Biochemical Pharmacology, Vol. 18, pp. 29–33. Pergamon Press. 1969. Printed in Great Britain

INACTIVATION OF COENZYME A BY ETHANOL—I
ACETALDEHYDE AS MEDIATOR OF THE INACTIVATION OF COENZYME A FOLLOWING THE ADMINISTRATION OF ETHANOL *IN VIVO*

H. P. T. AMMON, C.-J. ESTLER and F. HEIM

Pharmakologisches Institut der Universität Erlangen-Nürnberg

(*Received* 18 *June* 1968; *accepted* 24 *July* 1968)

Abstract—Ethanol inactivates coenzyme A in liver and brain of white mice *in vivo*. In order to determine whether this effect is caused by ethanol itself or by acetaldehyde its metabolite the influence of ethanol and acetaldehyde on brain and liver was studied *in vitro*. Acetaldehyde lowered coenzyme A activity in homogenates of brain and liver. The concentrations required were 0·1–1·6 mM for brain and 1–32 mM for liver. On the other hand ethanol (20–360 mM) inactivated coenzyme A only in liver but not in brain homogenates. Since brain tissue is not able to oxidize ethanol in measurable quantities and, therefore, acetaldehyde is formed only in liver homogenates it is concluded that the action of ethanol on coenzyme A is mediated by acetaldehyde.

A DECREASE of the coenzyme A activity in brain and liver of white mice following an i.v. injection of alcohol has been reported in earlier papers from this laboratory.[1–4] In later experiments a short lasting inactivation of coenzyme A in the liver by acetaldehyde could be shown.[4] Since both alcohol and acetaldehyde inactivate coenzyme A *in vivo* the question arose whether the decrease in the coenzyme A activity following the administration of ethanol is caused by the alcohol itself or is mediated through acetaldehyde, which is an intermediate product of ethanol metabolism. Acetaldehyde is formed mainly in the liver but not in brain tissue. *In vivo*, however, it is distributed throughout the body via the blood stream and is found also in the brain. The effects of acetaldehyde and ethanol can be differentiated, however, by *in vitro* comparison of the effects of ethanol on two different organ systems, which are selected in that manner that only one of them possesses the ability to oxidize ethanol to acetaldehyde. Therefore we carried out experiments in homogenates of brain and liver tissue and investigated the influence of ethanol and acetaldehyde on coenzyme A activity.

METHODS

Male NMRI-mice, which were kept at 24° and fed with standard diet (Altromin R supplied by Altromin GmbH, Lage/Lippe, Germany) and tap water *ad libitum* were used in all experiments. Homogenates of 20% of brain or 10% of liver tissue in 7 mM phosphate buffer pH 7·4 were prepared at 0°. After adjustment to 37° the homogenates were incubated for 1 min with increasing amounts of ethanol or acetaldehyde as indicated in Figs. 2 and 3. At the end of the incubation period samples were

A preliminary report of the results has been presented at the 6th Spring Meeting of the Deutsche Pharmakologische Gesellschaft at Mainz, 1965.

taken and placed into a boiling water bath for 3 min and then into an ice bath for 10 min. After centrifugation at 15,000 rpm for 15 min coenzyme A activity in the supernatant was estimated according to the method of Kaplan and Lipmann[5] as modified by Tabor and coworkers.[6] This method is based on the formation of acetyl-CoA from Coenzyme A and acetate in the presence of ATP and glutathion and the transfer of the acetyl moiety from acetyl-coA to p-nitroanilin. Both reactions are catalized by a crude enzyme system from pigeon liver. Coenzyme A can participate in the reaction only when it is present in its SH-form ("active state").

For *in vivo* experiments 1·5 mg/g ethanol were injected i.v. to white mice. Part of the animals were pretreated with 0·5 mg/g disulfiram p.o. 30 min previously Coenzyme A activity in the liver was measured 60 min after the alcohol injection.

The values, which are depicted in the figures, are mean values calculated from 6–10 single estimations. The results were checked by means of Student's *t*-test. Differences between any value and its control value were regarded to be significant if $P \leqq 0{\cdot}05$.

RESULTS AND DISCUSSION

Fig. 1 shows the time course of blood alcohol levels and coenzyme A inactivation in brain and liver *in vivo* following the administration of 1·5 and 4·1 mg/g ethanol i.v.[1–3] As soon as 10 min after the alcohol injection the coenzyme A activity in brain and liver is lowered markedly. Minimum activity is found 10–60 min after the injection of 1·5 mg/g ethanol and 60–120 min after the injection of 4·1 mg/g ethanol. It appears

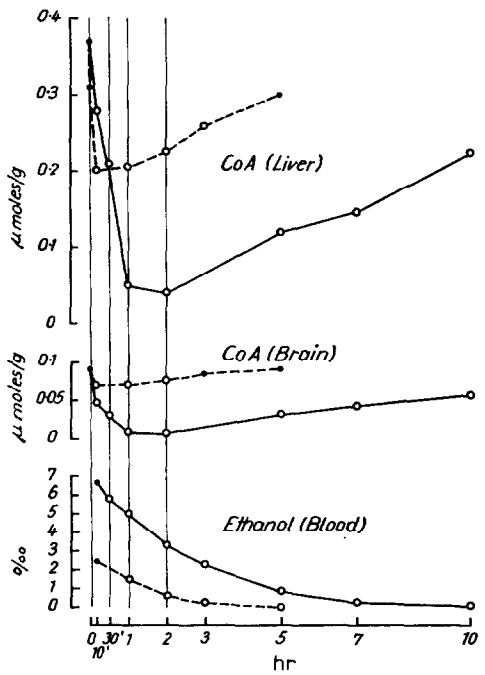

FIG. 1. Coenzyme A as measurable in Kaplan & Lipmann's test in liver and brain and blood alcohol levels in white mice after i.v. injection of —— 4·1 mg/g ethanol; – – – 1·5 mg/g ethanol; ○ = $P \leqq 0{\cdot}05$.

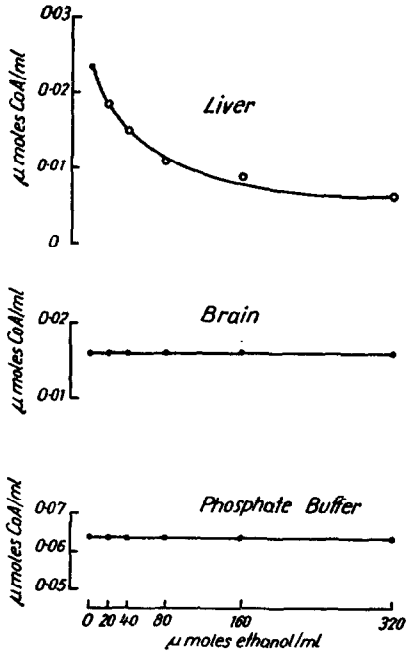

FIG. 2. Coenzyme A as measurable in Kaplan and Lipmann's test in liver and brain homogenates and in phosphate buffer (containing 0·063 μ mole co A-SH/ml) as function of the ethanol concentration in the medium. Co A was measured 1 min after the addition of ethanol. ○ = P ≦ 0·05.

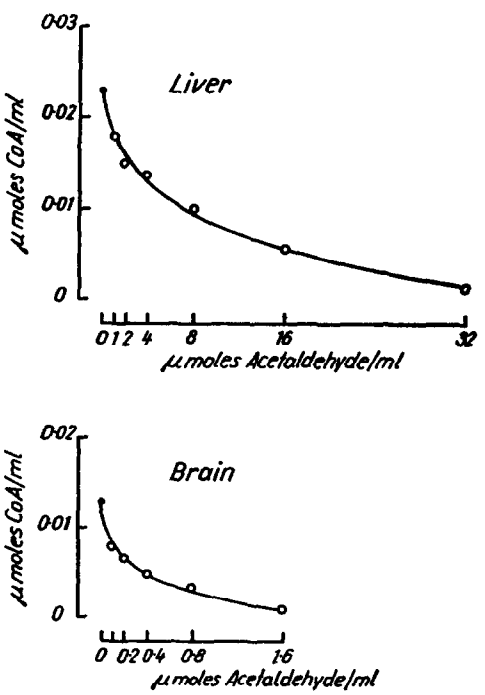

FIG. 3. Coenzyme A as measurable in Kaplan and Lipmann's test in liver and brain homogenates as function of the acetaldehyde concentration in the medium. Co A was estimated 1 min after addition of acetaldehyde. ○ = P ≦ 0·05.

that the coenzyme A activity is lowest at the same time when the rate of ethanol oxidation is highest, i.e. when the blood alcohol levels decline most rapidly. When the blood alcohol curve becomes flatter the coenzyme A activity tends to return to normal values. Thus the degree of coenzyme A inactivation seems to be dependent mostly on the amount of alcohol oxidized per time unit but not on the absolute amount of alcohol present in the blood.

This assumption is confirmed by *in vitro* experiments (Fig. 2). When increasing concentrations of ethanol are added to brain or liver homogenates the coenzyme A activity decreases only in liver homogenates whereas the coenzyme A activity of brain homogenates remains unchanged. In the same way alcohol has no effect on the activity of a solution of co A-SH* in phosphate buffer. Since only liver homogenates contain alcohol dehydrogenase, which is required for the dehydrogenation of ethanol, these experiments give further evidence that coenzyme A is not inactivated by alcohol itself but through the action of acetaldehyde, which can be formed only in the liver but not in brain tissue. In accordance with this view coenzyme A activity is lowered in brain homogenates as well as in liver homogenates when acetaldehyde is added instead of ethanol (Fig. 3). Therefore it may be concluded safely that the decrease of the coenzyme A activity in the brain *in vivo* is caused by acetaldehyde, which is formed in the liver and carried to the brain via the blood stream.

As shown in Fig. 3 in brain a 50 per cent inactivation of coenzyme A is achieved by as little at 0·2 μmole/ml acetaldehyde. According to Hulpieu *et al.*[7] in humans blood levels of 0·2 μmole/ml occur when blood alcohol levels are about 0·1 per cent. For the inactivation of coenzyme A in liver homogenates much higher concentrations of acetaldehyde are required. This may be due to the fact that in liver acetaldehyde is oxidized at a faster rate than in brain and that the acetaldehyde concentration in liver homogenates, therefore, declines more rapidly than in brain homogenates. On the other hand, the reason for the more marked decrease of the coenzyme A activity in the liver *in vivo* may be that probably the acetaldehyde concentration is higher in the liver where acetaldehyde is formed than in blood and brain.

The oxidation of acetaldehyde can be inhibited by disulfiram and higher levels of acetaldehyde in blood are found when disulfiram is given together with ethanol. In *in vivo* experiments disulfiram has no effect on the coenzyme A activity, but the inactivation of coenzyme A is more pronounced in animals treated with ethanol +

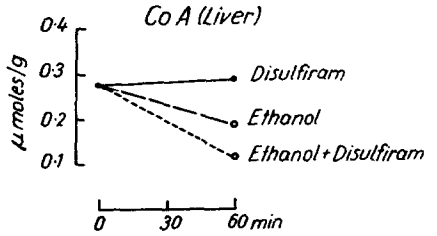

FIG. 4. Coenzyme A as measurable in Kaplan and Lipmann's test in the liver of white mice. —— 90 min after 0·5 mg/g disulfiram p.o.; — — 60 min after 1·5 mg/g ethanol i.v.; - - - - 60 min after 1·5 mg/g ethanol i.v. in animals treated with 0·5 mg/g disulfiram p.o. 30 min before alcohol injection; ○ = $P \leq 0{\cdot}05$.

* Co A-SH was a commercial product by Boehringer Mannheim GmbH, Mannheim, Germany.

disulfiram than in animals treated with ethanol alone (Fig. 4). This is another proof that acetaldehyde but not ethanol is responsible for the inactivation of coenzyme A that can be observed after the administration of ethanol to animals *in vivo*.

It is supposed that the mechanism of coenzyme A inactivation consists in the formation of a mercaptide or semimercaptide bond between acetaldehyde and the SH-group of coenzyme A whereby the active site of coenzyme A is blocked. Coenzyme A thus looses its transacetylating property, which is measured in Kaplan and Lipmann's test. This hypothesis is now subject to further investigation.

Acknowledgement—The authors thank Miss H. Darweger for technical assistence.

REFERENCES

1. H. P. T. Ammon, C.-J. Estler and F. Heim, *Archs int. Pharmacodyn. Thér.* **154**, 108 (1965).
2. H. P. T. Ammon, C.-J. Estler and F. Heim, *Archs int. Pharmacodyn. Thér.* **159**, 258 (1966).
3. F. Heim, H. P. T. Ammon, C.-J. Estler and D. Mikschiczek, *Med. Pharmac. exp.* **13**, 361 (1965).
4. H. P. T. Ammon, F. Heim, C.-J. Estler, G. Fickeis and M. Wagner, *Biochem. Pharmac.* **16**, 1953 (1967).
5. N. O. Kaplan and F. Lipmann, *J. biol. Chem.* **147**, 37 (1948).
6. H. Tabor, A. Mehler and E. Stadtmann, *J. biol. Chem.* **204**, 127 (1953).
7. H. R. Hulpieu, W. C. Clark and H. Onyett, unpublished data cited by R. B. Forney and R. N. Harger in J. R. DiPalma, *Drills Pharmacology in Medicine*, 3rd edn. McGraw-Hill, New York (1965).