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Plasma catecholamines in ethanol tolerance and withdrawal in mice

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Abstract

Plasma levels of catecholamines (noradrenaline and adrenaline) as well as the ratio of the two catecholamines were measured in experimental mice during various stages of acute and chronic ethanol treatment. Acute intraperitoneal (i.p.) ethanol administration and acute per os (p.o.) ethanol ingestion resulted in a similar elevation of the plasma levels of both catecholamines. During the development of ethanol tolerance/dependence (ingestion of drinking water containing ethanol for 14 days), plasma catecholamine concentrations returned to the control levels. During subsequent ethanol withdrawal, a highly significant increase was observed in plasma noradrenaline. The withdrawal-associated elevation of plasma adrenaline was also significant; however, the rise in plasma noradrenaline during withdrawal appeared to be higher than that found for adrenaline. Thus, the ratio of plasma noradrenaline to adrenaline was higher during withdrawal, and this ratio also exhibited an association with the severity of ethanol withdrawal symptoms. Since plasma noradrenaline derives to a great extent from the sympathetic nervous system—and the alcohol withdrawal syndrome is characterized by symptoms of overactivity of this system—a positive correlation may exist between noradrenaline and the severity of withdrawal symptomatology.
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Keywords: Noradrenaline plasma; Adrenaline; Catecholamine ratio; Ethanol tolerance; Ethanol dependence; Ethanol withdrawal; Adaptation

1. Introduction

It was recognized over 90 years ago that the secretion of adrenaline was increased by stress (Cannon and de la Paz, 1911). Unlike adrenaline, which is almost exclusively hormonal in origin, noradrenaline is secreted by both the adrenal medulla and postganglionic sympathetic neurons (for review, see Fink, 2000). There has been a long-standing belief that the secretion of these two catecholamines reflected different aspects of sympathoadrenal function and subserved different functions in adaptation to stress (Frankenhaeuser, 1971). The sympathetic response has many physiological and psychological concomitants, or consequences. These include accelerated heart rate, increased blood pressure and respiration rate, dilatation of pupils, etc. At the peak of the response, physiological resources are mobilized (for review, see Fink, 2000).

In alcoholics, disturbances of the autonomic nervous system are well known. Accordingly, alcohol-withdrawal syndrome is characterized by signs of overactivity of the sympathetic nervous system. An increased release of catecholamines (adrenaline and noradrenaline) is associated with certain symptoms of withdrawal, such as tremulousness, paroxysmal sweats, increased blood pressure and increased heart rate (Linnoila et al., 1987).

Abnormalities in central nervous catecholaminergic systems in alcoholism have been described, and significant attempts have been made to correlate results with various characteristics of alcoholism, such as severity, duration of abstinence, etc. (for review, see Fink, 2000). Low doses of ethanol given to mice or rats cause an increase in brain noradrenaline turnover, while higher doses cause a decrease in noradrenaline turnover (Hoffmann and Tabakoff, 1985). The changes in turnover of neurotransmitters in specific regions of the brain may reflect alterations in neuronal activity that result from the withdrawal of ethanol, or may be determinants of particular ethanol-withdrawal symptoms (Eisenhofer et al., 1990). In healthy human individuals, Borg et al. (1983) found an increased level of noradrenaline metabolites (presumably due to increased noradrenaline

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turnover) after acute ingestion of ethanol. Hawley et al. (1994) found a positive correlation of the elevated noradrenaline level in the cerebrospinal fluid from patients suffering from acute alcohol withdrawal with other central indicators of stress (e.g. corticotropin-releasing factor level) and the diastolic blood pressure. These findings indicate significant perturbations of the noradrenergic neuronal system during alcohol withdrawal. Altered neurotransmission of noradrenaline may subsequently also change central adrenoreceptor functions, although human data on the specific role of α_2-adrenoreceptors are contradictory (for review, see Fahlke et al., 1999).

The circulating level of noradrenaline derives from both the sympathetic nervous system and the adrenal medulla, while the adrenal medulla is the only known source of circulating adrenaline. An important function of the sympathetic nervous system, as well as of the adrenal medulla, is to maintain homeostasis by modulating the level of cellular activity in many diverse organ systems. For the development of alcohol-related or withdrawal-related symptoms, as well as for the diagnosis of these diseases, an understanding of the characteristics of peripheral (plasma) adrenaline and noradrenaline levels might be as important as that for the central catecholamines. In spite of the suggested roles of peripheral catecholamines in experimental animals and in human patients, little is known about plasma levels of noradrenaline and adrenaline during the development of tolerance to and dependence on ethanol. The association between peripheral levels of catecholamines and the ratio of noradrenaline to adrenaline during ethanol withdrawal have not been studied in detail either. The present experiments were undertaken to examine these questions in ethanol-addicted mice.

2. Materials and methods

2.1. Experimental animals and induction of ethanol tolerance/dependence

Male inbred CFLP mice (Gödöllõ, Hungary), weighing 35 ± 5 g, were used in the experiments (for details, see Kovács 1993, 2000a,b). Animals were kept in groups of 15. Fluid consumption/24 h was measured per groups of animals. Various treatment groups are described in Table 1.

Group 1. Intact control animals were decapitated following an adaptation period of 2 weeks, and trunk blood was collected for the measurement of plasma catecholamines.

Group 2. In order to test the effect of acute intraperitoneal (i.p.) ethanol treatment, mice were injected with 1.75 g/kg ethanol (15% v/v ethanol in physiological saline) and decapitated 2 h later.

Group 3. The effect of acute per os (p.o.) ethanol ingestion was studied. Following 14 days of drinking tap water, drinking water was replaced with 5% (v/v) ethanol, and mice were decapitated 24 h later.

Group 4. Drinking water was replaced with sucrose solution for 14 days. Sucrose was isocaloric to 5% or 7% ethanol for the first and the second week of treatment, respectively. Mice were decapitated on day 15 of the experiment.

Group 5. Animals rendered tolerant/dependent on ethanol received ethanol in the drinking water (5% for 1 week, then 7% for the second week). On day 15, mice also received 7% ethanol for drinking, and thus withdrawal was not precipitated.

Group 6. In ethanol-tolerant/dependent animals, ethanol was replaced with water on day 15, and 5 h later, trunk

Table 1
Summary table of treatments and experimental groups

Pretreatment in drinking water	Treatment of day 15	Withdrawal hyperexcitability tested	Decapitation on day 15[a]	Measured parameter	Group name (group names as they appear on the figures)
(1) Drinking water for 14 days	–	no	0 min	plasma catecholamines	control (control)
(2) Drinking water for 14 days	1.75 g/kg ethanol treatment i.p. (15% v/v ethanol in physiological saline)	no	2 h	plasma catecholamines; blood ethanol concentration	acute ethanol treatment i.p. (acetoh i.p.)
(3) Drinking water for 14 days, then 5% ethanol for 1 day	–	no	24 h after drinking	plasma catecholamines; blood ethanol concentration	acute ethanol (acetoh p.o.)
(4) Drinking isocaloric sucrose in water for 14 days	–	no	0 min	plasma catecholamines	isocaloric control (isocal)
(5) Drinking ethanol in drinking water (for details, see Materials and methods)	ethanol NOT replaced with water	no	0 min	plasma catecholamines; blood ethanol levels	tolerant/dependent (toler/dep)
(6) Drinking ethanol in drinking water (for details, see Materials and methods)	ethanol replaced with water for 5 h	no	5 h after replacement of ethanol with water	plasma catecholamines	withdrawal without scoring (with rest)
(7) Drinking ethanol in drinking water (for details, see Materials and methods)	ethanol replaced with water for 5 h	yes	5 h after replacement of ethanol with water	withdrawal hyperexcitability and plasma catecholamines	withdrawal with scoring (with test)

[a] All animals were decapitated between 11 AM and 1 PM.

blood was sampled for plasma catecholamines. This way, ethanol withdrawal was precipitated but not measured (withdrawal without scoring).

Group 7. In tolerant/dependent animals, ethanol withdrawal was precipitated by replacing ethanol with water on day 15 and withdrawal hyperexcitability (handling-induced convulsion) was scored according to Goldstein (1972). The scoring rate was as follows:

0: no convulsion,
1: facial grimace (after 180° spin),
2: tonic convulsion (after 180° spin),
3: tonic-clonic convulsion (after 180° spin),
4: tonic convulsion (when lifted by tail),
5: tonic-clonic convulsion (when lifted by tail),
6: severe tonic-clonic convulsions of long duration (when lifted by tail),
7: severe tonic-clonic convulsions of long duration (before lifted by tail),
8: severe tonic-clonic convulsions ending with death.

Mice were decapitated and blood was collected for plasma catecholamine measurements immediately after withdrawal testing (withdrawal with scoring).

2.2. Measurement of plasma catecholamines

Trunk blood was collected in heparinized tubes and immediately immersed in an ice bath. Then, 10 μl of 10% sodium metabisulfite solution was added to each blood sample to stabilize catecholamines. Blood was centrifuged at +4 °C with 2000 × *g* for 15 min. After removal of the plasma, an aliquot of 250-μl mouse plasma was diluted to 1:10 with stabilized drug-free serum (Bio-Rad, USA). Samples were stored at −70 °C until estimation, but for not longer than 14 days. Catecholamines were measured with high-performance liquid chromatography (HPLC) with electrochemical detection (ISCO, USA). The plasma catecholamine assay (PCAT) of Bio-Rad was used. A constant flow rate of 1.1 ml/min was maintained. The assay principle is based on the absorption to, and then subsequent extraction from alumina of catecholamines. The PCAT analytical column uses an isocratic mobile phase, where separation of adrenaline, noradrenaline and internal standards (IS) takes place. Quantition is based on the comparison of the adrenaline/IS and noradrenaline/IS peak height ratios in the unknown sample, to the corresponding ratios in the calibrated plasma samples. The detection limit for adrenaline was 4 pg (10 pg/ml plasma level), whereas that for noradrenaline was 10 pg (25 pg/ml plasma level). The within-run precision for adrenaline was 4% (coefficient of variation), and for noradrenaline, it was 3%. The run-to-run precision for adrenaline was 3%, whereas for noradrenaline, it was 2%. Plasma catecholamine levels are expressed in pg/ml ± standard deviation (S.D.) of the mean. Individual noradrenaline/adrenaline ratios are expressed as well.

2.3. Blood ethanol levels

Blood ethanol levels were measured with the UV photometric method of Boehringer Diagnostica Mannheim. The assay principle is based on measurement of an increase in NADH on enzymatic dehydrogenation of NAD. Samples were measured on an automated clinical chemistry analyzer (Hitachi-911) at a wavelength of Hg 365 nm. Results are expressed as mg ethanol/ml blood.

2.4. Statistical evaluation of the data

Data were analyzed with one-way analysis of variance (ANOVA) followed by the post hoc comparison with Scheffe's test, correlation and linear regression analysis. An SPSS-10 statistical program for Windows was used for statistical analysis. A probability level of less than 5% was accepted as indicating significant differences.

3. Results

Plasma adrenaline levels were significantly different in the various treatment groups ($F_{(6)}=21.9$, $P<0.001$). Acute i.p. ethanol treatment ($P<0.001$), as well as acute ethanol ingestion ($P<0.05$), significantly elevated plasma adrenaline levels. The rise in plasma adrenaline after acute i.p. ethanol treatment was significantly ($P<0.05$) higher than that after p.o. ethanol ingestion. Plasma adrenaline levels measured following ingestion of isocaloric sucrose or of ethanol for 14 days were not different from those measured in the intact control animals. Thus, the elevated plasma adrenaline following acute (p.o. or i.p.) ethanol ingestion was normalized in the 2-week period of regular p.o. ethanol intake. As compared to the levels in tolerant/dependent animals, plasma adrenaline levels significantly increased following ethanol withdrawal. This increase was significant irrespective of whether withdrawal symptoms were actually measured ($P<0.01$) or not ($P<0.05$). Thus, ethanol withdrawal itself—but not the testing procedure for withdrawal—elevated the plasma level of adrenaline (Fig. 1).

There was a significant difference in the noradrenaline levels as a result of various treatments ($F_{(6)}=33.6$, $P<0.001$). Acute i.p. ethanol treatment ($P<0.001$) and acute p.o. ethanol ingestion ($P<0.05$) resulted in significant increases in plasma noradrenaline. Acute i.p. ethanol treatment induced a significantly higher ($P<0.05$) increase than acute p.o. ethanol ingestion. Following sucrose ingestion, plasma noradrenaline levels were not statistically different from those of the intact controls. Neither was a difference observed between intact control and the tolerant/dependent animals after ethanol ingestion for 14 days. Compared to the levels of tolerant/dependent animals, plasma noradrenaline

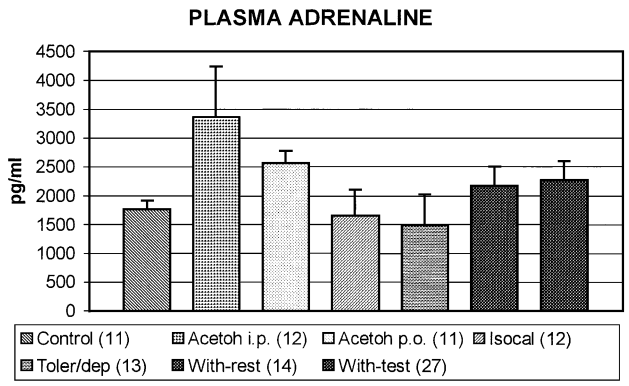

Fig. 1. Plasma adrenaline levels in mice following acute or chronic ethanol administration. The bars represent the means ± S.D. The number of investigations is shown in brackets. For abbreviations, see Table 1.

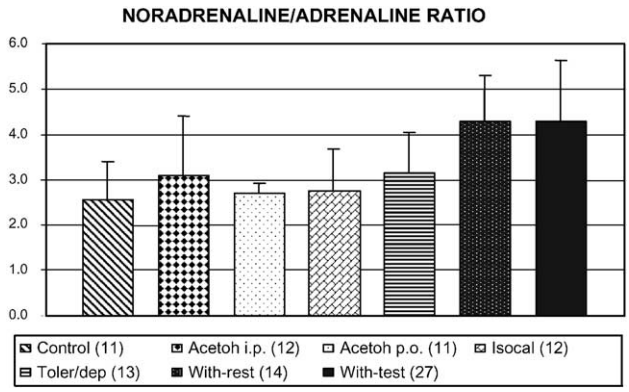

Fig. 3. Plasma noradrenaline to adrenaline ratios in mice following acute or chronic ethanol administration. For legend, see Fig. 1.

levels were significantly increased as a result of ethanol withdrawal ($P<0.001$). This withdrawal-induced elevation of plasma noradrenaline was present to a similar extent in mice tested, as well as in the animals not tested, for withdrawal (Fig. 2).

Fig. 3 summarizes the results of the ratios of noradrenaline to adrenaline in the various treatment groups. The ratio in the intact control animals was 2.56 ± 0.82 (mean ± S.D.). Significant differences were observed within the various treatment groups ($F_{(6)}=7.7$, $P<0.001$). The between-group comparison revealed that neither acute i.p. ethanol treatment, acute p.o. ethanol ingestion, isocaloric sucrose ingestion nor the development of ethanol tolerance/dependence was able to affect the noradrenaline/adrenaline ratios in a significant manner. However, the noradrenaline/adrenaline ratio during ethanol withdrawal was significantly increased ($P<0.05$ in mice without withdrawal testing; $P<0.001$ in mice with withdrawal testing). The increased ratio of noradrenaline to adrenaline revealed large individual variations during ethanol withdrawal.

Ethanol ingestion for 14 days resulted in medium to severe withdrawal hyperexcitability (withdrawal score: 3.1 ± 1.1). The withdrawal score of the animals treated with isocaloric sucrose was negligible (0.3 ± 0.1), whereas the theoretical maximum—supposing all the ethanol-dependent animals had died of withdrawal seizures—is 8.0. A significant positive correlation was found between withdrawal hyperexcitability and plasma noradrenaline/adrenaline ratios (coefficient: +0.893, $N=27$, $P<0.01$; regression analysis is illustrated in Fig. 4) and also between withdrawal hyperexcitability and plasma noradrenaline levels (coefficient: +0.811, $N=27$, $P<0.01$; data not illustrated). The correlation between withdrawal hyperexcitability and plasma adrenaline levels was not significant (coefficient: −0.336, $N=27$, $P>0.05$; data not illustrated). These findings indicate that ethanol-dependent mice with higher plasma noradrenaline levels and with subsequently increased noradrenaline/adrenaline ratios exhibit more severe withdrawal symptoms (Fig. 4).

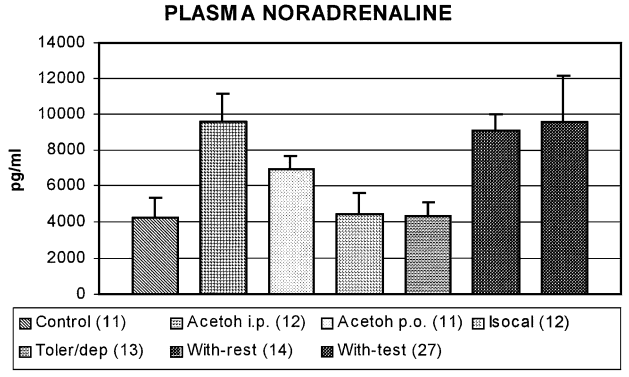

Fig. 2. Plasma noradrenaline levels in mice following acute or chronic ethanol administration. For legend, see Fig. 1.

Blood ethanol concentrations are depicted in Fig. 5. The blood ethanol levels varied significantly in the various treatment groups ($F_{(2)}=5.448$, $P<0.05$). Acute i.p. administration of 1.75 g/kg ethanol resulted in a blood ethanol concentration of 1.62 ± 0.49 mg/ml. Following ingestion of 5% ethanol for 1 day (average ethanol consumption of 13.8 g/kg/24 h), the blood ethanol concentration was also high,

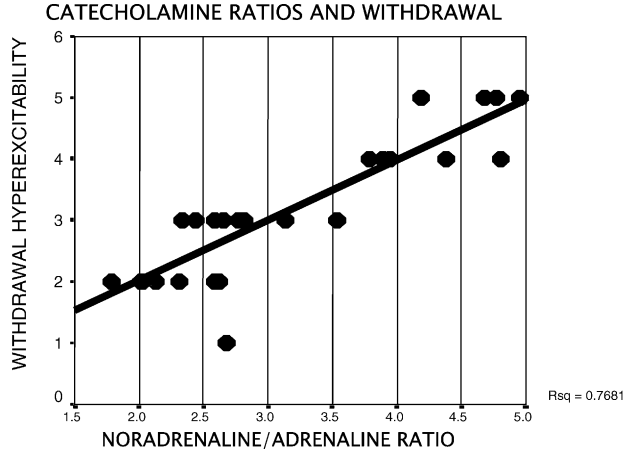

Fig. 4. The correlation of individual plasma noradrenaline/adrenaline ratios with the severity of withdrawal-induced hyperexcitability in ethanol-dependent mice.

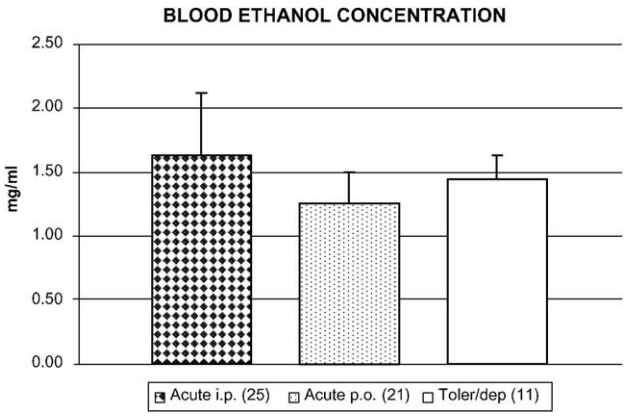

Fig. 5. Blood ethanol concentrations in mice following acute or chronic ethanol administration. For legend, see Fig. 1.

but significantly ($P < 0.05$) lower than that following acute i.p. treatment. Blood ethanol levels of the tolerant/dependent animals (following 14 days of ethanol ingestion) were not different from those after 1 day of ethanol drinking.

4. Discussion

In the present experiments, plasma levels of catecholamines (noradrenaline and adrenaline), as well as the ratio of the two catecholamines, were measured during acute ethanol treatment, ethanol tolerance/dependence and ethanol withdrawal in experimental mice.

Laso et al. (1990) in human patients found an elevated adrenaline level at the time of early withdrawal, patients with delirium showing greater values. Plasma noradrenaline concentrations were also increased at early withdrawal, but did not change significantly during the evolution of delirium tremens. Maki et al. (1990, 1998) also observed elevated plasma catecholamine levels at early withdrawal, as well as a rapid reversal of these changes during recovery from withdrawal. Other authors (Smith et al., 1990) also observed that plasma noradrenaline was significantly higher in patients undergoing ethanol withdrawal. Ehrenreich et al. (1997) found basal levels of adrenaline elevated in abstinent alcoholics (alcohol-free for at least 18 days), whereas noradrenaline levels tended to be lower than in nonalcoholic controls, resulting in a significantly decreased noradrenaline to adrenaline ratio in plasma. Despite the dramatically lowered plasma noradrenaline/adrenaline ratio, the noradrenaline response to stimuli was unaffected.

In ethanol-sensitive mice, the elevation of peripheral adrenaline and noradrenaline following ethanol challenge was tenfold higher than in nonsensitive animals (Zgombick et al., 1986). In a recent study, Kharchenko (2000) measured plasma catecholamine levels in rats with a disposition to alcohol consumption under conditions of free choice between alcohol and water. The author concluded that the effect of ethanol was centrally mediated through an increased sympathetic outflow, rather than by a direct effect of ethanol on the adrenal medulla, and that there are contact points between the ‘‘acetaldehyde’’ and the ‘‘catecholamine’’ hypotheses of the pathogenesis of alcoholism.

In the present experiments, the effect of acute i.p. administration of 1.75 g/kg ethanol on plasma catecholamines was significantly larger than that of the p.o. ingestion of 5% ethanol for 24 h. This is probably due to the fact that an intraperitoneal injection of ethanol per se may be considered as a stressful stimulus for animals. In both acute treatment models, noradrenaline and adrenaline levels were elevated to a similar extent; thus, the ratio of the two catecholamines was not different from that of the intact control animals. These findings are in agreement with those of other investigators (Forman et al., 1988), indicating that acute ethanol treatment elevates plasma catecholamines after exposure to continuous ethanol vapor, and suggesting that acute ethanol administration causes the release of glycogenolytic hormones. Following i.p. administration, ethanol is also known to increase blood levels of catecholamines (Zgombick and Erwin, 1988).

Previous research found that alcohol-preferring (C57BL/6) mice that voluntarily drank from a 10% (v/v) ethanol solution during a 24-h period consumed an average of 5 g/kg ethanol and had a blood ethanol level of 1.25 mg/ml (Dole and Gentry, 1984). The variation reported for various mouse strains is extremely large, i.e. some mouse strains—in particular, those genetically predisposed to drink alcohol—ingest 25 g/kg ethanol per day (Banks and Kastin, 1989; Plotkin et al., 2001). In our recent experiments, the average ethanol consumption in CFLP mice was 22.2 g/kg/24 h on the second week of ethanol ingestion, when the drinking water contained 7% (v/v) ethanol (data not illustrated). The observation that blood ethanol concentrations following the chronic ingestion of 7% (v/v) ethanol was not higher than those following the acute ingestion of 5% ethanol already indicates the appearance of metabolic tolerance in our 14-day alcohol treatment schedule.

Examples from both human and animal research indicate that high levels of ethanol drinking are often associated with resistance to the physiological effects of this drug (Schuckit, 1994; Kurtz et al., 1996). In our mice rendered tolerant to and dependent on ethanol, plasma adrenaline and noradrenaline levels were back to the range of the intact control animals and were not different from those of mice treated with isocaloric sucrose for 14 days either. Thus, it appears that tolerance developed to the catecholamine-increasing effect of acute ethanol treatment, as it does to many other physiological and behavioral effects of ethanol.

Ethanol withdrawal in the tolerant/dependent animals resulted in a significant rise in plasma adrenaline levels and even more in noradrenaline levels. As a consequence, the ratio of noradrenaline to adrenaline substantially increased in these animals. Neither the mechanism nor the importance of such a dissociated catecholamine increase is clearly understood. The selective elevation of one or the

other catecholamine may also be related to the differential time gradient of their secretion into and/or elimination from plasma, respectively (Kraemer et al., 1991). It has been concluded by Kjaer (1998) that trained individuals have a higher adrenaline secretion capacity compared to nontrained individuals and even more so when compared to individuals with a sedentary lifestyle. This indicates a development of long-term adaptation of the adrenal medulla. Such an adaptation is parallel to adaptations taking place in other tissues, such as skeletal muscle and the heart, and can be advantageous in stressful situations. In the present experiments, mice with a lower noradrenaline to adrenaline ratio exhibited milder ethanol withdrawal symptoms, and there was a close correlation of the catecholamine ratio to the severity of withdrawal hyperexcitability.

Recording parallel changes in biochemistry and behavior usually lead to the presumption that they are causally linked, and that the former is justified by the latter. However, until one can confirm that this is the case, we are unlikely to identify factors which increase vulnerability to ethanol addiction, or to develop ways of protecting susceptible individuals. The present experiments raise the possibility that the noradrenaline to adrenaline ratio in plasma might be an indicator of the adaptive capacity to chronic ethanol ingestion.

Acknowledgements

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References

Banks, W.A., Kastin, A.J., 1989. Inhibition of the brain to blood transport system for enkephalins and tyr-MIF-1 in mice addicted or genetically predisposed to drinking ethanol. Alcohol 6, 53–57.

Borg, S., Kvandle, H., Mossberg, D., Valverius, P., Sedvall, G., 1983. Central nervous system norepinephrine metabolism and alcohol consumption in man. Pharmacol. Biochem. Behav. 18, 375–378.

Cannon, W.B., de la Paz, D., 1911. Emotional stimulation of adrenal secretion. Am. J. Physiol. 28, 64–70.

Dole, V.P., Gentry, R.T., 1984. Toward an analogue of alcoholism in mice: scale factors in the model. Proc. Natl. Acad. Sci. 81, 3543–3546.

Ehrenreich, H., Schuck, J., Stender, N., Pilz, J., Gefeller, O., Schilling, L., Poser, W., Kaw, S., 1997. Endocrine and hemodynamic effects of stress versus systemic CRF in alcoholics during early and medium term abstinence. Alcohol Clin. Exp. Res. 21, 1285–1293.

Eisenhofer, G., Szabo, G., Hoffman, P.L., 1990. Opposite changes in turnover of noradrenaline and dopamine in the CNS of ethanol-dependent mice. Neuropharmacology 29, 37–45. Eur. J. Clin. Invest. 20, 313–316.

Fahlke, C., Berggren, U., Lundborg, C., Balldin, J., 1999. Psychopathology in alcohol withdrawal: relationship to α_2-adrenoreceptor function. Alcohol Alcohol. 34, 750–759.

Fink, G., 2000. Encyclopedia of Stress. Academic Press, San Diego, CA.

Forman, D.T., Bradford, B.U., Handler, J.A., Glassman, E.B., Thurman, R.G., 1988. Involvement of hormones in the swift increase in alcohol metabolism. Ann. Clin. Lab. Sci. 18, 318–325.

Frankenhaeuser, M., 1971. Behavior and circulating catecholamines. Brain Res. 31, 241–262.

Goldstein, D.B., 1972. Relationship of alcohol dose to intensity of withdrawal signs in mice. J. Pharmacol. Exp. Ther. 180, 203–215.

Hawley, R.J., Nemeroff, C.B., Bisette, G., Guidotti, A., Rawlings, R., Linnoila, M., 1994. Neurochemical correlates of sympathetic activation during severe alcohol withdrawal. Alcohol., Clin. Exp. Res. 18, 1312–1316.

Hoffmann, P.L., Tabakoff, B., 1985. Ethanol's action on brain biochemistry. In: Tarter, R.E., van Thiel, D.H. (Eds.), Alcohol and the Brain: Chronic Effects. Plenum, New York, pp. 19–68.

Kharchenko, N.K., 2000. Effects of glycine on the catecholamine levels and activities of alcohol-metabolizing enzymes in rats with alcohol intoxication and addiction. Neurophysiology 32, 312–320.

Kjaer, M., 1998. Adrenal medulla and exercise training. Eur. J. Appl. Physiol. Occup. Physiol. 77, 195–199.

Kovács, G.L., 1993. α-Atrial natriuretic peptide attenuates ethanol withdrawal symptoms. Eur. J. Pharmacol. 238, 417–419.

Kovács, G.L., 2000a. The role of atrial natriuretic peptide in alcohol withdrawal: a peripheral indicator or central modulator? Eur. J. Pharmacol. 405, 103–112.

Kovács, G.L., 2000b. Atrial natriuretic peptide modulates *N*-methyl-D-aspartate-induced hyperexcitability in ethanol-dependent mice. Eur. J. Pharmacol. 401, 343–347.

Kraemer, W.J., Patton, J.F., Knuttgen, H.G., Hannan, C.J., Kettler, T., Gordon, S.E., Dziados, J.E., Fry, A.C., Frykman, P.N., Harman, E.A., 1991. Effects of high-intensity cycle exercise on sympathoadrenal-medullary response patterns. J. Appl. Physiol. 70, 8–14.

Kurtz, D.L., Stewart, R.B., Zweifel, M., Li, T.K., Froehlich, J.C., 1996. Genetic differences in tolerance and sensitization to the sedative/hypnotic effects of alcohol. Pharmacol. Biochem. Behav. 53, 585–591.

Laso, F.J., Gonzales-Buitrago, J.M., Vicens, E., Moyano, J.C., 1990. Interrelationship between serum potassium and plasma catecholamines and 3′:5′ cyclic monophosphate in alcohol withdrawal. Drug Alcohol Depend. 26, 183–188.

Linnoila, M., Mefford, I., Nutt, D., Adinoff, B., 1987. Alcohol withdrawal and noradrenergic function. Ann. Intern. Med. 107, 875–889.

Maki, T., Toivonen, L., Koskinen, P., Naveri, H., Harkonen, M., Leinonen, H., 1998. Effect of ethanol drinking, hangover, and exercise on adrenergic activity and heart rate variability in patients with a history of alcohol-induced atrial fibrillation. Am. J. Cardiol. 82, 317–322.

Maki, T., Heikkonen, E., Harkonen, T., Kontula, K., Harkonen, M., Ylikahri, R., 1990. Reduction of lymphocytic beta-adrenoreceptor level in chronic alcoholics and rapid reversal after ethanol withdrawal. Eur. J. Clin. Investig. 20, 313–316.

Plotkin, S.R., Banks, W.A., Cohn, C.S., Kastin, A.J., 2001. Withdrawal from alcohol in withdrawal seizure-prone and -resistant mice: evidence for enkephalin resistance. Pharmacol. Biochem. Behav. 68, 379–387.

Schuckit, M.A., 1994. Low level of response to alcohol as a predictor of future alcoholism. Am. J. Psychiatry 151, 184–189.

Smith, A.J., Brent, P.J., Henry, D.A., Foy, A., 1990. Plasma noradrenaline, platelet alpha 2-adrenoceptors, and functional scores during ethanol withdrawal. Alcohol Clin. Exp. Res. 14, 497–502.

Zgombick, J.M., Erwin, V.G., 1988. Ethanol differentially enhances adrenocortical response in LS and SS mice. Alcohol 5, 287–294.

Zgombick, J.M., Erwin, V.G., Cornell, K., 1986. Ethanol-induced adrenomedullary catecholamine secretion in LS/Ibg and SS/Ibg mice. J. Pharmacol. Exp. Ther. 236, 634–640.