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EFFECTS OF CHRONIC ETHANOL TREATMENT ON AMINO ACID UPTAKE AND ENZYME ACTIVITIES IN THE LACTATING RAT MAMMARY GLAND

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SUMMARY

The effects of chronic ethanol consumption on mammary gland amino acid uptake at the 15th day of lactation in the rat have been studied. Ethanol treatment decreased the arterial levels of Ala, Asp, Gly, Pro, Lys and Met, and increased those of Gln and α-amino-butyrate. Chronic ethanol treatment produced a decrease in the arteriovenous differences of Asp, Thr, Arg, Met and Phe, and increased those of Ala, Gln, Gly, Pro and Tyr. The combination of the calculated values of relative extraction and the arteriovenous differences indicate that these alterations in amino acid uptake are related to changes in the transport process for Ala, Asp, Thr, Pro, Arg, Asn, Gly, Tyr, and Phe, and that the alterations in the arteriovenous differences of Gln, Lys and Met are due to the affected arterial levels of these amino acids. Measurements of enzymatic activities in the mammary gland show that these alterations in the amino acid transport process cannot be ascribed to changes in the γ-glutamyl cycle.

At the peak of lactation, the rat mammary gland takes up a considerable amount of amino acids due to its increasing importance as a site of protein synthesis (1). We have recently shown that chronic ethanol treatment profoundly alters normal mammary gland function, as indicated by changes in milk and mammary gland composition and impaired milk production (2). The aim of the present study was to determine whether mammary gland amino acid metabolism is affected by ethanol treatment by calculating the differences in arteriovenous amino acid levels in mammary gland of lactating rats and by measuring some enzymatic activities directly involved in amino acid metabolism. Results show that maternal ethanol consumption alters normal mammary gland amino acid metabolism in the rat.

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METHODS

Female Wistar rats from our colony were fed a standard diet (Panlab, Barcelona, Spain) "ad libitum". Ethanol was given diluted in the drinking water as previously described (2,3) in increasing doses starting four weeks before impregnation, reaching the highest dosis (25% v:v) at the beginning of pregnancy and maintaining it until the day of sacrifice (day 15 of lactation). The amounts of ingested food and liquid were determined daily on the last 4 experimental days. At parturition, all offspring were exchanged for litters of the same age from untreated mothers and adjusted to a number of 8 pups per litter. Experiments were performed at the beginning of the light cycle. Rats were anesthesized by intraperitoneal injection of sodium-pentobarbital (50mg/Kg). Arteriovenous differences were determined as described by Viña et al. (1). Blood was collected into heparinized syringes from the pudic-epigastric vein and then from the abdominal aorta of the same rat. Aliquots of plasma were deproteinized with trichloroacetic acid (10% w:v) and amino acids measured by autoanalyzer (Rank-Hilger, England). Liver and mammary gland samples were extracted immediately after blood collection and were quickly homogenized in 25 vol. of a Krebs-Ringer medium. Aliquots of the homogenates were used to measure the γ-glutamyl transferase (GGT) (E.C. 2.3.2.2.) (4), glutamate dehydrogenase (GDH) (E.C.1.4.1.3.) (5), aspartate amino transferase (GOT) (E.C.2.6.1.) (6), alanine amino transferase (GPT) (E.C.2.6.1.2.) (7) activities and protein concentration (8). Statistical analyses were performed with the Student's t test, the paired test for comparisons with zero and the unpaired test for comparisons between the experimental groups.

RESULTS AND DISCUSSION

From the 11th to the 15th day of lactation rats receiving ethanol in the drinking fluid had a mean daily ethanol intake of 24.6 $\pm$ 0.1 g/Kg body weight and a total caloric intake of 520.6 $\pm$ 21.9 Kcal/Kg body weight, whereas controls had a daily caloric intake of 540.1 $\pm$ 37.5 Kcal/Kg body weight (not significantly different from the ethanol treated rats). At the 15 day of lactation rats receiving ethanol showed a significant decrease in the arterial plasma levels of Ala, Asn, Gly, Pro, Lys and Met and an increase in plasma Gln concentration (table I). This pattern was similar to previously reported data (3), although the effects were greater in pregnant rats receiving similar doses of ethanol (9). These changes could be due to alterations in intestinal absorption of amino acids caused by ethanol (10) or to the modification of amino acid uptake by certain tissues including the mammary gland. This explanation would account for the increase in plasma Gln, which is a carrier of the ammonia derived from amino acid metabolism (11). Since to our knowledge the effects of ethanol on mammary gland amino acid uptake have not previously been explored, it was tested in lactating rats in which mammary gland arteriovenous levels of amino acids were measured. As shown in table II, mammary glands from control rats took up all amino acids except Gly, α-amino-butyrate and taurine. These values are similar to those in other studies (1,12) except for the small but significant release of Gly and the Ala uptake which was lower than the value reported by Viña et al. (1). This

Table I

EFFECT OF CHRONIC ETHANOL TREATMENT ON ARTERIAL PLASMA AMINO ACIDS OF 15-DAY LACTATING RATS

Amino acid	Control	Ethanol
Alanine	296±13	237±10 **
Glutamic acid	112±14	116±21
Glutamine	494±46	916±20 ***
Aspartic acid	22±3	26±2
Asparagine	53±2	44±3 *
Glycine	177±9	138±9 *
Serine	181±10	167±11
Threonine	189±9	160±9
Proline	144±10	111±10 *
Lysine	391±23	275±12 **
Arginine	68±7	58±6
Histidine	60±4	67±4
Citrulline	64±5	58±5
Valine	160±15	148±10
Leucine	123±13	121±9
Isoleucine	72±6	74±3
Cysteine	28±7	16±1
Methionine	71±5	41±2 ***
Taurine	121±10	118±6
Tyrosine	36±3	43±4
Phenylalanine	50±4	49±3
Tryptophan	68±5	65±3
α-amino-butyric acid	17±3	29±3 *

Values are mean + SEM of 6-8 animals/group, expressed as nmols/ml; Statistical omparisons vs. Controls: **=p<0.01, ***=p<0.001.

latter difference could be due to the low plasma Ala concentration in our animals (table I). Ethanol treatment enhanced mammary gland uptake of Ala, Gln, Gly, Pro, Tyr and α-amino-butyrate and reduced the uptake of Asp, Asn, Thr, Lys, Arg, Met and Phe as compared with controls (table II). When these values were expressed as a function of arterial amino acid levels (relative extraction), which allowed us to consider the amino acid transport process, Asn, Thr, Arg and Phe showed reduced values in ethanol-treated rats whereas the Ala, Pro and Tyr values were enhanced (table II). These changes indicate that ethanol intake modified the mammary gland amino acid transport process for all these amino acids although the underlying mechanism remains to be established because no amino acid transport systems have been described for mammary tissue except for the γ-glutamyl cycle (1,12). From these results it may also be concluded that the alteration in Gln, Met and Lys uptake was due to their modified arterial levels rather than to alterations of their transport mechanisms.

The effect of ethanol treatment on mammary gland enzyme activities related to amino acid metabolism was also investigated. To our knowledge, no such enzymatic activities except for GGT have been reported in the mammary gland. Therefore these enzymatic activities were measured also in the liver and used as controls for the data obtained in mammary tissue. As

Table II

EFFECT OF CHRONIC ETHANOL TREATMENT ON ARTERIOVENOUS DIFFERENCES AND RELATIVE EXTRACTION OF AMINO ACIDS BY 15-DAY LACTATING RAT MAMMARY GLAND

	A-V		R.E. (%)	
Amino acid	Control	Ethanol	Control	Ethanol
Alanine	7±0.4	30±2***	3±0.1	13±1***
Glutamic acid	18±3	32±8	16±3	28±7
Glutamine	224±25	381±19**	45±5	42±2
Aspartic acid	3±0.4	-7±1***	15±2	-
Asparagine	15±1	9±0.5***	28±1	21±1**
Glycine	-12±1	6±0.4***	-	4±0.3
Serine	39±3	41±3	21±2	25±2
Threonine	38±3	16±1***	20±1	10±0.6***
Proline	17±1	30±2**	12±1	27±2***
Lysine	41±3	31±2*	10±0.6	11±0.6
Arginine	32±4	14±1**	48±7	23±2*
Histidine	20±1	20±2	32±2	31±3
Citrulline	21±2	25±3	33±3	43±5
Valine	92±1	78±10	58±6	52±7
Leucine	84±9	70±6	68±8	58±5
Isoleucine	48±5	42±2	68±7	56±3
Methionine	27±2	16±1**	37±3	38±3
Taurine	-40±4	-10±1***	-	-
Tyrosine	8±0.7	18±3**	21±2	41±6*
Phenylalanine	28±2	18±1**	56±4	37±2**
Tryptophan	9±0.7	8±0.3	14±1	13±0.5
α-amino-butyrate	-10±2	6±0.7***	-	22±2

Means ± S.E.M. of 5-8 rats/group. Arteriovenous differences are expressed as nmols/ml. Relative extraction=((A)-(V)/(A))x100. Comparisons vs. Controls: *=p<0.05, **=p<0.01, ***=p<0.001. All values are different from zero (p<0.01).

Table III

EFFECT OF CHRONIC ETHANOL TREATMENT ON ENZYMATIC ACTIVITIES IN THE LIVER AND THE MAMMARY GLAND OF 15-DAY LACTATING RATS

	LIVER		MAMMARY GLAND	
Enzyme	Control	Ethanol	Control	Ethanol
GGT				
μKat/g.t.w.w.[1]	0.4±0.07	0.8±0.1**	12±1	10±1
μKat/g.t.prot[2]	2±0.4	4±0.6*	89±9	71±6
GDH				
μKat/100g.t.w.w.	252±42	185±28	3±0.4	2±0.4
μKat/g.t.prot	14±2	9±1	0.2±0.02	0.2±0.04
GPT				
μKat/100g.t.w.w.	52±4	74±6**	7±0.6	10±0.9*
μKat/g.t.prot	3±0.2	4±0.3	0.5±0.04	0.7±0.06*
GOT				
μKat/100g.t.w.w.	265±19	349±13**	70±4	50±3**
μKat/g.t.prot	15±1	17±0.7	5±0.2	4±0.2*

Means ± S.E.M. of 6-8 rats. 1=Tissue wet weight; 2=Tissue protein. Comparisons vs. Controls: *=p<0.05, **=p<0.01, ***=p<0.001.

shown in table III, ethanol treatment increased the activities of GGT, GPT and GOT in the liver while GDH activity remained unchanged. When these activities are expressed per gram of protein only GGT is increased, in agreement with an earlier study (13). In the mammary gland (table III), GGT and GDH were not modified by ethanol whereas GPT was enhanced and GOT decreased. These findings fit with the enhanced uptake of Ala and decreased uptake of Asp in treated rats. Moreover, the stable GGT activity indicates that the γ-glutamyl cycle is not involved in the observed changes of amino acid uptake, and suggests that other mechanism(s) of amino acid transport in addition to the γ-glutamyl cycle are active in mammary gland tissue, in agreement with previous proposals (12). In conclusion, chronic ethanol treatment produced alterations in mammary gland amino acid uptake which appear selective and different according to the amino acid studied. The higher and lower amino acid uptake were due to variations in afferent concentrations and/or linked to the transport processes. These alterations are in agreement with the disturbed milk synthesis and production caused by ethanol treatment (2) and appear unrelated to changes in the -glutamyl cycle.

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