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INHIBITION OF RAT OVARIAN ORNITHINE DECARBOXYLASE BY ETHANOL IN VIVO AND IN VITRO

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Summary

Intragastric administration of ethanol greatly inhibited ovarian ornithine decarboxylase (L-ornithine carboxy-lyase EC 4.1.1.17) stimulated by human chorionic gonadotropin in vivo The inhibition occurred only if the treatment with ethanol was started before the injection of hormone.

The use of inhibitors for alcohol dehydrogenase and aldehyde dehydrogenase clearly showed that the observed inhibition was a direct effect of ethanol itself.

When rat ovarian cells were incubated in vitro with human chorionic gonadotropin the activity of ornithine decarboxylase was also markedly stimulated This stimulation could also be inhibited by ethanol. Moreover, actinomycin D and α-amanitin inhibited the stimulation of ornithine decarboxylase, showing that the enhanced activity in vitro resulted from the synthesis of new mRNA for ornithine decarboxylase.

The time dependence of the inhibition caused by ethanol addition resembled that after addition of actinomycin D. This supports the view that one site where ethanol inhibits protein synthesis is at the transcriptional level.

Introduction

It has been shown that a moderate (2 g/kg) dose of ethanol inhibited the dramatic increase in the activity of ornithine decarboxylase, occurring after partial hepatectomy in rats, by 50—75% [1,2]. Similar findings have been reported in other experimental systems, like isolated liver cells [3], heart [4] and brain [5] tissues. Because the induction of ornithine decarboxylase after

partial hepatectomy is based on the synthesis of new protein [6], we have suggested that ornithine decarboxylase could be used as a specific marker of protein synthesis.

Partial hepatectomy, however, causes stress in rats and the effect of ethanol treatment can thus be partially due to some secondary effects. To avoid complications due to surgery we tested the effect of ethanol on the activity of ornithine decarboxylase in ovaries where its activity could be stimulated by exogenous hormones, like chorionic gonadotropin and luteinizing hormone, both in vivo [7] and in vitro [8]. Since ethanol is not oxidized in ovarian tissue, it is suitable for use in testing for the effective molecule in the inhibition of ornithine decarboxylase synthesis

Materials and Methods

Chemicals DL[1-^{14}C]Ornithine (spec. act. 53 mCi/mmol) was the product of Radiochemical Centre (Amersham, Bucks., U.K). Medium 199 (M199) and lactalbumin hydrolysate were obtained from Gibco. Bovine serum albumin, which was dialyzed overnight against 25 mM Tris-HCl buffer (pH 7.4) before use, and deoxyribonuclease were from Sigma Chemical Co. (St. Louis, MI). Collagenase was the product of Worthington Biochemical Corp. (Freehold, NJ). Antinomyocin D and α-amanitin were from Boehringer (Mannheim, F.R.G.) 4-Methylpyrazole was from Labkemi AB (Stockholm, Sweden) and cyanamide (Dipsan®) was obtained from Lederle (Montreal, Canada). Ethanol (grade A) was the product of Alko (Helsinki, Finland) and acetaldehyde was from BDH Chemicals Ltd. (Poole, U.K.). Human chorionic gonadotropin (Pregnyl®) which was dissolved in physiological saline was obtained from Organon.

Animals and treatments. Female rats 2 months of age and weighing 206 ± 23 g, from the mixed strain [9] bred at the Alko Laboratories (Helsinki, Finland) were used for the in vivo experiments. Immature female rats of age 28—31 days from the same strain were used for the preparation of ovarian cells for in vitro experiments.

In in vivo experiments ethanol was given by gastric intubation either 1 h before (3 g/kg; 15% (w/v) solution in water) and 2 h after (2 g/kg) human chorionic gonadotropin, or 2 h after (5 g/kg) human chorionic gonadotropin only. All the rats were killed by decapitation 4 h after the intraperitoneal administration of human chorionic gonadotropin (25 I U./rat). When 4-methylpyrazole (0.2 mmol/kg, intraperitoneal) and cyanamide (1 mg/kg, post-operative) were used they were given 15 min and 2 h before the first dose of ethanol, respectively. In experiments with 4-methylpyrazole ethanol doses were 2 g/kg and 1 g/kg. Samples for blood ethanol and acetaldehyde determinations were taken 5 min before death.

Experiment in vitro. The cells were obtained with collagenase treatment exactly as described earlier [8] for in vitro experiments. In vitro ovarian cells were incubated at 37°C in the air/CO_2 atmosphere for 6 h as described in 8. Samples for determinations of the concentrations of ethanol, lactate and pyruvate were taken 5 min before the end of the incubation.

Analytical methods. The activity of ornithine decarboxylase [10], concentration of proteins [11], concentrations of lactate and pyruvate [12] and that

of ethanol and acetaldehyde [13] were analyzed by the cited methods. Bovine plasma albumin was used as standard in protein determinations. Statistical differences were calculated with the Student's *t*-test.

Results

Effect of ethanol on ovarian ornithine decarboxylase in vivo As shown in Tables I and II, 4 h after a single injection of human chorionic gonadotropin ovarian ornithine decarboxylase was stimulated 13—17-fold. The intragastric intubation of ethanol 5 h (i.e., 1 h before the hormone) and 2 h before death (i.e., 2 h after the hormone) inhibitited the human chorionic gonadotropin-induced stimulation in the activity of ornithine decarboxylase about 70% ($P <$ 0 01). When ethanol was given only 2 h before death there was no inhibition of ornithine decarboxylase.

Table I shows that a combination of ethanol plus 4-methylpyrazole (an inhibitor of alcohol dehydrogenase; [14]) prevented the appearance of acetaldehyde in the tail-blood of the animals, while the inhibition of ornithine decarboxylase was similar to that seen after ethanol alone. When ethanol was given together with cyanamide (an inhibitor of aldehyde dehydrogenase; [15]) the accumulation of acetaldehyde was very obvious (Table II) and the inhibition of ornithine decarboxylase was somewhat less than after ethanol alone. Results from Tables I and II suggested that ethanol itself is most probably the inhibitory molecule for ornithine decarboxylase synthesis in ovarian tissue.

Effect of ethanol and acetaldehyde on ovarian ornithine decarboxylase in vitro Fig. 1 shows that the addition of ethanol to a system where ornithine decarboxylase can be stimulated in vitro [8] inhibited the activity of ornithine decarboxylase in a dose-dependent manner. The presence of ethanol in the incubation did not change the lactate-to-pyruvate ratio (8.2 in controls and 8.4 in incubations with 75 mM ethanol) 6 h after the addition of ethanol, and also the decrease in ethanol concentration which most probably was due to evapora-

TABLE I

EFFECT OF ETHANOL (EtOH), 4-METHYLPYRAZOLE (4-MePyr) OR ETHANOL PLUS 4-METHYLPYRAZOLE ON HUMAN CHORIONIC GONADOTROPIN (hCG)-INDUCED INCREASE IN THE ACTIVITY OF RAT OVARIAN ORNITHINE DECARBOXYLASE (ODC) IN VIVO

The rats were treated as described in Materials and Methods There were five animals in each group The results are means ± S D

Treatment	ODC activity (nmol/mg protein per 30 min)	Blood ethanol concentration (mmol/l)	Blood acetaldehyde (μmol/l)
Saline	2 0 ± 1 2 **	—	—
hCG	34 4 ± 10 7	—	—
hCG + EtOH 5 h and 2 h before death	10 3 ± 1 7 *	85 0 ± 12 3	3 4 ± 6 6
before death hCG + 4-MePyr	28 5 ± 6 0	129 1 ± 7 9	2 8 ± 4 2
hCG + 4-MePyr	28 5 ± 8 1	—	—
hCG + 4-MePyr + EtOH 5 h and 2 h before death	9 9 ± 4 6 **	91 9 ± 19 4	not detectable

* $P < 0$ 01

** $P < 0$ 001 when compared to group receiving human chorionic gonadotropin alone

TABLE II

EFFECT OF ETHANOL (EtOH), CALCIUM CYANAMIDE (CaNCN) OR ETHANOL PLUS CALCIUM CYANAMIDE ON HUMAN CHORIONIC GONADOTROPIN (hCG)-INDUCED INCREASE IN THE ACTIVITY OF RAT OVARIAN ORNITHINE DECARBOXYLASE (ODC) IN VIVO

The rats were treated as described in Materials and Methods Other details are as in Table I

Treatment	ODC activity (nmol/mg protein per 30 min)	Blood ethanol concentration (mmol/l)	Blood acetaldehyde (μmol/l)
Saline	1 7 ± 1 1 **	—	—
hCG	21 8 ± 6 8	—	—
hCG + EtOH 5 h and 2 h before death	6 1 ± 5 1 *	94 3 ± 6 9	1 8 ± 3 4
hCG + CaNCN	19 3 ± 4 3	—	—
hCG + CaNCN + EtOH 5 h and 2 h before death	8 2 ± 3 9 *	104 0 ± 14 1	724 ± 284 **

* $P < 0.01$

** $P < 0.001$ when compared to the group receiving human chorionic gonadotropin alone

tion was exactly the same as in control incubations without cells (about 20%). These results show that ethanol is not metabolized in this system. Furthermore, the addition of ethanol (50 mM) together with 4-methylpyrazole (0.2 mM) caused similar inhibition to ethanol alone, which means that the observed inhibitions are a direct effect of the ethanol molecule. Addition of acetaldehyde up to a concentration of 0.5 mM, which is higher than ever seen in vivo during ethanol oxidation [13], together with cyanamide (1 mg/l) did not inhibit the stimulated activity of ornithine decarboxylase (Fig. 1). When acetaldehyde concentration was elevated as high as 5 mM it totally prevented the

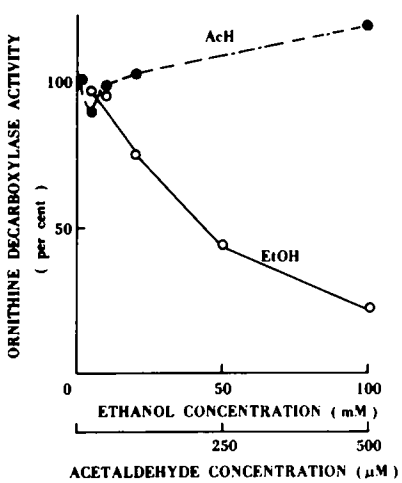

Fig 1. Effect of increasing concentration of ethanol (EtOH) and that of acetaldehyde (AcH) on human chorionic gonadotropin-induced stimulation of ovarian ornithine decarboxylase in vitro. Ethanol (10—100 mM concentration) was added alone and acetaldehyde (5—500 μM) with calcium cyanamide (1 mg/l) were added in the beginning of the incubation. Incubations were made in duplicates. The amount of human chorionic gonadotropin was 200 I.U /dish The cells for the experiment were obtained from 50 rats The activity of ornithine decarboxylase is expressed as %of control • — — •, acetaldehyde, ○———○, ethanol

stimulation of ornithine decarboxylase. Acetate up to a concentration of 5 mM did not affect the stimulation of ornithine decarboxylase after human chorionic gonadotropin treatment and 20 mM acetate, which is a much higher concentration that that seen in the liver during ethanol oxidation in vivo [16], partially prevented the rise in the activity of ornithine decarboxylase (44%).

Time dependence of the inhibition of ornithine decarboxylase by addition of ethanol in vitro. To test if the stimulation of ornithine decarboxylase by human chorionic gonadotropin was associated with the synthesis of new protein, actinomycin D and α-amanitin were used to inhibit the formation of mRNA for ornithine decarboxylase. It was found that α-amanitin (20 μg/ml) added at the same time as the hormone partially (79%) prevented the stimulation of ornithine decarboxylase after human chorionic gonadotropin in vitro. Fig. 2A shows that actinomycin D also completely inhibited the stimulatory effect of human chorionic gonadotropin when added simultaneously with the hormone or 2 h later. These results show that the synthesis of ornithine decarboxylase is preceded by the synthesis of mRNA for ornithine decarboxylase. When actinomycin D was added 4 h after the hormone the activity of ornithine decarboxylase did not markedly change from the stimulated control value, and addition 5 h after the hormone led to the superinduction of ornithine decarboxylase.

Fig. 2B shows that ethanol (50 mM) added during the period when actinomycin D was inhibitory (the possible time for the synthesis of mRNA for ornithine decarboxylase) partly (55—70%) inhibited the stimulation of ornithine decarboxylase. The addition of ethanol 4 h after human chorionic gonado-

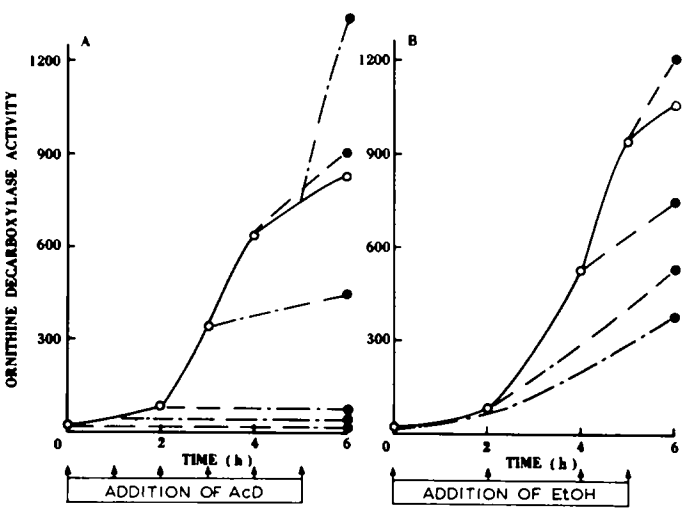

Fig 2 Effect of actinomycin D (AcD) (A) and ethanol (EtOH) (B) on human chorionic gonadotropin-induced stimulation of ovarian ornithine decarboxylase in vitro AcD (10 μg/ml) and EtOH (50 mM) were added at the time points indicated after the administration of human chorionic gonadotropin Incubations were made in duplicates (A) or in triplicates (B) and the values given are the means. The cells for the experiments were obtained from 100 rats The activity of ornithine decarboxylase is expressed as pmol of CO_2 produced by 10^8 cells per 30 min Other details are as in Fig 1 (A) ○———○, human chorionic gonadotropin, ● — — ●, human chorionic gonadotropin + actinomycin D, 0, 1, 2, 3, 4 and 5 h, respectively (B) ○———○, human chorionic gonadotropin, ● — — ●, human chorionic gonadotropin + ethanol, 0, 2, 4 and 5 h, respectively

tropin still inhibited ornithine decarboxylase a little, but 1 h before the end of the incubation the addition of ethanol even led to a small superinduction (Fig. 2B). Thus, the effect of ethanol resembled the action of actinomycin D on the activity of ornithine decarboxylase, giving further support to our suggestion of transcriptional inhibition of the synthesis of ornithine decarboxylase by ethanol [1].

Discussion

The effect of time dependence of ethanol administration on the activity of ovarian ornithine decarboxylase was similar to that seen in regenerating rat liver [1]. It appears that ethanol has to be given either befor or very soon after the stimulus for ornithine decarboxylase (e.g., partial hepatectomy or hormone), during an interval which in regenerating rat liver is approximately contemporaneous with the period during which mRNA for ornithine decarboxylase is synthetized [17,18]. Our results are also in agreement with previous results suggesting that ethanol itself is most probably the inhibitory molecule for ornithine decarboxylase synthesis in regenerating rat liver [1,2], rat kidney [1,2] and rat stomach amd small intestine [19]. The direct effect of ethanol on the synthesis of ovarian ornithine decarboxylase was also observed in vitro (Figs. 1 and 2). This findings is in striking contrast to that reported earlier [3] where ethanol oxidation was needed in vitro to inhibit the induction of ornithine decarboxylase by glucagon in isolated liver cells. One reason for this discrepancy could be the dramatic change in the redox state in vitro in systems which oxidize ethanol [20,21] and this abnormal change in the redox state could have an effect on protein synthesis not seen in vivo [20]. Another possibility is the accumulation of acetate in vitro since, we observed also that 20 mM acetate appeared to be inhibitory for the synthesis of ornithine decarboxylase in ovarian cells in vitro as in isolated liver cells [3]

As mentioned earlier the effect of ethanol on the activity of ornithine decarboxylase resembled the action of actinomycin D suggesting that ethanol may interfere with the synthesis of mRNA for ornithine decarboxylase. The reason for the observed inhibition could be at the nuclear level since we have shown that ethanol inhibits RNA synthesis in the beginning of the liver regeneration (Poso, H and Poso, A.R., unpublished data) Similarly ethanol inhibited the activity of RNA polymerase I and II in vitro when the activities of polymerases were assayed using purified nuclei as the source of the enzymes (Pöso, H. and Poso, A R , unpublished data). From these results and those discussed above, it is obvious that ethanol may inhibit protein synthesis at more than one site

Ethanol also caused a small superinduction of ornithine decarboxylase like actinomycin D (Fig. 2). Superinduction of this enzyme has been reported both in vivo [22] and in vitro [23,24]. The reason for the superinduction is not known [25,26] but it has been suggested that there is a protein inhibitor for ornithine decarboxylase 'antizyme'; [27], and if its synthesis is more sensitive for transcriptional inhibition than ornithine decarboxylase itself this situation could lead to the superinduction.

Our results, as far as we know, are the first to show that ethanol inhibits the

synthesis of ornithine decarboxylase (and protein synthesis) in reproductive organs Interestingly, it has been shown that rat ovarian ornithine decarboxylase flutuates during estrus cycle with a maximum immediately before ovulation [7]. Inhibition of rat ovarian ornithine decarboxylase has been reported to prevent ovulation (cited in Ref. 28). Specific inhibition of ornithine decarboxylase by nonphysiological diamines which are indirect inhibitors of ornithine decarboxylase [17,29], also partially prevented the rise in ovarian progesterone level seen after human chorionic gonadotropin treatment [30]. Furthermore, partial inhibition of ornithine decarboxylase during early embryogenic development in mice terminated pregnancy in all cases [31]. This phenomenon was partly reversed by a drug that inhibited putrescine catabolism, which indicates that putrescine, the end-product of the reaction catalyzed by ornithine decarboxylase, is essential for normal pregnancy [32]. Ethanol consumption also produced disturbances in ovarian function in rat [33]. These include histological changes and more importantly significantly lower progesterone levels in the alcohol-fed animals than in controls. Alcohol also disturbs female sexual function and fetal growth and development [34], and it is tempting to speculate that these deleterious effects seen after ethanol ingestion could in part be mediated via the inhibition of ornithine decarboxylase leading to a reduction of putrescine in reproductive organs. The inhibition of ornithine decarboxylase may thus represent a new mechanism for detrimental effects of ethanol in reproductive organs, and in embryos a mechanism for detrimental action of alcohol. This new mechanism differs from that of alcoholic liver injury, where continuous presence of ethanol is necessary [35].

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References

1 Poso, A R and Poso, H (1980) Biochim Biophys Acta 606, 338—346
2 Pösö, H. and Poso, A R (1980) Biochem. Pharmacol 29, 2799—2803
3 Lumeng, L (1979) Biochim. Biophys Acta 587, 556—566
4 Rettig, G., Gilson, K and Harris, P (1979) J Mol Cell Cardiol 11, 127—135
5 Thadani, P V , Lau, C , Slotkin, T.A and Schanberg, S M (1977) Biochem Pharmacol 26, 523—527
6 Obenrader, M F and Prouty, W F (1977) J Biol Chem 252, 2866—2872
7 Kobayashi, Y , Kupelian, J and Maudsley, D V (1971) Science 172, 379—380
8 Pilk, K , Poso, H and Janne, J (1979) Endocrine Res Commun 6, 107—121
9 Eriksson, K , Halkka, O , Lokki, J and Saura, A (1976) Heredity 37, 341—349
10 Janne, J and Williams-Asham, H G (1971) J Biol Chem. 246, 1725—1732
11 Lowry, O H , Rosebrough, N J , Farr, A L and Randall, R J (1951) J Biol Chem 193, 265—275
12 Bergmeyer, H U (1975) Methods of Enzymatic Analysis, 2nd edn , Academic Press, New York
13 Eriksson, C J P , Sippel, H.W and Forsander, O A (1977) Anal Biochem 80, 116—124
14 Li, T -K and Theorell, H (1969) Acta Chem Scand 23, 892—902
15 Marchner, H and Tottmar, O (1976) Acta Pharmacol Toxicol 39, 331—343
16 Crouse, J.R., Gerson, C D , DeCarli, L M and Lieber, C S (1968) J Lipid Res 9, 509—512
17 Janne, J , Pösö, H and Raina, A (1978) Biochim Biophys. Acta 473, 241—293
18 Russell, D H and Snyder, S H (1969) Mol Pharmacol 5, 253—262
19 Poso, H (1980) Med Biol 58, 117—122

20 Baraona, E , Pikkarainen, P , Salaspuro, M , Finkelman, F and Lieber, C S (1980) Gastroenterology 79, 104—111
21 Pikkarainen, P , Baraona, E , Finkelman, F and Lieber, C S (1979) Gastroenterology 77, A 32
22 Beck, W T., Bellantone, R A and Canellakis, E S (1973) Nature 241, 275—277
23 Clark, J L (1974) Biochemistry 13, 4668—4674
24 Heby, O and Emanuelsson, H (1980) Biochem Biophys Res Commun 92, 75—79
25 Stemberg, R A., Levinson, B B and Tomkins, G M (1975) Cell 5, 29—35
26 Kenney, F.T., Lee, K -L , Stilles, C D and Fritz, J E (1973) Nature 246, 208—210
27 Heller, J , Fong, W and Canellakis, E S (1976) Proc Natl. Acad Sci U S A 73, 1858—1862
28 Veldhuis, J.D. and Hammond, J M. (1979) Biochem Biophys Res Commun 91, 770—777
29 Poso, H (1977) Acta Chem. Scand B 32, 71—76
30 Guha, S K and Janne, J (1977) Biochem Biophys Res Commun 75, 136—142
31 Fozard, J R , Part, M -J , Prakash, N J , Grove, J , Schehter, P J , Sjoerdsma, A and Koch-Weser, J (1980) Science 208, 505—508
32 Fozard, J R , Grove, J , Part, M L and Prakash, N J (1979) Br J Pharmacol 66, 436P—437P
33 Van Thiel, D.H , Gavaler, J S and Lester, R (1978) J Clin. Invest 61, 624—632
34 Van Thiel, D.H and Lester, R (1979) Clin. Endocrinol. Metabol 8, 419—510
35 Pikkarainen, P., Lindros, K., Sipponen, P , Pekkanen, L , Lamminsivu, U Salaspuro, M (1979) Gastroenterology 76, 1295