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Chronic and Acute Ethanol Impair the *in vivo* Glucose Uptake by Lactating Rat Mammary Gland

S. Vilaró, O. Viñas, E. Herrera[1] and X. Remesar[2]

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Chronic and acute ethanol treatments increased the 3-hydroxybutyrate uptake by lactating rat mammary gland as a consequence of its high afferent concentration, without changing its relative extraction. The uptake of glucose was inhibited in the ethanol treated animals due to intrinsic alterations in the mammary gland metabolism as indicated by the decreased relative extraction and unchanged afferent concentration. These results would suggest that the elevated uptake of ketone bodies in ethanol-treated rats can be responsible, at least in part, for the decrease in glucose uptake by lactating rat mammary gland, although other direct effects of ethanol may be implied.

INTRODUCTION

We have recently reported that chronic ethanol ingestion in lactating rats produces important metabolic disturbances (1) in addition to a reduction in milk production and changes in milk composition (2) and an altered amino acid uptake by mammary gland (3). Glucose is a major substrate for energy production, lipogenesis and lactose synthesis in the lactating rat mammary gland (4). Therefore, it is possible that abnormal milk composition in ethanol-treated rats is related to changes in glucose metabolism in the gland. It has been reported that glucose transport is a rate-limiting

Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, 08071 Barcelona, and [1]Servicio de Bioquímica, Departamento de Investigación, Centro Ramón y Cajal, 28034 Madrid, Spain.
[2] To whom correspondence should be addressed.

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factor in carbohydrate utilization by the mammary gland (5) and that this transport is also regulated by circulating ketone bodies in different physiological states (4,6). For these reasons we have studied the effects of both chronic and acute ethanol treatment on glucose and ketone body uptake by the lactating rat mammary gland by measuring their arteriovenous differences across this tissue.

MATERIALS AND METHODS

Female Wistar rats from our colony fed *ad libitum* purina chow diet (A04, Panlab, Barcelona, Spain) were used. *Chronic ethanol* was administered as previously described (1) diluted in the drinking water in increasing doses from four weeks before impregnation and attaining the highest doses (25% volume:volume) at the beginning of pregnancy that were maintained until the day of experiment (day 15 of lactation). Control animals were kept under the same conditions but drank tap water. At parturition all the litters were exchanged for litters of the same age from untreated mothers and adjusted to a number of 8 pups per litter. *Acute ethanol* was administered by oral intubation (40% weight:volume) without anesthesia, at a dose of 3 g/kg body weight to a group of 15-day lactating rats that had not been previously treated. Control rats were administered a saline solution (NaCl 0.9%). The arteriovenous differences were determined 2.5 hours after saline or ethanol administration.

Arteriovenous Determinations

Rats were anesthesized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). Arteriovenous differences were determined as described by Viña *et al.* (7). Blood was collected into heparinized syringes from the pudic epigastric vein and then from the aorta of the same rat. After blood deproteinization (8) glucose (9), acetoacetate and 3-hydroxybutyrate (10) were measured in the supernatants. Other blood aliquots were collected with thiourea (40 mM) and used for ethanol determination by a gas chromatographic method (11) (Perkin Elmer, UK). Relative extraction was calculated as follows:

$$ER = \frac{[\text{substrate in arteria}] - [\text{substrate in vein}]}{[\text{substrate in arteria}]} \times 100$$

Results are expressed as means ± SEM, and comparisons between groups were performed using Student's *t* test.

RESULTS AND DISCUSSION

Present findings show that ethanol treatment impaired glucose uptake by the lactating rat mammary gland. This effect was not caused by changes in the afferent blood concentration of glucose, but instead seemed to be related to intrinsic changes in the mammary gland metabolism as indicated by the reduced relative glucose

Table 1. Effects of chronic and acute ethanol treatment on arterial blood, arteriovenous (A-V) differences and relative extraction of glucose and ketone bodies by 15-day lactating rat mammary gland

	Acetoacetate nmols/ml	3-OH-butyrate nmols/ml	Glucose μmols/ml
CHRONIC			
Arterial blood			
Control	3.5 ± 0.9	132 ± 33	5.9 ± 0.4
Ethanol	4.2 ± 0.9	841 ± 4.7***	5.7 ± 0.2
A-V differences			
Control	2.9 ± 0.9	79 ± 16	3.3 ± 0.3
Ethanol	2.6 ± 0.8	517 ± 22***	2.4 ± 0.1*
% Relative extraction			
Control	83 ± 28	60 ± 13	55 ± 5.6
Ethanol	63 ± 20	62 ± 3	42 ± 2*
ACUTE			
Arterial blood			
Control	4.3 ± 0.4	62 ± 17	7.4 ± 0.3
Ethanol	2.1 ± 0.4**	173 ± 25**	7.0 ± 0.7
A-V differences			
Control	2.5 ± 0.3	49 ± 19	4.3 ± 0.3
Ethanol	1.4 ± 0.3*	116 ± 21*	1.9 ± 0.2***
% Relative extraction			
Control	58 ± 8	78 ± 30	58 ± 4
Ethanol	68 ± 12	67 ± 12	28 ± 3***

Significance versus controls: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Results are the means ± SEM of 6–8 animals.

extraction (Table 1). Chronically treated rats also showed a marked increase in both arterial concentrations and arteriovenous differences across the mammary gland of 3-hydroxybutyrate, although its relative extraction value was not affected. This fact indicated that 3-hydroxybutyrate was taken up more extensively in ethanol treated rats because of the increased afferent concentration, which agrees with the effects of ethanol in the increase of ketone body concentrations in blood (12,13). It is likely that these two changes, the inhibition of glucose uptake and the increase in ketone body uptake, are interrelated, since ketone bodies are known to decrease mammary gland glucose uptake (6); thus, in starvation, when ketone bodies in the blood are increased, it has been shown that glucose uptake by the gland is decreased (4). It is proposed that the observed changes are specifically produced by the ethanol treatment rather than as a consequence of the reduced food intake (in spite of a similar energetic intake: 520 ± 22 kcal/kg body weight in treated rats, and 540 ± 37 kcal/kg body weight in controls) which occurs in rats under chronic ethanol treatment (1) since it has been shown that other kinds of underfeeding fail to increase the circulating ketone body levels (14). This hypothesis is also supported by the present findings under acute ethanol treatment. In this condition, blood ethanol concentration reached similar, but lower, levels to those in chronically treated rats (20 ± 2 mM in acute vs 37 ± 2 mM in chronically treated rats) and produced essentially the same effects, that is reducing glucose and enhancing ketone body uptake by the lactating rat mammary gland. Nevertheless, the fact that the uptake of glucose was greated inhibited (54%) in the presence of lower increases of the 3-hydroxybutyrate levels (2.4 times) as when compared to chronic treated rats

(28% and 6.5 times respectively) (Table 1) suggests that, in addition to the inhibitory effect of ketone bodies on glucose uptake, other causes may be involved.

A possible explanation would be that the ethanol has affected the insulin levels, since this hormone is known to play a regulatory role in mammary gland glucose uptake and lipogenesis (4,15), but we have previously reported that rats under the same ethanol treatments showed unmodified circulating insulin levels (1,16). Other possibilities would involve a direct ethanol effect on membrane fluidity. Direct inhibitory effects of ethanol on basal and stimulated glucose uptake in adipocytes have been described previously (17) and disturbances at receptor and post-receptor insulin levels in hepatocytes have also been reported (18). If similar direct effects of ethanol are involved in the impairment of glucose uptake by mammary gland of treated rats, it remains to be established.

It is proposed that the impairment of mammary gland glucose uptake caused by ethanol intake could contribute to the alterations in milk composition previously found (2) in rats chronically treated with ethanol.

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REFERENCES

1. Viñas, O., Vilaró, S., Remesar, X. and Herrera, E. (1986). Effect of chronic ethanol ingestion on circulating metabolites and liver composition in the lactating rat. *Gen. Pharmacol.* **17**:197–202.
2. Vilaró, S., Viñas, O., Remesar, X. and Herrera, E. (1987). Effects of chronic ethanol consumption on lactational performance in rat: mammary gland and milk composition and pup's growth and metabolism. *Pharmacol. Biochem. Behav.* **27**:333–399.
3. Viñas, O., Vilaró, S., Herrera, E. and Remesar, X. (1987). Effects of chronic ethanol treatment on amino acid uptake and enzyme activities in the lactating rat mammary gland. *Life Sci.* **40**:1745–1749.
4. Robinson, A. M. and Williamson, D. H. (1977). Comparison of glucose metabolism in the lactating mammary gland of the rat *in vivo* and *in vitro*. Effects of starvation, prolactin or insulin deficiency. *Biochem. J.* **164**:153–159.
5. Threadgold, F. C. and Kuhn, N. J. (1984). Monosaccharide transport in the mammary gland of the intact lactating rat. *Biochem. J.* **218**:213–219.
6. Hawkins, R. S. and Williamson, D. H. (1972). Measurements of substrate uptake by mammary gland of the rat. *Biochem. J.* **129**:1171–1173.
7. Viña, J., Puertes, I. R., Estrela, J. M., Viña, J. R. and Galbis, J. L. (1981). Involvement of γ-glutamyltransferase in amino-acid uptake by the lactating mammary gland of the rat. *Biochem. J.* **194**:99–102.
8. Somogyi, M. (1945). Determination of blood sugar. *J. Biol. Chem.* **60**:69–73.
9. Hugget, A. St. G. and Nixon, D. A. (1957). Use of glucose-oxidase peroxidase and O-dianisidine in determination of blood and urinary glucose. *Lancet* **ii**:368–379.
10. Williamson, D. H., Mellanby, J. and Krebs, H. A. (1962). Enzymatic determination of D-(−)-β-hydroxybutyric acid and acetoacetic acid in blood. *Biochem. J.* **82**:90–96.
11. Espinet, C. and Argilés, J. M. (1984). The use of thiourea in the determination of tissue ethanol and acetaldehyde concentrations. *IRCS Med. Sci.* **12**:291–292.
12. Reitz, R. C. (1979). Effects of ethanol on the intermediary metabolism of liver and brain. In:

Biochemistry and Pharmacology of Ethanol (E. Majchorowicz and P. E. Noble, Eds.), Vol. 1, Plenum Press, New York, p. 353.
13. Lefevre, A., Adler, H. and Lieber, C. S. (1970). Effect of ethanol on ketone metabolism. *J. Clin. Invest.* **49**:1775–1782.
14. Masoro, E. J., Compton, C., Yu, B. and Bertran, H. (1983). Temporal and compositional dietary restrictions modulate age-related changes in serum lipids. *J. Nutr.* **113**:880–892.
15. Robinson, A. M., Girard, J. R. and Williamson, D. H. (1978). Evidence for a role of insulin in the regulation of lipogenesis in lactating rat mammary gland. *Biochem. J.* **176**:343–346.
16. Villarroya, F., Mampel, T. and Herrera, E. (1985). Similar metabolic response to acute ethanol intake in pregnant and non pregnant rats either fed or fasted. *Gen. Pharmacol.* **16**:537–540.
17. Sauerheber, R. D., Esgate, J. A. and Kuhn, L. E. (1982). Alcohols inhibit adipocyte basal and insulin-stimulated glucose uptake and increase the membrane lipid fluidity. *Biochim. Biophys. Acta* **691**:115–124.
18. Rifkin, R. M., Todd, W. W., Toothaker, D. R., Sussman, A., Trownbridge, M. and Draznin, B. (1983). Effects of *in vivo* and *in vitro* alcohol administration on insulin binding and glycogenesis in isolateral hepatocytes. *Ann. Nutr. Metab.* **27**:313–319.