Pharmacology Biochemistry & Behavior, Vol. 32, pp. 1071–1074. © Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

BRIEF COMMUNICATION

Effect of Ethanol on Plasma Amino Acids and Related Compounds of Stressed Male Rats

LOUIS MILAKOFSKY,*[1] JOANNE M. MILLER† AND WOLFGANG H. VOGEL†

**Chemistry Department, Penn State University, Berks Campus, Reading, PA 19610*
†Department of Pharmacology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107

Received 30 November 1987

MILAKOFSKY, L., J. M. MILLER AND W. H. VOGEL. *Effect of ethanol on plasma amino acids and related compounds of stressed male rats.* PHARMACOL BIOCHEM BEHAV **32**(4) 1071–1074, 1989.—Plasma amino acid levels in rats are known to be affected by ethanol or by immobilization stress. This paper investigated the effect of ethanol on plasma amino acid levels of stressed rats. Rats received ethanol (2 g/kg, IP) 15 minutes prior to a 30-min immobilization period. Blood samples were obtained from individual rats before, during and after stress. Ethanol lowered the concentration of most plasma amino acids (AA) or related compounds in stressed rats (e.g., aspartic acid, threonine, serine, glycine, alanine, valine, tyrosine, phenylalanine, tryptophan). Some compounds remained unaffected (e.g., taurine, cystine, ethanolamine and methylhistidines) and one (phosphoethanolamine) increased initially. A comparison of the effects of ethanol on plasma AA and related compounds in resting and stressed rats shows similarities and differences. In general, ethanol tends to change the concentrations of these compounds away from normal levels in nonstressed rats, whereas in stressed rats, ethanol tends to antagonize stress-induced changes. This study shows that ethanol can affect individual AA and related compounds differently in nonstressed and stressed rats and that ethanol reduces stress-induced changes. The latter finding supports the "tension-reduction hypothesis" of ethanol.

Plasma amino acids HPLC Ethanol Immobilization Stress Male rats

PLASMA amino acid concentrations in animals and humans are affected by ethanol and stress. The administration of ethanol decreases significantly the levels of most plasma amino acids (AA) and related compounds in resting rats within 15 min and these reductions remain unchanged for up to 3 hr (5, 14, 19). In contrast, stress alone has been shown to have a more variable effect on plasma amino acid concentrations in rats (1, 3, 8, 9, 13, 15). Milakofsky and co-workers (13), using the catheterized rat and immobilization as the stressor, found that some AA, such as taurine, aspartic acid, glutamic acid and alanine increase significantly, whereas other AA, such as valine, tryptophan and arginine, show significant decreases. The levels of most AA were restored to baseline concentrations 30 min after the animal was released from the immobilization.

Recently, it has become apparent that ethanol can affect certain biochemicals differently in resting versus stressed animals. For instance, moderate doses of ethanol have no effect on plasma catecholamine levels in nonstressed rats, but strongly decrease and antagonize stress-induced increases in these biochemicals in stressed rats (4,18). Thus, it was of interest to investigate whether ethanol would also differentially affect plasma AA and related compounds in resting and stressed rats. It was hypothesized that ethanol would reduce stress-induced increases in certain plasma AA. This hypothesis was shown to be correct.

METHOD

Methodology used in this study is essentially identical to that used in previous studies (4, 12, 13, 18).

Animals and Catheterization

Seven male Sprague-Dawley rats (Perfection Breeders, Douglassville, PA), weighing 235 to 275 g, were fitted with chronic indwelling jugular catheters (silastic tubing) and were allowed to

[1]Requests for reprints should be addressed to Louis Milakofsky, Chemistry Department, Penn State University, Berks Campus, Tulpehocken Rd., P.O. Box 7009, Reading, PA 19610.

TABLE 1

THE EFFECT OF ETHANOL ON PLASMA AMINO ACIDS OF STRESSED MALE RATS

Amino Acids and Related Compounds	Time After Ethanol Injection (min) 0[a]	15	30[b]	45[c]	75	120	180
Taurine	57.4 ± 7.7	68.1 ± 8.9	74.0 ± 11.2	49.0 ± 7.2	56.0 ± 8.3	60.0 ± 9.6	85.5 ± 33.0
Phosphoethanolamine	3.4 ± 0.2	4.7 ± 0.3*	5.0 ± 0.7†	3.2 ± 0.3	3.5 ± 0.3	3.5 ± 0.3	3.9 ± 0.4
Aspartic Acid	6.3 ± 0.5	5.7 ± 0.6	5.5 ± 0.7	4.1 ± 0.4†	4.1 ± 0.3†	3.7 ± 0.3†	4.1 ± 0.4†
Threonine	202 ± 15	141 ± 15†	121 ± 12†	101 ± 9†	108 ± 12†	111 ± 11†	129 ± 14†
Serine	172 ± 16	117 ± 14†	97.4 ± 8.0†	78.9 ± 6.6†	88.9 ± 8.9†	84.3 ± 8.7†	102 ± 9†
Asparagine	58.3 ± 4.5	37.6 ± 3.9†	32.6 ± 2.4†	25.4 ± 1.7†	27.4 ± 2.5†	29.0 ± 2.3†	36.5 ± 3.5†
Glutamic Acid	45.4 ± 9.2	40.8 ± 10.4	37.1 ± 8.7	26.7 ± 6.4†	27.7 ± 6.0†	26.5 ± 4.9†	38.6 ± 8.6
Glutamine	412 ± 18	344 ± 18	328 ± 20*	268 ± 18†	320 ± 32*	365 ± 38	453 ± 37
α-Aminoadipic Acid	11.5 ± 0.8	9.59 ± 0.78*	9.06 ± 0.8†	7.36 ± 0.50†	8.44 ± 0.87†	8.70 ± 0.81†	10.6 ± 0.7
Glutathione (oxidized)	0.56 ± 0.12	0.45 ± 0.08	0.41 ± 0.13	0.69 ± 0.15	0.47 ± 0.10	0.50 ± 0.07	0.44 ± 0.18
Glycine	272 ± 20	197 ± 18†	170 ± 15†	128 ± 11†	154 ± 18†	161 ± 16†	208 ± 13†
Alanine	354 ± 28	256 ± 23†	225 ± 23†	160 ± 12†	172 ± 19†	142 ± 13†	170 ± 16†
Citrulline	68.8 ± 3.3	53.1 ± 3.8†	49.8 ± 4.5†	40.8 ± 3.2†	47.8 ± 5.2†	47.4 ± 4.6†	57.8 ± 5.6†
α-Amino-n-Butyric Acid	6.3 ± 1.0	4.9 ± 0.7	4.9 ± 0.7	4.4 ± 0.9	5.7 ± 1.5	7.1 ± 1.8	9.5 ± 2.2†
Valine	190 ± 9	145 ± 12†	135 ± 12†	120 ± 11†	120 ± 10†	132 ± 6†	158 ± 13†
Cystine	12.3 ± 0.7	12.0 ± 1.2	11.2 ± 0.9	10.8 ± 1.2	11.1 ± 1.8	10.2 ± 1.4	11.1 ± 1.5
Methionine	47.0 ± 0.8	36.1 ± 2.6†	34.2 ± 1.8†	27.6 ± 1.8†	27.9 ± 2.3†	27.3 ± 2.2†	32.2 ± 2.2†
Cystathionine	1.4 ± 0.1	1.5 ± 0.3	1.4 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
Isoleucine	88.5 ± 3.2	64.4 ± 5.5†	64.5 ± 6.3†	55.2 ± 3.2†	55.5 ± 2.6†	64.8 ± 3.2†	70.5 ± 7.4†
Leucine	148 ± 3	115 ± 10†	113 ± 13†	91.9 ± 9.4†	94.0 ± 7.0†	106 ± 5†	127 ± 8*
Tyrosine	67.8 ± 4.7	53.2 ± 3.9†	50.1 ± 4.2†	40.4 ± 2.8†	36.3 ± 4.0†	32.8 ± 2.6†	35.5 ± 1.9†
Phenylalanine	56.0 ± 2.3	41.3 ± 3.0†	38.7 ± 2.2†	31.5 ± 2.0†	33.6 ± 2.3†	38.3 ± 1.7†	47.5 ± 1.5†
β-Alanine	1.1 ± 0.2	1.0 ± 0.1	0.89 ± 0.09	0.73 ± 0.09*	0.86 ± 0.16	0.84 ± 0.11	0.97 ± 0.09
Tryptophan	49.4 ± 3.5	35.1 ± 4.5†	28.4 ± 2.7†	23.8 ± 2.8†	25.9 ± 2.5†	24.5 ± 3.4†	31.8 ± 2.7†
Ethanolamine	6.09 ± 0.63	7.74 ± 0.54*	7.40 ± 0.52	6.16 ± 0.59	6.40 ± 0.68	5.27 ± 0.58	6.10 ± 0.77
Ammonia	88.6 ± 16.3	52.3 ± 5.0*	64.1 ± 10.2	65.3 ± 15.6	47.2 ± 5.4*	49.5 ± 6.8*	30.3 ± 4.4†
Hydroxylysine	2.1 ± 0.1	1.9 ± 0.2	1.6 ± 0.1*	1.6 ± 0.1*	1.6 ± 0.2*	1.8 ± 0.2	1.5 ± 0.2*
Ornithine	39.5 ± 3.7	30.9 ± 3.5†	24.2 ± 1.9†	20.3 ± 2.0†	23.3 ± 2.7†	21.3 ± 2.0†	26.3 ± 1.8†
Lysine	275 ± 17	228 ± 23†	192 ± 10†	169 ± 16†	191 ± 20†	183 ± 17†	213 ± 12†
Histidine	47.4 ± 3.4	39.0 ± 3.4*	35.7 ± 1.6†	29.7 ± 2.3†	36.8 ± 3.9†	39.4 ± 3.9*	45.7 ± 2.4
1-Methylhistidine	2.9 ± 0.5	2.5 ± 0.4	2.3 ± 0.4	2.2 ± 0.4*	2.6 ± 0.6	2.6 ± 0.5	2.9 ± 0.4
3-Methylhistidine	3.0 ± 0.5	2.4 ± 0.3	2.4 ± 0.4	2.3 ± 0.5	2.9 ± 0.5	2.9 ± 0.5	3.3 ± 0.5
Arginine	163 ± 12	119 ± 12†	99.2 ± 5.9†	85.6 ± 8.6†	97.5 ± 11.0†	88.8 ± 8.3†	92.1 ± 3.5†

Values are mean ± S.E.M. (nmol/ml), N = 7.

The following amino acids were not detectable at any time: glutathione (reduced), β-aminoisobutyric acid, γ-aminobutyric acid, anserine, carnosine and homocarnosine.

[a]Baseline followed by ethanol injection (2 g/kg); [b]15 min of immobilization stress; [c]30 min of immobilization stress.

*$p<0.05$ with respect to time = 0 min; †$p<0.01$ with respect to time = 0 min.

recover from surgery for 24 hr before the experiment.

Ethanol Injection, Immobilization and Preparation of Plasma Samples

Blood (0.3 ml) was first drawn from the catheterized animals in their home cages (t=0 min). Rats were then injected with ethanol (20%, w/v, solution, 2 g/kg, IP). After 15 minutes (t=15 min) a second blood sample was obtained from the resting animals. Thereafter, rats were immobilized by taping their paws to a laboratory bench for a period of 30 min. Blood samples were collected after 15 (t=30 min) and 30 (t=45 min) minutes of immobilization. Following release, animals were returned to their home cages and blood samples were drawn at 30 (t=75 min), 75 (t=120 min) and 135 (t=180 min) minutes. All blood samples were centrifuged to obtain plasma and the plasma was stored at −80° until assayed.

Amino Acid Analysis

Deproteinized plasma amino acid levels were determined by ion-exchange HPLC with fluorometric detection as previously described (12).

TABLE 2

RATIOS OF TRYPTOPHAN, TYROSINE AND PHENYLALANINE TO 5 AMINO ACIDS WHICH INFLUENCE THEIR PENETRATION INTO THE BRAIN

Time (min)	Ratios TRY (V+I+L+T+P)	T (V+I+L+P+TRY)	P (V+I+L+T+TRY)
0[a]	0.0903 ± 0.0067	0.128 ± 0.010	0.103 ± 0.005
15	0.0837 ± 0.0069	0.135 ± 0.010	0.101 ± 0.005
30[b]	0.0721 ± 0.0062†	0.135 ± 0.010	0.101 ± 0.005
45[c]	0.0701 ± 0.0055†	0.128 ± 0.009	0.0961 ± 0.0043
75	0.0773 ± 0.0059*	0.110 ± 0.008*	0.102 ± 0.003
120	0.0647 ± 0.0069†	0.0894 ± 0.0036†	0.107 ± 0.002
180	0.0733 ± 0.0077†	0.0825 ± 0.0042†	0.115 ± 0.007*

Ratios are mean ± S.E.M., N = 7.

TRY = tryptophan; V = valine; I = isoleucine; L = leucine; T = tyrosine; P = phenylalanine.

[a]Baseline followed by ethanol injection (2 g/kg); [b]15 min of immobilization stress; [c]30 min of immobilization stress.

*$p<0.05$ with respect to time = 0 min; †$p<0.01$ with respect to time = 0 min.

Evaluation

Individual concentrations of AA were quantitatively calculated by relating their chromagraphic peak heights to the peak heights from a known amount of a physiological AA standard and an internal standard (m-fluorophenylalanine) as described previously (12). Statistical analysis of the ethanol-stress data was performed by a one-way analysis of variance for repeated measurements with a Newman-Keul post hoc test.

RESULTS

The effects of acute ethanol administration and a 30-min immobilization stress on plasma amino acid levels and related compounds are shown in Table 1. The results indicate that the levels of many of the detectable plasma AA and related compounds (22 out of 33) decrease significantly with time. Such decreases in plasma AA are evident throughout the immobilization period and even at rest in the home cage. Threonine, serine, glycine, alanine and tryptophan showed the largest reduction from baseline values. However, the plasma concentrations of some AA such as glutamic acid, glutamine, α-aminoadipic acid and histidine return to baseline values by 180 minutes. A few AA including taurine, cystine, ethanolamine, 1-methylhistidine and 3-methylhistidine do not change during the ethanol-stress treatment. Only phosphoethanolamine shows an initial increase but returns quickly to its baseline value 45 min after ethanol injection.

Table 2 shows the ratios of tryptophan, tyrosine and phenylalanine over the sum of 5 amino acids which control the penetration of the first 3 AA into the brain (6). The tryptophan ratios are significantly decreased during and after stress. The ratios of tyrosine are decreased after stress and the ratios of phenylalanine are slightly increased at the end of the experiment.

DISCUSSION

The results clearly show that ethanol affects the levels of some but not all plasma AA and related compounds in stressed rats. The mode of action of ethanol, either directly or indirectly, is still uncertain and is probably different for individual AA. However, a relatively clear association exists between ethanol levels and changes in AA; plasma ethanol peak levels occur at 15 min after injection (18), which coincides with the onset of changes of many of the plasma AA. As levels of blood ethanol fall, many of the changes seen (e.g., glutamic acid, glycine, alanine, valine, phenylalanine) start to return towards normal. However, this is not true for all biochemicals studied. For instance, the concentrations of arginine, tyrosine, and tryptophan are still markedly depressed at 180 min at a time when ethanol levels have dropped considerably. Thus, in the stressed organism, ethanol affects AA in a highly individual matter.

It now seems appropriate to compare the results of our present study on the effects of ethanol on plasma AA in stressed rats with those found in animals which were only stressed (13) or which received ethanol in the resting state (14). A comparison seems possible since all rats were handled in a similar fashion and since baseline values for AA and related compounds were rather similar.

First, the effects of ethanol on plasma AA and related compounds in nonstressed (14) vs. stressed animals can be summarized as follows. In resting and stressed rats, ethanol markedly lowers the plasma levels of most AA and related compounds. However, it is important to emphasize the existence of quantitative and qualitative differences between these 2 emotional states. For instance, alcohol reduced aspartic acid by 22% in the nonstressed rat but only by about 8% in the stressed rat. Ethanol reduces plasma glutamic acid levels by about 9% in the resting but lowers this AA by 40% in the stressed rat. Taurine and phosphoethanolamine fall markedly after ethanol administration in the nonstressed but increase initially in the stressed rat. Thus, the effects of ethanol are similar but not identical in resting and stressed organism and caution must be exercised when extrapolating results obtained in resting animals to humans who are often exposed to stressful experiences.

Second, a comparison of the plasma amino acid levels during stress alone (13) and during stress after ethanol pretreatment leads to the following observations. In general, stress alone increases the concentrations of most plasma AA and related compounds and rather marked increases are seen with alanine, cystine, β-alanine, ethanolamine, taurine, aspartic acid and glutamic acid. These observed stress-induced increases in AA and related compounds are usually not seen in stressed rats which are pretreated with ethanol. Here, ethanol prevents stress-induced increases and reduces or normalizes plasma levels of certain compounds. For instance, plasma levels of taurine rise up to 300% above baseline levels during stress alone but rise only transiently (about 30%) during stress after ethanol pretreatment. Alanine and aspartic acid rise up to 50 and 100% above baseline in the stressed animal without ethanol but fall about 50% below baseline levels in stressed animals under the influence of ethanol. Threonine and tyrosine do not change during stress alone but are reduced significantly by ethanol during stress. Other AA, such as the methylhistidines, are little affected by stress or ethanol and stress. Thus, ethanol prevents or antagonizes many stress-induced increases in the above biochemicals. This is quite similar to the effects of alcohol on plasma corticosterone and catecholamines; all of these biochemicals rise during stress but these stress-induced increases are markedly antagonized by ethanol (4, 17, 18).

The reduction of the stress responses by ethanol has led to the formulation of the "tension-reduction hypothesis" of alcoholism (2) which states that people drink to reduce stress-induced tension. Investigations on a behavioral level are ambiguous and either support or refute this hypothesis (16). However, our results on a biological level add evidence that ethanol can indeed reduce biological stress. It may be this property of ethanol which makes people use and abuse ethanol to reduce the effects of stress.

Ethanol and stress have each been shown to produce significant reductions in the brain levels of norepinephrine and serotonin (4, 7, 10, 11). Ethanol pretreatment before stress reduces many of

these changes and normalizes the levels of these neurotransmitters (4,10). Since stress alone decreases the ratios of tryptophan over its five controlling AA (6), the decrease in brain serotonin can easily be explained by the reduction in its supply. The normalization of serotonin levels by alcohol during stress (10) cannot be explained by an increase in supply since the ratios of tryptophan are decreased (Table 2). Thus, alcohol must normalize brain serotonin levels in a different way perhaps by altering blood-brain barrier characteristics. Similarly, the ratios of tyrosine to its controlling AA (Table 2) do not explain the fall in brain norepinephrine during stress or the normalization by ethanol. Thus, the ratios seem to control brain levels during rest, but not during stress, under the influence of alcohol.

In conclusion, ethanol affects certain plasma AA and related compounds quite selectively and differently in resting vs. stressed animals. In nonstressed rats, ethanol tends to shift plasma AA and related compounds away from normal baseline levels, whereas in stressed rats stress-induced changes in AA are reduced. This latter finding adds biological support to the tension-reduction hypothesis; ethanol indeed reduces and antagonizes stress-induced biochemical changes. The reduction of this biological stress response could be the cause why certain, highly stress-susceptible individuals, abuse alcohol.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Penn State University Biomedical Research Support Grant, the Penn State University-Berks Campus Faculty Development Fund and the Public Health Grant (AA06017) for financial support. The authors would also like to thank Dr. H. Menduke for help with the statistical evaluation of the data.

REFERENCES

1. Arola, L. L.; Palou, A.; Remesar, X.; Herrera, E.; Alemany, M. Effect of stress and sampling site on metabolite concentration in rat plasma. Arch. Int. Physiol. Biochim. 88:99–105; 1980.
2. Cappell, H.; Herman, P. Alcohol and tension reduction: A review. Q. J. Stud. Alcohol 33:33–64; 1972.
3. Curzon, G.; Joseph, M. H.; Knott, P. J. Effects of immobilization and food deprivation on rat brain tryptophan metabolism. J. Neurochem. 19:1967–1974; 1972.
4. Deturck, K. H.; Vogel, W. H. Effects of acute ethanol on plasma and brain catecholamine levels in stressed and unstressed rats: Evidence for an ethanol-stress interaction. J. Pharmacol. Exp. Ther. 223: 348–354; 1982.
5. Eriksson, T.; Carlsson, A.; Liljequist, S.; Hagman, M.; Jagenburg, R. Decrease in plasma amino acids in rat after acute administration of ethanol. J. Pharm. Pharmacol. 32:512–513; 1980.
6. Fernstrom, J. D.; Wurtman, R. J. Brain seretonic content: physiological regeneration by plasma neutral amino acids. Science 178:414–416; 1972.
7. Glavin, G. B.; Tanaka, M.; Tsuda, A.; Kohno, Y.; Hoaki, Y.; Nagasaki, N. Regional rat brain noradrenaline turnover in response to restraint stress. Pharmacol. Biochem. Behav. 19:287–290; 1983.
8. Kennett, G. A.; Curzon, G.; Patel, A. J. Immobilization decreases amino acids concentrations in plasma but maintains or increases them in brain. J. Neurochem. 46:208–212; 1986.
9. Kennett, G. A.; Joseph, M. H. The functional importance of increased brain tryptophan in the serotonergic response to restraint stress. Neuropharmacology 20:39–43; 1981.
10. Kuriyama, K.; Kanmori, K.; Yoneda, Y. Preventive effect of alcohol against stress-induced alteration in content of monoamines in brain and adrenal gland. Neuropharmacology 23:649–654; 1984.
11. Lehnert, H.; Reinstein, D. K.; Strowbridge, B. W.; Wurtman, R. J. Neurochemical and behavioral consequences of acute, uncontrollable stress: Effects of dietary tyrosine. Brain Res. 303:215–223; 1984.
12. Milakofsky, L.; Hare, T. A.; Miller, J. M.; Vogel, W. H. Comparison of amino acid levels in rat blood obtained by catheterization and decapitation. Life Sci. 34:1334–1340; 1984.
13. Milakofsky, L.; Hare, T. A.; Miller, J. M.; Vogel, W. H. Rat plasma levels of amino acids and related compounds during stress. Life Sci. 36:753–761; 1985.
14. Milakofsky, L.; Miller, J. M.; Vogel, W. H. Effects of acute ethanol administration on rat plasma amino acids and related compounds. Biochem. Pharmacol. 35:3885–3888; 1986.
15. Pare, W. P.; Glavin, G. B. Restraint stress in biomedical research: A review. Neurosci. Biobehav. Rev. 10:339–370; 1986.
16. Pohorecky, L. A. The interaction of alcohol and stress—A review. Neurosci. Biobehav. Rev. 5:209–238; 1981.
17. Pohorecky, L. A.; Rassi, E.; Weiss, J. M.; Michalak, V. Biochemical evidence for an interaction of ethanol and stress: Preliminary studies. Alcohol.: Clin. Exp. Res. 4:423–426; 1980.
18. Vogel, W. H.; DeTurck, K.; Miller, J. M. Differential effects of ethanol on plasma catecholamine levels in rats. Biochem. Pharmacol. 35:3983–3087; 1986.
19. Ward, L. C. Effect of ethanol and dietary protein on plasma amino acids in the rat. Nutr. Rep. Int. 31:273–280; 1985.