Vol. 112, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 29, 1983 Pages 361-365

ETHANOL POTENTIATES THE INHIBITORY EFFECT OF D-GALACTOSAMINE ON PROTEIN SYNTHESIS IN ISOLATED HEPATOCYTES

Jozsef Mandl[X], Tamas Garzo, Ferenc Antoni

Ist Institute of Biochemistry, Semmelweis University Medical School, Budapest H-1444 P.O.Box 260, Hungary

Received March 9, 1983

The effect of the combined addition of D-galactosamine and ethanol on hepatic protein synthesis was studied in isolated mouse hepatocytes. 2.5 mM D-galactosamine or 40 mM ethanol alone caused slight or no inhibition of amino acid incorporation into proteins. However, a profound inhibition (about 80%) was observed if D-galactosamine of the same dose was added after a preincubation of the cells with 40 mM ethanol and vice versa. It shows that there is a strong mutual potentiation between D-galactosamine and ethanol in the inhibition of protein synthesis.

Ethanol enhances the damaging effect of several hepatotoxic agents (1). D-galactosamine induces specific hepatic injury (for review see 2), data concerning the combined action of ethanol and galactosamine in the liver are relatively scarce (3,4). Both ethanol and galactosamine are damaging the membrane of the hepatocytes and at the same time they are known to inhibit the hepatic protein synthesis.

The inhibition of protein synthesis by galactosamine in vivo has been reported by Reutter et al (5), in liver slices by Meszaros et al (6) and in isolated hepatocytes by Hofmann et al (7) and Mandl et al (8). Similar effect of ethanol has been found in vivo (9), in liver slices (10) and in isolated hepatocytes (11).

The aim of our present work was to investigate the combined action of ethanol and galactosamine on protein synthesis in isolated hepatocytes. It was found that galactosamine potentiated the effect of ethanol and vice versa on protein synthesis inhibition.

[X]Present address: Boston Biomedical Research Institute
20 Staniford Street
Boston, Massachusetts 02114

0006-291X/83 $1.50

MATERIALS AND METHODS

Hepatocytes were isolated from CFLP mice (20-25 g, fed ad libitum), according to the method of Berry and Friend (12) and Krebs et al (13) as detailed earlier (8). The isolated cells (5 x 10^6 cells/ml) were incubated in Krebs-Henseleit bicarbonate buffer (14) containing 8.5 mM glucose, 5 mM lactate, amino acids necessary for protein synthesis (1 mM each) except valine, 40 ug/ml streptomycin, 20 IU/ml penicillin and 1.0% albumin. The cell suspension was stirred by vigorous, constant bubbling with O_2:CO_2 (95:5, v/v) at 37°C. Viability of isolated hepatocytes checked by the trypan blue exclusion test was about 90%.

Protein synthesis of isolated hepatocytes was examined by the measurement of the incorporation of [^{14}C]-labeled valine (sp. act. 65 TBq/mole) into hot trichloroacetic acid insoluble fraction of the cells as described previously (8). The results were expressed as cpm/ug DNA. The DNA content of the cells was measured by the method of Burton (15).

Collagenase type II (129 U/mg) was purchased from Worthington Biochem. Corp.(Freehold, N.J.), D-galactosamine from Calbiochem AG (Lucerne).

RESULTS AND DISCUSSION

In our earlier experiments it has been shown that 10 mM D-galactosamine inhibits amino acid incorporation into proteins by 67% (8). The inhibition induced by ethanol on protein synthesis was investigated in the presence of 40 mM and 80 mM ethanol. These concentrations were in the range of blood ethanol levels measured in mice after oral administration (1).

40 mM ethanol did not cause significant inhibition of amino acid incorporation into proteins of isolated hepatocytes, whereas 80 mM ethanol inhibited amino acid incorporation by approximately 40% (Table 1). The mild

TABLE I

Effect of ethanol on amino acid incorporation into proteins of isolated hepatocytes.

concentration of ethanol (mM)	incubation time (min)	amino acid incorporation (% ± S.E.M.)
40	60	79.7 ± 8.1
40	120	72.0 ±14.1
80	60	60.0 ± 9.4
80	120	52.3 ± 5.3

Control: 100%. Number of observations : 4.

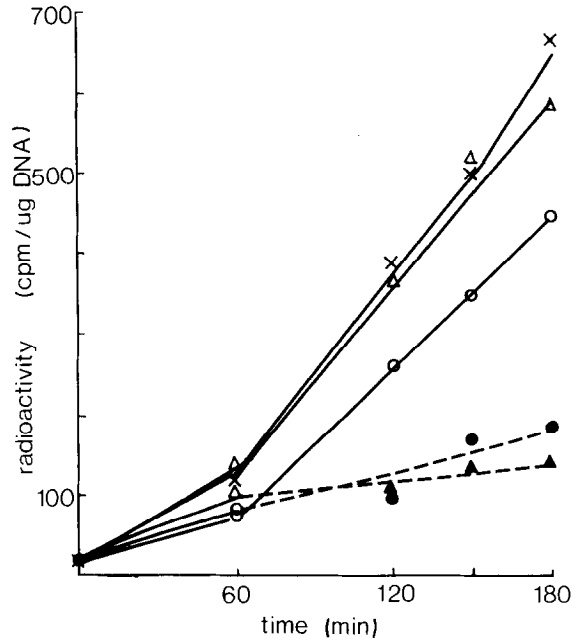

Fig.1: Combined effect of ethanol and D-galactosamine on protein synthesis in isolated hepatocytes. Incorporation of [^{14}C]-valine (9.25 KBq/ml) into proteins was measured as described in Materials and Methods. x——x control; o——o 2.5 mM D-galactosamine.HCl; Δ——Δ 40 mM ethanol; o——o---● 2.5 mM D-galactosamine.HCl + 40 mM ethanol added at 60. min of the incubation time; Δ——Δ---▲ 40 mM ethanol + 2.5 mM D-galactosamine.HCl added at 60. min of the incubation time.

inhibitory effect of 40 mM ethanol on protein synthesis in isolated hepatocytes is in accordance with the data of Dich and Tonnesen (16), while a more profound inhibition (about 60%) has been reported by Morland et al (11,17).

To demonstrate the combined effect of galactosamine and ethanol on protein synthesis 40 mM ethanol was added and a smaller dose of galactosamine (2.5 mM instead of 10 mM) was chosen. The hepatocytes were first incubated in the presence of 40 mM ethanol or 2.5 mM D-galactosamine for 60 min. Then one-half of the samples was complemented with the other agent (2.5 mM galactosamine or 40 mM ethanol, respectively); the remaining part of the samples was regarded as control. Fig. 1 shows that 40 mM ethanol alone did not decrease the amino acid incorporation into proteins, while a small inhibitory effect of 2.5 mM D-galactosamine was found. However, the combined administration of these agents resulted in a marked inhibition (79.4% ± 2.9%,

mean ± S.E.M. n = 8) of amino acid incorporation independent on the sequence of their administration.

Besides inhibiting protein synthesis in indirect ways (for review see 18) there are evidences that ethanol may exert its effect directly on the protein synthesizing machinery, as well. Ethanol administration leads to a disaggregation of membrane bound polysomes (19,20). The direct effect of galactosamine addition on protein synthesis at the translational level has been investigated in our laboratory in cell free systems (21) and in isolated hepatocytes (8,22). It has been evidenced that in these cases the formation of aminoglycogen (a polysaccharide of basic character) which causes aggregation of ribosomes and endoplasmic membranes (21,23) is the main reason of the inhibition of protein synthesis by galactosamine (8,22).

Our experimental results concerning the potentiating effect of ethanol are in agreement with the data of Schanne et al (4) obtained in primary cultures of adult rat hepatocytes in a long term culture. The potentiation by galactosamine of ethanol induced cell death (as determined by Trypan blue exclusion test) has been dependent on calcium. The calcium requirement is thought to be related to the membrane damaging effect of the two molecules (24). However, the potentiating effects on protein synthesis inhibition observed in our experiments were independent of the concentration of calcium ions in the culture medium (data not shown).

Chronic liver injury caused by ethanol commences with a long "asymptomatic" period, which is converted suddenly to a state with the pronounced signs of developing necrosis (4). Isolated hepatocytes are supposed to be very useful tools in studying this conversion (4). We observed the potentiating effect of ethanol on galactosamine induced inhibition of protein synthesis in short term experiments (180 min). Therefore, we think that the protein synthesizing machinery of the hepatocytes might be one of the first targets of ethanol, and the potentiating effect of galactosamine is one of the first signs of the developing cell injury.

ACKNOWLEDGEMENTS

Thanks are due to Mrs Gizella Ferencz for skillful technical assistance. This work was supported by the Ministry of Health, Hungary (1-56-0306-01-0G).

REFERENCES

1. Strubelt, O. (1980) Biochem. Pharmacol. 29, 1445-1449.
2. Decker, K. and Keppler, D. (1974) Rev. Biochem. Pharmacol. 71, 77-106.
3. Strubelt, O., Obermeier, F. and Siegers, C.P. (1978) Acta Pharmac. (Kbh) 43, 211-218.
4. Schanne, F.A.X., Zucker, A.H., Farber, J.L. and Rubin, E. (1981) Science 212, 338-340.
5. Reutter, W., Keppler, D., Lesch, R. and Decker,K. (1969) Verh. Deutsch. Ges. Inn. Med. 75, 363.
6. Meszaros, K., Antoni, F., Hrabak, A., Szikla, Ch., Garzo, T., Tompa, A. and Lapis, K. (1973) FEBS Lett. 35, 1-3.
7. Hofmann, S.R., Wagle, S.R. and Decker, K. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 1395-1400.
8. Mandl, J., Garzo, T., Meszaros, K. and Antoni, F. (1979) Biochim. Biophys. Acta 586, 560-567.
9. Princen, J.M.G., Mol-Backx, G.P. and Yap, S.H. (1981) Biochim. Biophys. Acta 655, 119-127.
10. Perin, A., Scalabrino, G., Sessa, A. and Arnaboldi, A. (1974) Biochim. Biophys. Acta 366, 101-108.
11. Morland, J. and Bessessen, A. (1977) Biochim.Biophys. Acta 474, 312-320.
12. Berry, M.N. and Friend, D.S. (1969) J. Cell Biol. 43, 506-520.
13. Krebs, H.A., Cornell, N.W., Lund, P. and Hems, R. (1974) in Alfred Benson Symposium VI (Lundquist, F. and Tygstrup, N. eds.) pp. 726-750, Munksgaard, Copenhagen.
14. Krebs, H.A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33-66.
15. Burton, K. (1956) Biochem. J. 62, 315-323.
16. Dich, J. and Tonnesen, I.C. (1980) Arch. Biochem. Biophys. 204, 640-647.
17. Morland, J., Rotschild, M.A., Oratz, M., Mongelli, J., Donor, D. and Schreiber, S.S. (1981) Gastroenterology 80, 159-165.
18. Comporti, M. (1981) in Biochemical Mechanisms of Liver Injury (Slater, T.F. ed.) pp. 469-516, Academic Press, London, New York and San Francisco.
19. Oratz, M., Rotschild, M.A. and Schreiber, S.S. (1976) Gastroenterology 71, 123-127.
20. Oratz, M., Rotschild, M.A. and Schreiber, S.S. (1978) Gastroenterology 74, 672-676.
21. Meszaros, K., Mandl, J., Garzo, T. and Antoni, F. (1976) FEBS Lett. 71, 215-219.
22. Mandl,J., Meszaros, K., Antoni, F. and Garzo, T. (1982) Mol. Cell. Biochem. 46, 25-30.
23. Meszaros, K., Antoni, F., Mandl, J., Hrabak, A. and Garzo, T. (1974) FEBS Lett. 44, 141-145
24. Schanne, F.A.X., Kane, A.B., Young, E.E. and Farber, J.L. (1979) Science 206, 700-702.