrazole was infused and 0–30 min in the saline control experiment. After these times, blood ethanol concentrations determined by the direct and indirect methods correlated extremely well; the linear regression equation for blood ethanol determined from breath analysis (Y) plotted against blood ethanol determined by direct analysis (X) being $Y = 1.1X - 0.55$ and the correlation coefficient was 0.93 (N = 35).

In all experiments, peak blood and breath acetaldehyde concentrations were separated from blood and breath ethanol peaks by no more than 5 min and acetaldehyde and ethanol concentrations were undetectable in blank blood and breath samples taken immediately before ethanol ingestion.

As can be seen by comparison of Fig. 1a with Fig. 1b, 4-methyl pyrazole infusion resulted in a rapid and parallel decline in blood and breath acetaldehyde concentrations.

Blood acetaldehyde concentrations following an ethanol dose of 1.2 *g/kg.* Figure 2 shows that under conditions of normal ethanol metabolism, blood acetaldehyde concentrations determined from blood and breath analysis follow a similar pattern even if their absolute magnitudes are significantly different. No large peak in acetaldehyde production was seen and no correlation between peak blood ethanol and peak blood acetaldehyde was observed, the acetaldehyde peak occurring between 10 and 70 min before the ethanol peak. In the absence of calcium carbimide treatment, blood acetaldehyde concentrations were much less variable, with peak concentrations ranging from 6 to 11 μM when measured directly, and from 6 to 16 μM when calculated from breath analysis.

The linear regression equation for blood acetaldehyde determined from breath analysis (Y) plotted against blood acetaldehyde concentration determined by direct analysis (X) was $Y = 0.54X + 4.4$ and the correlation coefficient, 0.46, was highly significant ($P < 0.002$).

DISCUSSION

Accurate estimations of blood ethanol concentrations in man may be carried out using breath ethanol analysis because of the relatively constant relation-

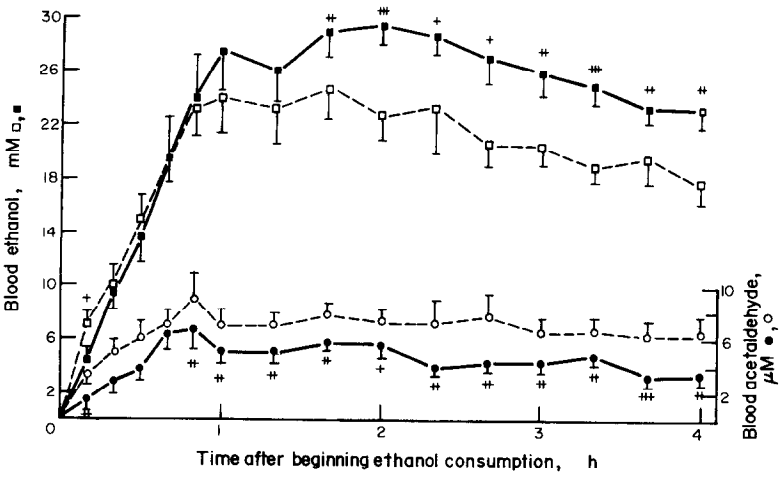

Fig. 2. Mean blood ethanol and acetaldehyde concentrations of subjects during and after oral administration of ethanol (1.2 g/kg). Closed symbols represent blood concentrations determined by direct assay and open symbols represent blood concentrations determined from breath analysis as described in Material and Methods. The bar lines represent S.E.M.s and each point is the mean of single determinations from five subjects. The matched pair *t*-test was used to calculate the significance of the differences between mean values obtained for both blood ethanol and acetaldehyde. Significance levels are denoted as follows: + = $P < 0.05$, ++ = $P < 0.025$, +++ = $P < 0.01$.

ship between the concentrations of ethanol in blood and breath [18]. The results of the present study suggest that breath analysis is also useful in predicting blood acetaldehyde concentrations.

During absorption of the low dose of ethanol, pulmonary blood ethanol and acetaldehyde concentrations calculated from breath analysis were higher than those in antecubital venous blood (see Figs. 1a and b). This difference would be expected for ethanol at least, as it takes some time for ethanol to equilibrate throughout the body tissues so that venous blood has a lower ethanol concentration than arterial blood until equilibration is complete [19]. The present results suggest that some time is also required for the equilibration of acetaldehyde. Since from a pharmacological viewpoint it is more important to know the concentrations of acetaldehyde reaching the brain rather that the peripheral tissues [20–22], the measurement of arterial rather than venous blood acetaldehyde concentrations is desirable. Such a measurement is most simply carried out by breath analysis as pulmonary blood would be expected to closely reflect arterial blood with respect to acetaldehyde concentrations [23].

When estimating blood ethanol levels from breath concentrations, residual alcohol remaining in the mouth must be cleared before breath sampling or it will give a positive bias to the breath alcohol concentrations [24]. This effect is seen in Figs. 1a and b. Our unpublished observations have indicated that an elapsed time of 15 min between drinking 10% (v/v) ethanol and breath sampling is sufficient to avoid contamination of breath by ethanol in the mouth, and this conclusion is supported by the work of Caddy *et al.* [24]. Five minutes was sufficient to avoid acetaldehyde interference, therefore, in this study, all breath acetaldehyde and most breath ethanol measurements were made on samples not contaminated by ethanol or acetaldehyde originating from the mouth. The possibility exists that some of the breath acetaldehyde may have originated from metabolism of ethanol in the tissues of the lungs and air passages and such alternative sources of breath acetaldehyde may have been responsible for the differences observed between the low blood acetaldehyde concentrations measured by the direct and indirect methods after subjects were given 1.2 g/kg ethanol (Fig. 2). However, these differences could also be caused by extrahepatic metabolism of acetaldehyde. Many extrahepatic mammalian tissues possess aldehyde dehydrogenase activity [25] and this could be responsible for the decrease in blood acetaldehyde between the arterial and venous systems. Venous tail-blood of rats metabolizing ethanol has been shown to have significantly lower concentrations of acetaldehyde than arterial blood [23]. The marked effect of 4-methyl pyrazole in lowering the artificially elevated blood and breath acetaldehyde concentrations in parallel provides good evidence for the origin of breath acetaldehyde being the blood rather than local ethanol metabolism in the lungs and air passages. However, when blood acetaldehyde concentrations are very low, such alternative sources of breath acetaldehyde may account for a greater proportion of the total breath acetaldehyde and could make estimations of blood acetaldehyde concentrations from breath less accurate under such circumstances.

The dose of 4-methyl pyrazole used in this study was 2/3 of a dose which has previously been shown to inhibit ethanol oxidation in humans by 21 per cent [26]. It is therefore clear that only a relatively low degree of alcohol dehydrogenase inhibition is necessary to greatly reduce blood acetaldehyde levels, a fact which has been shown to be true for rats [27].

Significant post-absorptive differences between blood ethanol concentrations measured by the direct and indirect methods occurred after the 1.2 g/kg

ethanol dose. These differences were not observed after the low ethanol dose and it is not clear why they were more marked than those observed by other investigators under similar experimental conditions [18, 28].

The finding of a rapid and transient increase in blood acetaldehyde after calcium carbimide and ethanol treatment (Fig. 1a) confirms the recent results of Brien *et al.* [29]. The initial increase in blood acetaldehyde was probably due to its more rapid production with increasing ethanol concentration and its subsequent rapid decay may have resulted from a recovery of aldehyde dehydrogenase from calcium carbimide inhibition [30], possibly in combination with a slight decrease in the rate of ethanol metabolism.

In conclusion, it may be stated that breath acetaldehyde levels not only reflect those in antecubital venous blood but probably also reflect those in pulmonary blood in such a way that these can be determined with reasonable accuracy by breath analysis. It is also clear that under normal conditions of ethanol metabolism, acetaldehyde is present in human blood in much lower concentrations than many previous studies have indicated (see ref. 1).

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