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# Effect of Glucagon on the Metabolism of Ethanol in Rat Liver

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Administration of low doses of glucagon resulted in a significantly increased rate of ethanol metabolism by rat liver slices. At high doses of glucagon this effect was not observed. *In vitro* addition of glucagon to the liver slices, did not alter the rate of ethanol metabolism. Glucagon administration resulted in an increased concentration ratio of lactate/pyruvate, at low as well as high doses of glucagon. Addition of ethanol to liver slices from glucagon treated animals resulted in a further increase in this ratio. The doses of glucagon which caused increased rate of ethanol metabolism by liver slices also resulted in a decrease in the concentration of plasma free fatty acids, while the doses of glucagon which resulted in an increase in the concentration of plasma free fatty acids, caused a small decrease in the rate of ethanol metabolism by rat liver slices. The possibility of an indirect effect of glucagon on ethanol metabolism at low doses, through the stimulation of insulin secretion is discussed.

The accelerating effect of insulin on the rate of alcohol oxidation has been explained [1] at least partly on the basis of the decrease in the concentration of plasma free fatty acids (FFA). The concentration of plasma FFA has been observed to increase significantly upon the administration of ethanol and the FFA have been observed to be inhibitors of hepatic alcohol and aldehyde dehydrogenases.[1] In view of the antagonistic effect of glucagon to insulin in several respects, it was considered desirable to investigate the effect of glucagon administration on the rate of ethanol oxidation by rat liver.

In the two step oxidation of ethanol to acetate one molecule of NADH is generated at each step and it has been suggested [2] that under some conditions, the limitation of ethanol oxidation may depend on the capacity of the liver to reoxidize the NADH formed.

In the present investigation an attempt was made to evaluate the accelerating effect of glucagon on the rate of ethanol metabolism, on the basis of changes produced by the hormone in the oxidation-reduction state of cytoplasmic redox pairs and the concentration of plasma FFA.

## MATERIAL AND METHODS

Fed male Wistar rats were bred in the laboratory and were maintained on water and laboratory chow *ad libitum*. Rats weighing 250–300 g were used.

The animals were anesthetized by intraperitoneal injection of Nembutal, 40 mg/kg. Artifical respiration was applied by a rodent respirator. The experimental animals were devided into two groups. To the one group 30 μg glucagon was infused in the first minute followed by 5 μg during the subsequent 30 min. The other group was given 200 μg followed by 30 μg given continuously. Blood samples were taken for plasma FFA estimation after 10, 20, and 30 min of glucagon infusion. Glucagon was dissolved in Krebs-bicarbonate buffer. After 30 min, the abdomen was opened, the liver removed and rapidly chilled in ice. The liver was blotted and slices 0.3 mm thick were cut at 4° by a McIlwain-Buddle tissue slicer. The slices (0.3–0.5 g per flask) were incubated for 60 min at 37° in 10 ml of Krebs-Henseleit bicarbonate buffer, pH 7.4. The flasks were gassed with $O_2+CO_2$ (95:5). Samples were removed at 15 min intervals and deproteinized with 6 % perchloric acid. The concentration of ethanol was estimated enzymatically.[3] The concentrations of lactate [4] and pyruvate [5] in the incubation medium were determined enzymatically after 60 min of incubation. Concentration of FFA was estimated titrimetrically.[6]

The enzymes and cofactors were obtained from Boehringer & Söhne (Mannheim, Germany). Insulin free glucagon (D.Sp. nr. 2514) was obtained from NOVO industri A/S, Copenhagen.

## RESULTS

*Rate of ethanol oxidation by liver slices.* As shown in Table 1, administration of low doses of glucagon (30 + 5 μg) to rats resulted in a significantly increased rate of ethanol oxidation by the liver slices. At higher doses of glucagon (200 + 30 μg) this increase in the rate of ethanol oxidation could not be observed;

*Table 1.* Effect of glucagon on the rate of ethanol oxidation in rat liver slices. The details of procedure are given in materials and methods. The results are expressed as average ± S.E.M. with the number of animals in parentheses.

| Treatment | Additions | Rate of ethanol oxidation (μmoles/g/h) | Lactate (μmoles/g/h) | Pyruvate (μmoles/g/h) | (Lactate)/(Pyruvate) |
|---|---|---|---|---|---|
| 1. Control | Nil | Nil | 1.40 ± 0.41 | 0.03 ± 0.01 | 46.6 |
| | Ethanol | 45.6 ± 4.2 (4) | 3.65 ± 0.50 | 0.05 ± 0.01 | 73.0 |
| 2. Glucagon (30 μg followed by 5 μg) | Nil | Nil | 2.00 ± 0.45 | 0.03 ± 0.01 | 66.6 |
| | Ethanol | 65.0 ± 6.5 (4) | 4.01 ± 0.50 | 0.05 ± 0.02 | 80.0 |
| 3. Glucagon (200 μg followed by 30 μg) | Nil | Nil | 2.20 ± 0.30 | 0.03 ± 0.01 | 73.0 |
| | Ethanol | 40.0 ± 4.8 (4) | 3.95 ± 0.56 | 0.04 ± 0.01 | 98.7 |
| 4. Control | Glucagon | Nil | 1.60 ± 0.41 | 0.03 ± 0.01 | 53.0 |
| | Ethanol + glucagon | 44.8 ± 4.3 (4) | 3.80 ± 0.50 | 0.05 ± 0.01 | 78.0 |

on the contrary a small decrease (N.S.) in the rate was obtained. Similarly no increase in the rate of ethanol oxidation was observed when glucagon (1.4 μM) was added *in vitro* to the liver slices.

*Effect of glucagon on the lactate/pyruvate ratio.* The concentration ratio of lactate/pyruvate was increased in the livers from glucagon treated animals as compared to control animals. Addition of ethanol increased this ratio in the livers from normal and glucagon treated animals. The changes in the concentration ratio of lactate/pyruvate after ethanol administration or after glucagon administration were mainly due to changes in the concentration of lactate (Table 1). Addition of glucagon, however, to the liver slices resulted in small alterations (N.S.) in the lactate/pyruvate ratio.

*Effect of glucagon on the concentration of plasma free fatty acids.* Low doses of glucagon (30 μg followed by 5 μg) after 10 min resulted in a fall in the concentration of plasma FFA. In the second case where 200 μg of glucagon was infused followed by 30 μg, an increase in the plasma FFA occurred in 10, 20, and 30 min blood samples (Table 2).

*Table 2.* Effect of glucagon administration on the concentration of plasma free acids. The plasma free fatty acids were determined titrimetrically, in the plasma from controls and from animals treated with two different doses of glucagon. Blood samples were taken for FFA estimation at 10 min intervals. The statistical treatment was the same as in Table 1.

| Treatment | Plasma free fatty acids, μequiv. $l^{-1}$ 10 min | 20 min | 30 min |
|---|---|---|---|
| 1. Control | 294 ± 34 (4) | 300 ± 32 (4) | 306 ± 35 (4) |
| 2. Glucagon (30 μg followed by 5 μg) | 217 ± 28 (4) | 210 ± 20 (4) | 203 ± 24 (4) |
| 3. Glucagon (200 μg followed by 30 μg) | 693 ± 48 (4) | 780 ± 60 (4) | 900 ± 73( 4) |

## DISCUSSION

Glucagon administration to dogs has been observed previously to cause a small but significant increase in the rate of blood alcohol disappearance.[7,8] However, these reports have been rather brief and attempts to explain the mechanism of action of the hormone on the observed effects were not made. A direct or indirect relationship between the alcohol and carbohydrate metabolism is indicated by the hypoglycaemic effect of alcohol in starved animals or human subjects [9,10] and a hyperglycemic effect in the fed experimental subjects.[11,12] The stimulatory effect of insulin on alcohol metabolism has also been given as evidence for an interrelation between alcohol and carbohydrate metabolism.

These findings, along with several other observations, *e.g. in vitro* inhibition of alcohol and aldehyde dehydrogenases by FFA, increased alcohol oxidation upon decreased FFA levels, produced upon insulin administration,[2] and

decreased alcohol oxidation upon increased plasma FFA concentrations as produced in conditions of starvation,[14] alloxan diabetes,[1] and antiinsulin serum treatment,[1] suggest that FFA may be important in the regulation of ethanol metabolism. The decline in plasma FFA induced by glucagon is similar to that caused by glucose and insulin.[15] Since the only direct effect of glucagon on adipose tissue so far demonstrated is stimulation of fatty acid release,[16] it is generally agreed that the depression of FFA levels observed *in vivo* is an indirect effect, presumably resulting from the insulin secretion. As regards alcohol metabolism low doses of glucagon act similar to insulin administration.

Furthermore two observations, namely the unaltered rate of ethanol oxidation upon glucagon addition to liver slices, and decreased rate of ethanol oxidation(N.S.) by livers from rats given high doses of glucagon which result in increased FFA level, suggest that the secretion of insulin was probably the reason for the stimulation of ethanol oxidation observed at low doses of glucagon.

A more reduced state of the cytoplasmic compartment, produced upon glucagon administration as evidenced by the increase in the lactate/pyruvate concentration ratio, suggests that the effect of the hormone on ethanol metabolism is not due to a facilitation of the reoxidation of NADH. It has previously been reported[13] that glucagon administration resulted in 1.4, 1.8, and 1.5 fold increase, respectively, in the concentration ratio of lactate/pyruvate, malate/oxaloacetate, and α-glycerophosphate/dihydroxyacetone phosphate in the perfused rat liver. Furthermore, though both low and high doses of glucagon resulted in a more reduced state of the cytoplasmic redox-pairs, only the low doses resulted in an increased rate of ethanol oxidation. These observations suggest that, perhaps, some other mechanism is responsible for the observed stimulatory effect of low doses of glucagon on the rate of ethanol metabolism. The observations recorded in Table 2, show that the doses of the hormone which result in a decrease in the concentration of plasma FFA also result in a stimulatory effect on the rate of ethanol oxidation, and the dose of the hormone which results in increased concentration of plasma FFA also results in a decrease in the rate of ethanol oxidation.

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## REFERENCES

1. Rawat, A. K. *European J. Biochem.* **9** (1969) 93.
2. Rawat, A. K. and Lundquist, F. *European J. Biochem.* **5** (1969) 17.
3. Lundquist, F. *Methods Biochem. Analy.* **7** (1959) 217.
4. Hohorst, H. J. In Bergmeyer, H. U. *Methoden der enzymatischen Analyse*, Verlag Chemie, Weinheim/Bergstr. 1962, p. 266.
5. Bücher, T., Czok, R., Lamprecht, W. and Latzko, E. In Bergmeyer, H. U. *Methoden der enzymatischen Analyse*, Verlag Chemie, Weinheim/Bergstr. 1962, p. 253.
6. Dole, V. P. and Meinertz, H. *J. Biol. Chem.* **235** (1960) 2595.
7. Nelson, D. and Jensen, C. E. *Federation Proc.* **20** (1961) 189.
8. Clark, W. C. and Owens, P. A. *Arch. Intern. Pharmacodyn.* **162** (1966) 255.
9. Lochner, A., Wulf, J. and Madison, L. L. *Metab. Clin. Exptl.* **16** (1967) 1.
10. Freinkel, N., Cohen, A. K., Arky, R. A. and Foster, A. E. *J. Clin. Endocrinol. Metab.* **25** (1965) 76.

11. Connor, C. L. *Arch. Pathol.* **30** (1940) 165.
12. Rawat, A. K. *European J. Biochem.* **6** (1968) 592.
13. Williamson, J. R. *Biochem. J.* **101** (1966) 11c.
14. Forsander, O. A., Räihä, N., Salaspuro, M. and Mäenpää, P. *Biochem. J.* **94** (1965) 259.
15. Dole, V. P. *J. Clin. Invest.* **35** (1956) 150.
16. Steinberg, D. M., Shafrir, E. and Vaughan, M. *Clin. Res.* **7** (1959) 250.

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