PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 226-229 (1978)

Interaction of Ethanol and Thyroxine on Hepatic Oxygen Consumption[1] (40320)

SANT P. SINGH AND ANN K. SNYDER

Medical Research Service, Veterans Administration Hospital and the Department of Medicine, Chicago Medical School, North Chicago, Illinois 60064

Chronic feeding of ethanol to rats has been shown to stimulate respiration by liver slices through an increase in conversion of ATP to ADP by the (Na + K)-ATPase system (1, 2). The calorigenic effect of thyroid hormones also involves stimulation of (Na + K)-ATPase (3). However, some studies have suggested that the availability of mitochondrial substrate and not ADP may determine the rate of respiration and that thyroxine (T_4) enhances the availability of the substrate for mitochondrial oxidation (4).

The present study was done to investigate interrelationship between the effects of chronic ethanol ingestion and T_4 treatment on O_2 consumption by rat liver slices and isolated mitochondria. Further, the influence of the available oxidizable substrate for the ethanol and T_4 effects on respiration of rat liver slices was studied.

Materials and Methods. Thirty-two Sprague–Dawley male rats weighing 150 to 200 g were divided equally into four groups at random. Group A received tap water and group B received 20% (v/v) ethanol as the only drinking solution *ad libitum.* Group C was rendered thyrotoxic by daily ip injection of l-T_4 (150 μg/100 g body wt) for 14 days. Group D received 20% (v/v) ethanol as drinking solution and T_4 treatment as outlined for group C. All animals were housed in individual cages, fed regular Purina Chow *ad libitum,* and weighed at regular intervals. Animals in group D lost considerable weight (see Table I) and appeared sick, although none died. In eight relatively young rats, average weight 100 g, a 25% mortality rate was observed during 20% ethanol + T_4 treatment and therefore present studies involved relatively larger animals.

After 14 days the animals were fasted for 18 hr and then sacrificed by decapitation. Blood was collected for the estimation of serum T_4 levels (5). Livers were removed and placed immediately in ice-cold oxygenated medium containing 135 m*M* NaCl, 5 m*M* KH_2PO_4, 0.5 m*M* $MgCl_2$, 5 m*M* Tris–HCl, and 10 m*M* glucose, pH 7.4. Liver slices 0.5 mm thick were prepared and their respiration was determined in a Warburg apparatus (Precision Scientific). Each Warburg flask contained approximately 60 mg of tissue in 3 ml of the oxygenated medium mentioned above. Respiration was measured for three consecutive 30-min periods. Thereafter 50 μl of 0.4 *M* succinate was added to the medium from the side arm to give a final concentration of 7 m*M* and respiration of the liver slices was estimated for three additional 10-min periods.

To determine oxygen consumption of isolated mitochondria instead of liver slices, mitochondria were isolated from the same livers according to the technique of Johnson and Lardy (6). An aliquot, 0.05 ml, of the mitochondrial suspension was placed in a Warburg flask containing 3 ml of incubation medium which contained 62.5 m*M* sucrose, 185.5 m*M* mannitol, 10 m*M* KCl, 10 m*M* Tris–HCl, pH 7.4, 5 m*M* K_2HPO_4, 5 m*M* $MgCl_2$, 0.2 m*M* EDTA, 7 m*M* succinate, and 83.3 μ*M* ADP. Respiration was measured for three consecutive 10-min periods.

Respiration estimations for liver slices and mitochondria were done in triplicate for each liver. The protein content of the liver slices and of each mitochondrial suspension was determined by the Lowry method (7). The data were expressed as microliters of O_2 consumed per minute per milligram of protein and statistically analyzed by Student's *t* test.

Results. Table I shows mean ± SEM values of body weight and serum thyroxine levels in rats receiving ethanol, thyroxine, or a combination of these two substances (n = 8 for each group of animals). Rats that received

[1] This work was supported by the Medical Research Service, Veterans Administrationand was presented, in part, at the American Physiological Society 28th Annual Fall Meeting, October 1977, Hollywood, Florida.

0037-9727/78/1592-0226$01.00/0

TABLE I. EFFECT OF ETHANOL INGESTION ON BODY WEIGHTS AND SERUM T_4 LEVELS OF NORMAL AND THYROXINE-TREATED RATS.

Treatment	Initial body weight (g)	Final body weight (g)	Difference	Serum thyroxine (μg%)
A. Saline	166 ± 8	238 ± 8	72 ± 6	4.6 ± 0.4
B. Ethanol	179 ± 8	194 ± 9	15 ± 4*	3.9 ± 0.3
C. T_4	176 ± 10	229 ± 12	53 ± 8	16.6 ± 2.4*
D. Ethanol + T_4	179 ± 7	132 ± 7	−47 ± 4*	14.1 ± 2.9*

* $p < 0.001$ compared to controls. (saline).

either ethanol or T_4 gained significantly less weight than controls ($p < 0.05$). Simultaneous treatment with ethanol and thyroxine produced a marked loss in body weight as compared to controls (176.2 ± 7 vs 127 ± 7; $p < 0.01$). Serum T_4 levels were significantly higher ($p < 0.01$) in T_4-treated animals and ethanol ingestion exerted no discernible effect on serum T_4 values.

Effect of ethanol on O_2 consumption by liver slices of euthyroid and thyrotoxic rats. Figure 1 shows that chronic ethanol ingestion decreased the rate of oxygen utilization from a control value of 0.098 ± 0.004 to 0.082 ± 0.004 μg of O_2/min/mg of protein in liver slices of euthyroid rats. The results were significant at the 2% level. Addition of succinate to liver slices produced a marked increase in O_2 consumption to 0.183 ± 0.01 and 0.243 ± 0.01 μl of O_2/min/mg of protein in controls and ethanol-treated animals, respectively. Furthermore, with succinate as oxidizable substrate, ethanol pretreatment produced an increase ($p < 0.001$) in the rate of respiration instead of a depression of respiration observed with glucose as the substrate.

Figure 2 shows that in thyrotoxic rat liver slices the O_2 consumption was 50% greater than in euthyroid rat liver slices ($p < 0.001$). Chronic ethanol ingestion decreased O_2 consumption by nearly 50% from 0.147 ± 0.004 to 0.071 ± 0.005 μl of O_2/min/mg of protein ($p < 0.001$) with glucose as substrate. Addition of succinate increased respiration of T_4-treated rat liver slices and chronic ethanol ingestion enhanced the rate of respiration further from 0.282 ± 0.015 to 0.367 ± 0.028 μl of O_2/min/mg of protein ($p < 0.02$).

Effect of ethanol and thyroxine on O_2 consumption of isolated mitochondria of rat liver. As shown in Fig. 3, mitochondria isolated from euthyroid rat liver showed no significant difference in respiration after chronic ethanol treatment as compared to control values.

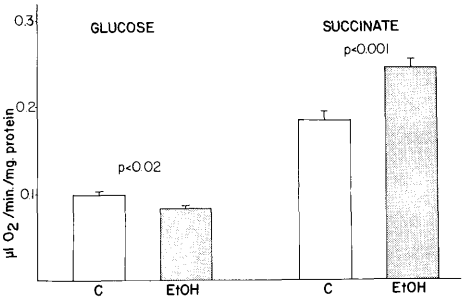

FIG. 1. Mean ± SEM (n = 8) oxygen consumption rate by liver slices of rats fed 20% ethanol as drinking solution or tap water (controls) for 14 days. The rate of O_2 consumption was estimated with liver slices in media containing glucose before and after the addition of succinate.

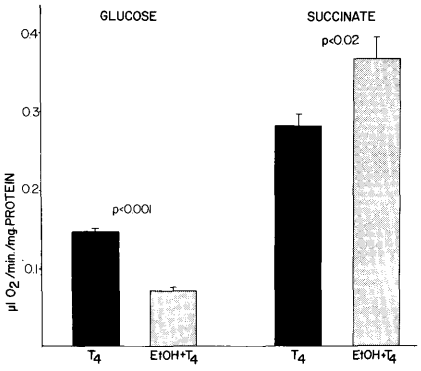

FIG. 2. Mean ± SEM (n = 8) oxygen consumption by liver slices of T_4-treated rats that received 20% ethanol or tap water *ad libitum* for 14 days. Oxygen estimation was done as described under Fig. 1 and T_4 injections were given as described in the text.

However, in mitochondria isolated from thyrotoxic rat livers it was observed that chronic ethanol treatment enhanced O_2 consumption significantly from 1.27 ± 0.032 to 1.57 ± 0.118 μl of O_2/min/mg of protein ($p < 0.05$).

Discussion. Previously it has been shown that daily ingestion of ethanol (35% caloriewise) for 21 to 27 days enhanced oxygen consumption by rat liver slices. The under-

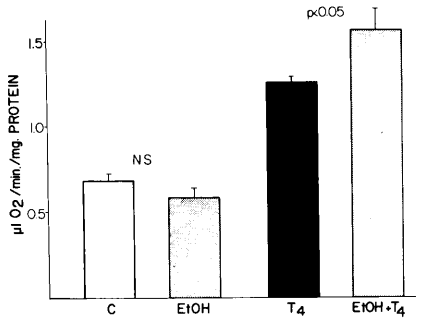

FIG. 3. Mean ± SEM (n = 8) oxygen consumption by mitochondria isolated from livers of euthyroid and T_4-treated rats. Both groups of euthyroid or T_4-treated rats were given 20% ethanol or tap water *ad libitum* for 14 days. Injections of T_4 were given as described in the text.

lying mechanism was reported to be an increased activity of the (Na + K)ATPase activity (1, 2). The present data derived from rats consuming relatively less ethanol (i.e., 20% (v/v) as drinking solution *ad libitum* for 14 days) show that the ethanol effect on respiration of rat liver slices is dependent on the available oxidizable substrate. The O_2 consumption by liver slices was increased in medium containing succinate as substrate but decreased when glucose was used instead of succinate.

Substrates can provide electrons to the respiratory chain at the beginning (the level of NADH dehydrogenase), at the middle (ubiquinone level), and at the terminus (cytochrome *c* level). Succinate which is flavin-linked provides electrons at the cytochrome *b*–ubiquinone segment and therefore bypasses energy coupling site I at the level of NADH dehydrogenase. The utilization of electrons from glucose is partly NAD-linked and thus involves energy coupling site I. The present results might be explained by an inhibitory effect of ethanol on coupling site I or on some steps prior to it. In fact, Cederbaum *et al.* (8) have shown that chronic ethanol ingestion (36% caloriewise) depresses mitochondrial respiration by damaging coupling site I.

Whereas ethanol enhanced O_2 consumption in rat liver slices incubated with succinate, it did not exhibit a similar effect when isolated mitochondria from the same livers were studied. Other studies have reported a depression of mitochondrial respiration by chronic ethanol ingestion and ascribed the effect to a damage to the respiratory chain. Furthermore, structural changes in mitochondria including swelling, disfiguration, disorientation of cristae, and intramitochondrial crystalline inclusion are observed after chronic ethanol treatment (9). Concomitant fat infiltration of hepatocytes has also been shown (10). In the present study rats ingested less ethanol, and any morphological changes in mitochondria, although not documented, were perhaps insufficient to depress respiration. In fact, the O_2 consumption of mitochondria isolated from thyrotoxic rat liver was enhanced by chronic ethanol ingestion. Therefore, it is unlikely that the respiratory chain was damaged by ethanol as fed to the rats in this study.

The calorigenic effect of thyroid hormones on liver is ascribed to an increase in ADP production due to stimulation of (Na + K)-ATPase activity (3). However, Primack and Buchanan (4) showed O_2 consumption of rat liver slices was greater with succinate than with glucose and suggested that the availability of oxidizable substrate rather than ADP controls the rate of O_2 consumption. The present data show that chronic ethanol treatment decreased O_2 consumption of thyrotoxic rat liver slices when glucose was available as substrate but a converse effect occurred when succinate was added. It seems that ethanol inhibition of energy coupling site I, as discussed above, was sufficient to block T_4 effect on liver slices respiration in a glucose-containing medium. On the other hand, the increase in succinate-supported respiration of the same liver slices might be related to increased (Na + K)-ATPase activity (1, 2).

It needs to be emphasized that rats in group D receiving ethanol + T_4 lost considerable weight and appeared sick. A decrease in food intake might have resulted in a limited supply of substrate for O_2 utilization. Thus, such a conceivable effect of malnutrition in addition to that of ethanol should be considered in interpretation of data derived from group D rats. Nonetheless, individual rat liver served its own control in terms of comparison of the rates of O_2 utilization during two different substrates, i.e., glucose and succinate.

Summary. Interrelationship between the

effects of chronic ethanol ingestion and T_4 treatment on O_2 consumption by rat liver slices and isolated mitochondria was investigated. The data showed that ethanol influence on O_2 consumption by liver slices was dependent on the available oxidizable substrate as it was decreased when estimated in media containing glucose but increased in media containing succinate as oxidizable substrate. The respiration of thyrotoxic rat liver slices was altered by ethanol in a manner similar to that observed with euthyroid rat liver slices. Whereas ethanol ingestion enhanced succinate-supported respiration of euthyroid and thyrotoxic rat liver slices, it produced a similar effect in isolated mitochondria of thyrotoxic rat livers but not of euthyroid rat livers.

The authors wish to thank Mrs. Ruth M. Bonovich for typing this manuscript.

1. Israel, Y., Videla, L., and Bernstein, J., Fed. Proc. **34,** 2052 (1975).
2. Israel, Y., Videla, L., MacDonald, A., and Bernstein, J., Biochem. J. **134,** 523 (1973).
3. Ismail-Beigi, F., and Edelman, S., Proc. Nat. Acad. Sci. USA **67,** 1071 (1970).
4. Primack, P. M., and Buchanan, J. L., Endocrinology **95,** 619 (1974).
5. T_4 RIA (PEG) Diagnostic Kit, Abbott Laboratories, North Chicago, Ill.
6. Johnson, D., and Lardy, H., *in* "Methods in Enzymology," Vol. 10, p. 94. Academic Press, New York (1967).
7. Lowry, O. H., Rosebrough, A. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
8. Cederbaum, A. I., Leiber, C. S., and Rubin, E., Arch. Biochem. Biophys. **165,** 560 (1974).
9. Leiber, C. S., and DeCarli, L. M., *in* "Metabolic Aspects of Alcoholism" (C. S. Leiber, Ed.), p. 31. Univ. Park Press, Baltimore (1977).
10. Gordon, E. R., J. Biol. Chem. **248,** 8271 (1973).

Received January 16, 1978. P.S.E.B.M. 1978, Vol. 159.