THE OCCURRENCE OF ACETALDEHYDE BINDING IN RAT BLOOD BUT NOT IN HUMAN BLOOD

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1. Introduction

Recent improvements in the determination of acetaldehyde (AcH) in biological samples [1–3] have generated an increasing number of investigations into in vivo AcH metabolism, which may be of importance in the toxic actions of ethanol. AcH is a reactive compound and it has been proposed that it can readily bind to sulphydryl groups [4–6]. Nevertheless, despite many speculations, it has not been conclusively shown that AcH actually exists in a bound form in blood in vivo.

In previous experiments [3] using head-space chromatography we found that the recovery of AcH added to rat blood decreased with the time between the AcH addition and the precipitation of protein with perchloric acid (PCA). No decrease of the AcH recovery was found, however, if the blood samples were hemolysed with water, instead of being treated with PCA. This implied that binding of AcH occurred in the blood and that some of the bound AcH had been lost with the proteins when these were removed during the PCA procedure. We now report our examination of this binding phenomenon.

AcH was found to be unevenly distributed in rat blood during ethanol oxidation because of binding in the erythrocytes. The calculated in vivo free AcH concentration in the plasma was about 40% of the whole blood AcH concentration. The capacity of the rat blood to bind AcH in vitro correlated positively with the blood hemoglobin concentration. The calculated maximum binding capacity of 1 molecule of hemoglobin was 4 molecules of AcH. In contrast no significant binding of AcH was found in human blood.

2. Materials and methods

In the in vivo part of the investigations different doses of ethanol (0.75 g/kg, 1.5 g/kg or 3.0 g/kg) were given intraperitoneally as a 15% (v/v) solution in saline to fed rats of both sexes of different rat strains (Wistar, Sprague-Dawley or Long-Evans). These ethanol doses gave AcH levels between 20 μM and 300 μM in the whole heparinized blood of the rats decapitated 15–40 min after the ethanol administration. The AcH concentrations were determined by head-space chromatography directly from the whole blood samples diluted with water, as previously described [3]. Part of the blood was kept on ice for up to 40 min and at intervals, plasma samples were obtained by 2 min centrifugation of the whole blood at 24°C and 1500 X g. The plasma and the remaining erythrocyte fraction AcH was measured in the same way as for the whole blood samples. Control experiments were performed in vitro, in which the total blood of decapitated fed rats of both sexes was added to AcH and ethanol in saline (containing heparin) to give the same initial whole blood AcH and ethanol concentrations (in about 100 parts blood and 1 part saline) as those in the in vivo experiments.

Male and female human blood was obtained from antecubital veins. The treatment of the blood was, however, complicated by the fact that hemolysed human blood in the presence of ethanol causes a strong non-enzymatic AcH formation. Also the normal procedure of ice-cold PCA precipitation followed by centrifugation results, at least in rats, in a loss of some of the bound AcH [3]. In preliminary experiments we found that the recovery of rat blood

AcH increased if the temperature of the precipitating PCA was raised, apparently because of a more complete liberation of the bound AcH. At 25°C practically all the bound AcH was released.
Thus in the human blood studies, which were similar in design to the rat experiments in vitro, we precipitated the blood, plasma and cell samples with 25°C PCA. The AcH was determined from the supernatants as described previously [3].

The AcH binding capacity of the rat blood was investigated by the addition of increasing AcH concentrations to rat blood from fed male and female Sprague-Dawley animals to final total AcH concentrations of 3–16 mM. Plasma was separated by centrifugation after 40 min incubation at 4°C and the AcH was measured from the whole blood, plasma and erythrocyte fraction diluted with water, as described previously [3]. The concentration of bound AcH in whole blood, i.e., $[AcH]_{whole\ blood} - [AcH]_{free}$, divided by the free AcH concentration, was plotted against the concentration of bound AcH in whole blood. The concentration of free AcH in whole blood was taken to be 0.88 $[AcH]_{plasma}$. This coefficient came from the calculation of the total amount of free AcH per volume of blood, considering the difference in the water volumes of the plasma and erythrocyte fraction. According to Scatchard [7], the intercept on the $[AcH]_{bound}$ axis estimates the theoretical maximum amount of bound AcH in whole blood.

In the last part of the investigations the ferric citrate (the only source of iron) was withdrawn from the salt mixture of the diet used for the rats (male Sprague-Dawley), in order to produce a gradual decrease of the blood hemoglobin concentration within 2 weeks. The blood hemoglobin concentrations were measured using the methods described by Hainline [8,9].

3. Results and discussion

3.1. *Occurrence of acetaldehyde binding*

The various AcH levels (20–300 μM) in the whole blood obtained in the in vivo part of the experiments were found to be steady during the first 40 min at 4°C after decapitation. The AcH concentration in the separated plasma, however, decreased with the time between decapitation and centrifugation (curve A in fig.1). The decrease was independent of sex, strain, the initial AcH concentration produced by the different ethanol doses and the duration of the ethanol oxidation (the data points in fig.1 are means of the pooled values). The decreased plasma AcH concentrations with time were also found to be matched by simultaneous increases in the AcH concentrations of the corresponding erythrocyte fractions. This indicated that the decrease of the plasma AcH was not due to any physical losses during the treatment but rather to binding in the erythrocyte fraction. The extrapolated time function of curve A in fig.1 gave a plasma AcH concentration of only 40% of the corresponding whole blood AcH concentration at zero time, i.e., the time of decapitation.

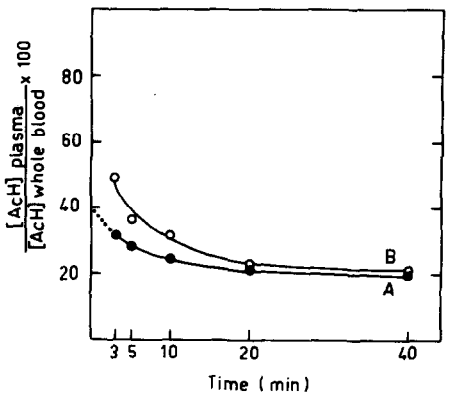

Fig.1. Relation between plasma and whole blood acetaldehyde concentration as a function of time. The time scale expresses the times before removing the plasma and (A) the decapitation of rats to which different ethanol doses had been given in vivo and (B) the in vitro addition of acetaldehyde and ethanol to blood of decapitated rats. For details see Materials and methods section. The values are the means of 14–15 rats (SD = 5–20%). The dotted line is the extrapolation of curve A to zero time.

Curve B of fig.1 shows the results of the experiment performed in vitro in order to control the consistency of the in vivo binding of AcH. Within 20 min the binding effect reached the same equilibrium state as in the in vivo studies, with the plasma AcH concentration being only 20% of the whole blood AcH concentration. A comparison of the curves obtained from the in vivo (A) and in vitro (B) experiments confirmed that the AcH in vivo (at the time of decapitation) was partly bound in the rat

blood. This follows since, if the shape of curve A would have only been the result of some very fast initial reaction instead of partial binding at time zero, the same reaction should have appeared in curve B.

An advantage with rat blood is that water-diluted blood, plasma or cell samples can be used directly for the head-space analyses and any bound AcH is liberated during the preheating prior to the analyses. With human blood the situation is more complex, because of the strong non-enzymatic AcH formation occurring in hemolysed human blood in the presence of ethanol [3]. This was not considered in two earlier studies [10,11], which probably resulted in erroneous conclusions about the binding of AcH in human blood. The present method using PCA precipitation also causes a slight non-enzymatic AcH formation dependent on the ethanol concentration [3]. Because ethanol was present in our samples, we obtained artificially higher AcH levels in whole blood and the erythrocyte fraction than in the corresponding plasma. The result was a steady $[AcH]_{plasma} \times 100/[AcH]_{whole\ blood}$ ratio of 75, which after correction for the 'spontaneous' formation became approximately 100. The time-dependent decrease in the plasma AcH concentration, caused by binding in the erythrocytes that was seen with the rat blood, could not be demonstrated with the human blood.

3.2. *Characterization of the acetaldehyde binding*

A Scatchard plot gave an estimated maximum binding capacity of 8.19 ± 0.92 μmol AcH/ml blood (mean ± SD, $n = 18$) for the rats. This extremely high capacity of the rat blood to bind AcH made us focus attention on the possible role of the hemoglobin in the binding effect. Accordingly, we withdrew the ferric citrate from the food given to the rats. This caused a gradual decrease of the blood hemoglobin concentration (from about 2.3–1.8 mM within 2 weeks) and a simultaneous decrease in the maximum capacity of the blood to bind AcH (fig.2). The significance ($p < 0.05$) of the correlation presented in fig.2 strongly supports the view that the blood hemoglobin is the protein responsible for the binding of the AcH. The molar ratio of the theoretical maximum concentration of bound AcH and the hemoglobin concentration was 4.06 ± 0.38, showing that each molecule of hemoglobin can bind at most 4 molecules of AcH. It is probably of significance

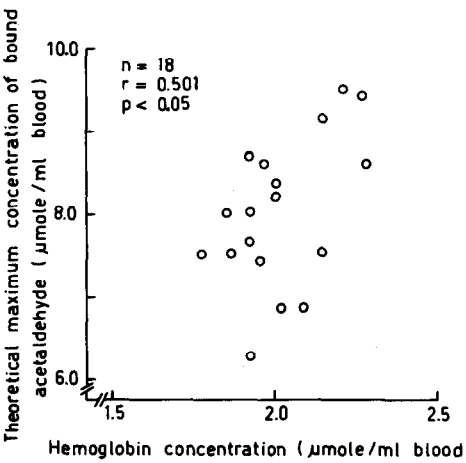

Fig.2. Correlation between the estimated theoretical maximum capacity of acetaldehyde binding and the hemoglobin concentration in rat blood. For details see Materials and methods section.

that native rat hemoglobin has 4 reactive sulphydryl groups/molecule [12]. Why the human blood, containing 2 reactive sulphydryl/hemoglobin molecule [12], did not bind any acetaldehyde could not be explained.

The physiological consequences of the AcH binding in rat blood might be important. The effect could, for example, partly explain why the brain AcH concentrations during ethanol oxidation in rats do not rise until very high arterial blood AcH concentrations are reached [13,14]. The demonstration of one type of AcH binding in rat blood raises the important possibility that other AcH bindings with potent pharmacological effects might occur in the brain or other tissues during and after the ethanol metabolism in vivo.

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