Biochemical Pharmacology, Vol. 30, pp. 542–545.
© Pergamon Press Ltd. 1981. Printed in Great Britain.
0006-2952/81/050542-04 $02.00/0

Mucosal alcohol dehydrogenase- and aldehyde dehydrogenase-mediated ethanol oxidation in the digestive tract of the rat*

(*Received* 16 *June* 1980; *accepted* 24 *September* 1980)

Numerous tissues contain appreciable concentrations of ethanol metabolism enzymes [1–3]. Their nature and role has been the subject of much discussion and several hypotheses have been proposed: degradation of alcohols and aldehydes originating from the gut flora [4] or from endogenous sources [5–7] notably serotonine [8, 9]. The role of these enzymes with respect to ethanol has of course been studied particularly in the case of modifications in these enzymes seen during alcoholism and withdrawal [10, 11] and also during anti-alcohol treatment [12, 13]. It can therefore be assumed that the enzymes of alcohol metabolism in organs other than the liver also take part in alcohol catabolism: from this arises the problem of interference between ethanol oxidation and the normal processes dealt with by these enzymes [14]. An interesting example of this is the digestive tube: the gastric and intestinal mucosa contain high alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AldDH) activities [13]; furthermore they constitute sites of important serotonine synthesis and storage [15]; finally, and above all, they can be suddenly brought into contact with large quantities of alcohol crossing the cells during absorption. It can therefore be thought that as far as ethanol oxidation is concerned, the digestive mucosa have potentially a very important action owing to the high concentrations of substrate, the easy permeation of alcohol, the adequate enzyme apparatus and the abundant vascularization rapidly removing the degradation products.

The aim of the present work, carried out on the rat, is to demonstrate that the enzymes ADH and AldDH, located in the digestive mucosa, can really act on ethanol during its absorption, proving the existence of alcohol metabolism performed by the digestive mucosa. This metabolism was studied by measuring the levels of the metabolites appearing in the gastric and intestinal blood after intraluminal administration of ethanol.

Methods

All the experiments were carried out on male, Sprague–Dawley, O.F.A. strain rats (250 to 350 g) after overnight starvation.

Gastric ethanol catabolism evaluation. The animals were anaesthetized with sodium pentobarbital (50 mg/kg s.c.). The abdominal cavity was opened, the mesenteric vein and the duodenal branch of the pyloric vein ligated and the vena porta cannulated to collect blood arising entirely from the stomach. A pump was used to regulate the flow at 0.2 ml/min – a value which is compatible with the total normal gastric blood flow in the rat [16, 17]; the samples were collected over 1.5 ml of 0.5 M perchloric acid solution and 20 mM thiourea at 1° with constant stirring. After a control period of 3 min the pyloric sphincter was ligated and a dose of ethanol introduced through the oesophagus (25 mmoles in a volume of 5 ml/kg); the oesophagus was then itself ligated. The blood was collected in 3 min samples over a 15 min period and chilled for the subsequent operations. The samples were centrifuged for 15 min at 7000 *g* and after elimination of the pellet, the supernatants were neutralized with KOH before the measurements were undertaken.

Intestinal ethanol catabolism evaluation. The method used was identical to that above except for the surgical preparation where the left gastric and right gastro epiploic veins were ligated, the ligation of the mesenteric vein was placed in such a way as to maintain blood circulation in the arterio-venous duodenal loop and so to collect the efferent duodenal blood; the dose of ethanol was administered by an intraduodenal pyloric fistula.

Concentration measurements of ethanol and its metabolites. The level of ethanol was determined using the method of Bonninschen and Theorell [18], the concentration of acetaldehyde with that of Lundquist [19] and the level of acetate by Bergmeyer and Mollering's method [20].

Expression of the results. The values given in the figures and the tables are the means of the experimental results ± the standard error of the mean; comparisons were made with Students *t*-test.

Results

Experiment I. Figure 1 gives the concentrations of alcohol, acetaldehyde and acetate in the efferent gastric blood, after intragastric administration of 25 mmoles ethanol/kg b.w., for the various conditions of treatment.

The curves denoted 'A' were obtained after administration of ethanol alone: alcohol appears rapidly in the blood, its concentration becomes stabilized at values of approximately 20 mM; the level of acetaldehyde rose slightly but significantly (21 ± 3.9 to 44 ± 5.6 μM $P<0.01$) the concentration of acetate also rose to 300 μM ($P<0.001$). The curves denoted 'B' were obtained under the same conditions but one hour before the experiment, the animals received a dose 2.4 mmoles cyanamide/kg b.w.; large differences appear with respect to the preceding curves: a 6-fold increase was seen in the acetaldehyde concentrations whereas the acetate concentrations decreased (difference with respect to group 'A' $P<0.001$).

A third group of animals (curves C) received, 1 hour before experimentation, 1.5 mmoles pyrazole/kg b.w. Compared with the animals of group 'A', acetaldehyde gradation remained low whereas acetate production was slightly decreased ($P<0.05$).

After simultaneous administration of pyrazole and cyanamide in the same conditions as above (curves 'D') neither significant acetate production observed after ethanol nor the large increase in the level of acetaldehyde induced by cyanamide followed by the dose of ethanol was observed. So the high levels of acetaldehyde observed in group 'B', resulting from the blockage of AldDH, are due to the action of ADH on ethanol.

Experiment II. The results obtained with a second series of animals are given in Table 1. (The efferent intestinal blood was collected after intraduodenal administration of 25 mmoles ethanol/kg b.w.).

The results of the control group are comparable with those of the previous experiment except that there is a large increase in the concentrations of alcohol and also of

* This work was supported by grant C.R.L. no. 807018 from I.N.S.E.R.M.

Table 1. Variations of the concentrations of alcohol, acetaldehyde and acetate in the efferent intestinal blood before and 12 min after ethanol administration

	Variations of the concentrations after treatment with:			
	Initial concentrations	Ethanol alone	Cyanamide + ethanol	Pyrazole + cyanamide + ethanol
Alcohol (mM)	2.67 ± 3.542	59.67 ± 17.035	43.12 ± 4.295	52.37 ± 9.13
Acetaldehyde (μM)	17 ± 3.9	114 ± 18.8	251 ± 35*	100 ± 23.4
Acetate (μM)	228 ± 13.6	147 ± 80.4	−30 ± 32.1	7 ± 23.2

Student's *t* test: 5 animals per group.
* P<0.01 compared to the ethanol alone group.

acetaldehyde, at the same time, the rise in the acetate concentration is lesser.

Cynamide treatment also induces an increase in the level of acetaldehyde with respect to the control group (P< 0.01); acetate production however remains constant. The addition of both cyanamide and pyrazole treatments prevents the specific action of cyanamide on the level of acetaldehyde.

Control experiments. (a) The validity of the experiments and therefore the accuracy of the conclusions depends on whether the metabolites measured in the blood are produced in the mucosa and not in the blood itself. We checked this possibility by adding varying amounts of ethanol (up to 250 mM) to venous blood; after a one minute incubation the usual measurements were made following the above protocol. Spontaneous formation of acetaldehyde was observed to be in relation with the concentration of alcohol [21] but it remained low (20 μM). It was noticed that thiourea reduces this phenomenon and also decreases the yield; this inconvenience is partially removed by using, for the calculation of the concentrations, a standard which is also treated with thiourea. The difficulties met in accurately determining the level of acetaldehyde have been recently reviewed by Ericksson and it can be considered that under our working conditions (blood dilution roughly 1/5, maximal alcohol concentration about 30 mM, deproteinization of the blood on collection, working temperature 1°) interference phenomena are reduced to a minimum [22]. The low basal values can be affected but the high values found for the cyanamide alcohol association are only altered to a negligible extent; the data given in the tables therefore are not corrected from any spontaneous formation of acetaldehyde. Apart from this phenomenon, directly related to the method used, the incubations showed no formation of acetaldehyde or acetate arising from blood metabolism.

(b) In order to demonstrate the metabolic origin of the investigated substances it must be ascertained that under our experimental conditions neither alcohol nor acetaldehyde can reach the other organs where they could be degraded and introduce inaccuracy on recirculation of the metabolic products. In the stomach and intestine it is technically difficult to carry out successful once through perfusion *in situ* and it is for this reason that the described method was chosen. It was verified that the method filled the required conditions by carrying out, at the end of the experiment, an intracardiac puncture and measuring the concentrations of alcohol, acetaldehyde and acetate in the collected sample.

Table 2 gives the results obtained in the control experiment concerning the stomach: 12 min after the administration of ethanol the concentration of alcohol and its metabolites in the general circulation was seen to be indentical to that observed before administration whereas the concentrations in the efferent blood were seen to change as already described. With the method used for the intestine the results were seen to be comparable.

(c) Furthermore other checks were made to verify, *in vitro*, the ability of the mucosa to oxidize ethanol and acetaldehyde in experimental conditions comparable with our *in vivo* findings. In a previous study we characterized the presence of ADH and AldDH in the digestive mucosa [13]. Using conditions in which the activities have the maximal velocity (0.1 M pH 10.5 glycine buffer, 0.47 mM NAD [12]), 100,000 *g* supernatants obtained from the mucosa were incubated with ethanol (50 mM), acetaldehyde (50 μM) and acetate (300 μM) in concentrations of the same order of magnitude as those found in the blood leaving the stomach or the intestine after alcohol administration. The optical density measurements observed at 340 nm, were corrected with a blank incubated without substrate. The two enzymes, ADH and AldDH, are present in our incubation mixture and the increase in the optical density indicates, if they both operate, the sum of the two reactions.

Previous heating of the supernatants (10 min, 60°) completely inhibits the process. The presence of 4-methyl pyrazole (10 mM) causes an 85 per cent inhibition of the reaction. Semi carbazide (aldehyde acceptor 75 mM) or preincubation of the enzyme fraction with disulfiram (1 mM) inhibits the phenomenom by 20 per cent.

Table 2. Blood concentrations of alcohol, acetaldehyde and acetate at time zero and 12 min after ethanol administration in the general circulation and in the efferent gastric blood

	Time zero	12 min	
	Initial concentration	Final concentration in the general circulation	Final concentration in the gastric blood
Alcohol (mM)	0.99 ± 0.257	1.4 ± 0.62	14.6 ± 1.98*
Acetaldehyde (μM)	12 ± 3.4	15 ± 3.4	56 ± 3.5*
Acetate (μM)	185 ± 11.2	192 ± 15.7	285 ± 10.9*

Student's *t*-test: 4 animals per group.
* P<0.001 compared to the initial concentration.

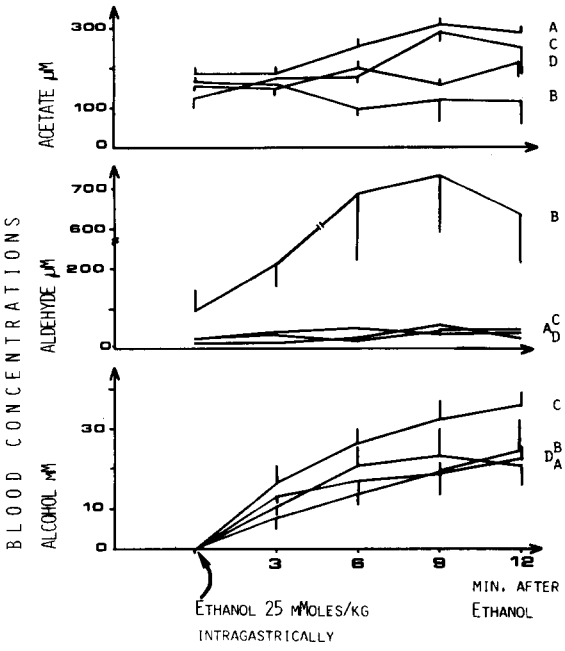

Fig. 1. Concentrations of alcohol, acetaldehyde and acetate in the gastric efferent blood after various treatments. (6 animals per group). (A) Ethanol alone (25 mmoles/kg b.w.). (B) Cyanamide (2.4 mmoles/kg b.w. 1 hr prior ethanol). (C) Pyrazole (1.5 mmoles/kg b.w. 1 hr prior ethanol). (D) Cyanamide (2.4 mmoles/kg b.w.) and pyrazole (1.5 mmoles/kg b.w.) 1 hr prior ethanol.

In the presence of ethanol, acetaldehyde and acetate, at the actual concentration found after intraluminal administration of ethanol, the use of inhibitors shows that the two enzymes in these conditions oxidize their substrate.

Discussion

The results obtained in the experiments show that the rise in the level of ethanol leaving the digestive tract after ethanol administration, is accompanied by the appearance of acetate: the metabolic link between ethanol and acetate is acetaldehyde; we can avoid interferences from microbial flora as shown by Carter and Isselbacher using germ-free animals [23] so the observations can be attributed to an action located in the mucosa. The alcohol concentration increase is higher for the intestine; this phemonenon is in agreement with the permeability differences of the barriers [24]: intestinal absorption is much faster than gastric absorption. As for acetaldehyde the initial concentrations are low—10 to 20 μM—near the limits of accuracy of the method and so can be considered negligible; the administration of ethanol induces a rise in acetaldehyde up to values comparable to those observed in the peripheral blood under similar conditions of administration [25–28]. The concentrations of acetate rise simultaneously with the level of alcohol.

Cyanamide is well known for its anti-alcohol properties: its action is thought to be linked to its capacity to inhibits AldDH [29, 30]. These properties have been demonstrated *in vivo* for the AldDH of the liver and the brain [31] and also for numerous other organs including the digestive mucosa [13]. In the present report, the treatment carried out with cyanamide increases the basal levels of acetaldehyde: indeed numerous aldehydes are oxidized throughout the body by AldDH [5–7], small quantities of ethanol could be produced by the gut flora [4] so the blockade of AldDH induces a relative accumulation of aldehydes. With these cyanamide-treated animals, ethanol leads to a very large increase, especially for the stomach, in the quantities of acetaldehyde leaving the digestive zone in the venous blood. This result, which is in agreement with previous data, indicates the possibility of an AldDH-dependent metabolic step.

In vivo pyrazole treatment causes, at the dose used, very strong inhibition of ADH [32]; under our conditions can be seen stagnation of the level of acetate and, above all, lowering of the strong acetaldehyde concentrations brought about by cyanamide; this confirms the role of ADH in the phenomenon.

The *in vitro* measurements, carried out in the presence of concentrations in the blood similar to those found *in vivo* which themselves reflect the cellular concentrations, show that an NAD-dependent oxidative phenomenon exists. The use of inhibitors also allows the role of ADH and AldDH to be defined in the metabolism.

The various checks carried out show firstly the impossibility of recirculation of the substances leaving the digestive tube by way of the veins and secondly the absence of any appreciable action of the blood on ethanol. So the presence of acetaldehyde and acetate in the efferent blood of the digestive tract on exposure of the mucosa to ethanol, is demonstrated to be the consequence of ethanol catabolism during its digestive absorption. The metabolism occurs through the enzyme couple ADH–AldDH which is present in the mucosa. The quantitative contribution of gastrointestinal ethanol oxidation to the ethanol metabolism of the whole body is small but our data show the early formation of acetaldehyde in tissues other than the liver.

In summary, ethanol oxidation was seen to occur in the stomach and intestine from the moment ethanol was absorbed leading to the appearance of alcohol metabolites in the portal blood. The use of pyrazole and cyanamide showed that this catabolism normally occurs via acetaldehyde which is then oxidized to acetate. These reactions are mediated by alcohol dehydrogenase and aldehyde dehydrogenase present in the mucous membrane.

Group de Recherches sur la Toxicologie des Aliments et des Boissons I.N.S.E.R.M. U-87
Institut de Physiologie
2, rue François Magendie
31400 *Toulouse, France*

Y. LAMBOEUF
G. DE SAINT BLANQUAT
R. DERACHE

REFERENCES

1. J. P. Von Wartburg in *The Biology of Alcoholism* (Eds B. Kissin and H. Begleiter) p. 63. Plenum Press, New York (1971).
2. V. G. Erwin and R. A. Deitrich, *Biochem. Pharmac.* **21**, 2915 (1972).
3. R. A. Deitrich, *Biochem. Pharmac.* **15**, 1911 (1966).
4. H. A. Krebs and J. R. Perkins, *Biochem. J.* **118**, 635 (1970).
5. R. D. Kissun, C. N. Graymore and P. J. Newhouse, *Expl Eye Res.* **14**, 150 (1972).
6. K. Okada and N. Takigawa, *Biochem. biophys. Acta.* **220**, 141 (1970).
7. R. Pietruszko and F. F. Chen, *Biochem. Pharmac.* **25**, 2721 (1976).
8. F. A. Seixas, *Ann. N.Y. Acad. Sci.* **215**, 89 (1973).
9. F. S. Messiha, *Pharmac. Biochem. Behav.* **9**, 379 (1978).
10. I. G. Fazekas and B. Rengei, *Enzymologia* **34**, 231 (1968).
11. Y. Lamboeuf, G. de Saint Blanquat and R. Derache, *Experientia* **36**, 286 (1980).

12. Y. Lamboeuf, G de Saint Blanquat and R. Derache, *Clin. exp. Pharmac. Physiol.* **1**, 361 (1974).
13. Y. Lamboeuf and G. de Saint Blanquat, *Archs. Int. Pharmcodyn. Ther.* **243**, 17 (1980).
14. R. A. Deitrich, *Psychoneuroendocrinology* **1**, 325 (1976).
15. J. H. Thompson, *Res. Commun. Path. Pharmac.* **2**, 687 (1971).
16. O. Özturkcan, G. de Saint Blanquat and R. Derache, *J. Physiol. Paris* **64**, 355 (1972).
17. E. D. Jacobson, *Gastroenterology* **54**, 434 (1968).
18. R. K. Bonninschen and H. Theorell, *Scand. J. Clin. Lab. Invest.* **3**, 58 (1951).
19. F. Lundquist, *Biochem. J.* **68**, 172 (1958).
20. H. U. Bergmeyer and H. Möllering, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer) p. 1520. Academic Press, New York (1974).
21. H. W. Sippel, *Acta Chem. Scand.* **27**, 541 (1973).
22. C. J. P. Eriksson, *Alcoholism Clin. exp. Res.* **4**, 22 (1980).
23. E. A. Carter and K. J. Isselbacher, *Proc. Soc. exp. Biol. Med.* **138**, 817 (1971).
24. H. Wallgreen, in *International Encyclopedia of Pharmacology and Therapeutics* **20**, (Ed. J. Tremolieres) p. 161. Pergamon Press, London (1970).
25. B. Lichtenstein and D. Winne, *Naunyn-Schmiedberg's Arch. Pharmac.* **279**, 153 (1973).
26. D. R. Petersen, A. C. Collins and R. A. Deitrich, *J. Pharmac. exp. Ther.* **201**, 471 (1977).
27. H. Peterson and K. H. Kiessling, *Biochem. Pharmac.* **26**, 237 (1977).
28. O. Tottmar, H. Marchner and N. Karlson, *Br. J. Nutr.* **39**, 957 (1960).
29. H. Marchner and O. Tottmar, *Acta Pharmac. Tox.* **39**, 331 (1976).
30. T. M. Kitson and K,. E. Crow, *Biochem. Pharmac.* **28**, 2551 (1979).
31. R. A. Deitrich, P. A. Troxell, N. S. Worth and V. G. Erwin, *Biochem. Pharmac.* **25**, 2733 (1976).
32. U. Rydberg, *Opusc. Med.* **26**, 1 (1972).

Biochemical Pharmacology, Vol. 30, pp. 545–547.
© Pergamon Press Ltd. 1981. Printed in Great Britain.
0006-2952/81/050545-03 $02.00/0

Effect of chronic administration of phencyclidine on hepatic mixed-function oxidases in the mouse

(*Received* 25 *April* 1980; *accepted* 15 *August* 1980)

Phencyclidine (PCP), a dissociative anesthetic, is known to be a potent psychotomimetic agent that resembles its pharmacologic analog, ketamine [1]. The recent literature suggests that PCP is one of the major drugs of abuse and also is used chronically [2, 3]. Phencyclidine is commonly sold in a mixture with other drugs of abuse [4, 5]. If the administration of PCP caused an alteration in the rate of metabolism of the compound that is mixed with PCP, both diagnosis and treatment of overdose cases would be complicated. For example, the acute administration of PCP has been reported to enhance both ethanol and hexobarbital sleeping time in the mouse [6]. On the other hand, significant shortening of hexobarbital [7] or pentobarbital [8] sleeping time was observed after chronic treatment of PCP. Radzialowski and Oppermann [7] further demonstrated that treatment of male rats for 4 days with PCP (50 $mg \cdot kg^{-1} \cdot day^{-1}$, i.p.) caused significant increases in hexobarbital, aminopyrine, and zoxazolamine metabolism *in vitro*. It has also been reported, however, that chronic administration of PCP (1 mg/kg) for 6 days failed to change cholesterol metabolism in rats [9]. In view of these findings, we systematically studied the effect of chronic administration of PCP on hepatic mixed-function oxidase systems.

Male ICR mice (30–36 g), obtained from the Charles River Breeding Laboratories (Wilmington, MA), were used. Three groups of fifteen mice each were treated as follows: group 1 (control) received saline daily for 7 days followed by Saline twice daily for 7 more days; group 2 (low dose, 1 week) received saline daily for 7 days, followed by the daily administration of PCP (40 mg/kg, i.p.) in the morning and saline in the evening for 7 days; group 3 (low dose, 1 week, then high dose, 1 week) received PCP (40 mg/kg, i.p.) daily for 7 days, followed by two daily doses of PCP (40 mg/kg, i.p.) for 7 more days. Body weight of each animal was monitored every day for the entire period of treatment.

All animals were decapitated 24 hr after the last injection. The livers were removed, weighed, and transferred to ice-cold isotonic (1.15 M) KCl solution. Three livers were pooled and assayed. All subsequent preparative procedures were carried out at 0–4°. Livers were homogenized in 3 vol. of KCl solution using a Potter–Elvehjem homogenizer (10 strokes). The homogenate was centrifuged successively to remove nuclear and mitochondrial fractions. Part of the resultant 9000 *g* supernatant fraction was centrifuged at 105,000 *g* for 60 min to obtain the microsomal pellet, which was resuspended in the original volume of KCl solution.

Each individual sample was assayed in duplicate incubations. Aniline [10], pentobarbital, and hexobarbital [11] hydroxylase activities, and aminopyrine [12] and ethylmorphine [10] demethylase activities were measured using the 9000 *g* supernatant fractions. Concentrations of cytochromes b_5 and P-450 were determined according to Omura and Sato [13]. NADPH dehydrogenase [14] and NADPH-cytochrome *c* reductase [15] activities were assayed using procedures described previously. Protein was measured