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The effect of ethanol on the biosynthesis and regulation of opioid peptides

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Summary. Alcoholism and alcohol abuse are serious health problems. Alcohol is known to influence the activity of a number of biological systems, for example the hormonal and neuronal systems. One of the biological systems whose activity is greatly influenced by alcohol is the endogenous opiate system. Alcohol modifies the function of both opiate receptors and opioid peptides. In fact it has been proposed that many of the effects of ethanol are mediated by its effects on the endogenous opiate system. This review will present results from various laboratories on the effects of acute and chronic ethanol treatments on various species, and on the release, biosynthesis and post-translational processing of the endorphins, enkephalins and dynorphins, the three known families of endogenous opioid peptides. Furthermore, the effect of acute and chronic ethanol consumption on the β-endorphin system in man, and the possible implications of the functional activity of the endogenous opiate system for the genetic predisposition to alcoholism will be discussed.
Key words. Acute ethanol; chronic ethanol; endorphins; enkephalin; dynorphins; release; biosynthesis.

Alcohol is both a nutrient and a dangerous drug, depending on the amount consumed and the duration of its consumption. Prolonged ingestion of large amounts of alcoholic beverages leads to the development of alcoholism. At the present time alcoholism may be considered as the most popular and most widespread type of drug addiction, and it is characterized by the development of tolerance and physical dependence symptoms which are also found in other types of drug addition, for instance opiate addiction. Furthermore, it is known that several behavioural and pharmacological effects of ethanol are similar to those produced by opiates [39] and cross-tolerance develops between ethanol and morphine with respect to some of these effects [42, 61]. However, in contrast to opiates, which act through specific receptors, ethanol is believed to have a non-specific effect on lipid components of cellular membranes [31, 34]. The effects of ethanol on membrane lipids may in turn influence the activity of proteins which reside within the lipid environment of the membranes (receptors, enzymes) as well as the rate of release of certain cellular products (hormones, neurohormones and neurotransmitters). With the discovery of the endogenous opiate system (opioid peptides and receptors) it seemed reasonable to suggest that ethanol may exert some of its effects, like the reinforcing [5] and epileptogenic [69] effects, via interactions with the endogenous opiate system. Ethanol may interact with the endogenous opiate system by: a) the production of certain ethanol metabolites, the isoquinolines which bind to opiate receptors [17]; b) altering the binding properties of opiate receptors [11, 21], and c) altering the release synthesis and post-translational processing of endogenous opioid peptides [16, 27, 66, 67]. The objective of this review is to examine the effect of ethanol on the release, synthesis and post-translational processing of the endogenous opioid peptides.

Endogenous opioid peptides: origin, location, function

Since 1975, when the presence of enkephalins in brain extracts was reported [35], a number of peptides having an opiate-like function have been isolated from brain, the pituitary gland, the adrenal gland, the placenta and the gastrointestinal system [2]. Extensive research in a number of laboratories has elucidated the biosynthetic origin of all known opioid peptides at the protein, messenger RNA and gene levels [38, 55, 56]. The known opioid peptides come from one of three precursors: the *endorphins* from the β-endorphin/ACTH precursor known as pro-opiomelanocortin [55]; the *enkephalins* from the proenkephalin precursor [56]; and the *dynorphins* and *neoendorphins* from the prodynorphin precursor [38]. POMC can be synthesized in the anterior and intermediate lobes of the pituitary gland, the arcuate nucleus of the hypothalamus [15, 20, 48] and the nucleus tractus solitarious [3, 19]; β-EPLPS are also found in the gut [73], the placenta [24] and the gonads [45]. The proenkephalin-derived peptides and the peptides derived from the prodynorphin precursor are also found widely distributed in the brain, posterior pituitary, gut and adrenal gland [40, 50].

The final forms of opioid peptides produced and stored within a given tissue depend not only on the genetic code for the precursor, but also on the specific enzymes present in the tissue, which direct the post-translational processing of the precursor in certain ways, e.g. by cutting off specific peptides out of the precursor protein, and modifying these peptides by acetylation, amidation, phosphorylation, methylation, glycosylation or further cleavage. Thus in spite of the existence of a common gene for the precursor, its post-translational processing appears to vary from tissue to tissue. For example, in the anterior lobe POMC is processed mainly to β-LPH and β-EP, while in the NIL the final maturation products are the opiate-inactive α-N-acetylated forms of β-EP1-27 and β-EP1-31, with smaller quantities of the opiate-active non-acetylated forms [75]. In the hypothalamus, the midbrain and the amygdala, the opiate-active non-acetyl forms of β-EP1-31 predominate, while in the hippocampus, dorsal collicula and brain stem the opiate-inactive α-N-acetylated forms of β-EP predominate [75]. Since α-N-acetylation produces peptides devoid of opioid activity [4], whereas cleavage at the carboxyl terminal of β-EP produces peptides with decreased opiate activity [4], it is possible that these modifications serve a physiological function (a subject previously mentioned in this multi-author review). The processing of β-EP to its various fragments may be sensitive to environmental stimuli, or stress and drug treatments, so that the relative proportions of the β-EP forms in a region may be modified by these agents. In fact haloperidol, stress, acute and chronic ethanol treatment alter the relative proportions of the β-EP forms in the NIL [1, 25, 33, 66]. Thus measurements of total content of opioid peptides in a tissue may not be a sufficient indicator of its activity; but rather measurements of the relative proportions of the various forms of β-EP peptides may provide a better index of the functional activity of the opioid system in the tissue.

The existence of different types of opioid peptides is associated with the existence of multiple types of opiate binding sites, which are classified as μ, δ, κ, ε and σ depending on their affinity for specific opiate ligands. Thus, morphine binds preferentially to the μ type of binding site, enkephalins to δ, endorphins to ε and dynorphins to κ [51, 52, 74]. However, though opioid peptides interact preferentially with a particular class of opiate binding sites, they may also interact with one or more of the other types of receptors [12, 14]. Thus the true action of any opioid peptide depends upon the type of receptor present in the synapse of a specific opioid neuron.

A careful examination of the origin and distribution of endogenous opioid peptides clearly reveals that peptides of all three opioid families are found in the hypothalamus, pituitary and adrenal medulla, systems known to regulate the body's responses to stress. Similarly, the autonomic nervous system contains both endogenous

opioid peptides[63] and opiate receptors[52]. Thus an important function of the endogenous opiate system is to regulate the organism's responses to physiological and environmental demands, including physical and psychological stress. Various types of stress have been shown to induce the release and biosynthesis of endogenous opioid peptides. This increased activity of the endogenous opioid peptides following stress may help the individual to cope with the stressful situation. Alcohol has long been known as an anxiolytic agent. In fact it has been suggested that alcohol use and abuse becomes reinforcing because of its tension-reducing properties. According to this hypothesis, ethanol consumption relieves anxiety and may thus serve as an alternative response to cope with stress. The evidence for this hypothesis is both confirmatory and contradictory. Experimental data show that ethanol can reduce anxiety, but it can also produce anxiety. Since endogenous opioid peptides are important for mood changes and behaviour, some of the effects of ethanol may be mediated by its effects on the activity of the endogenous opiate system. Therefore the effects of both acute and chronic ethanol treatment on the activity of the endogenous opiate system have been studied by a number of laboratories.

Effects of acute ethanol exposure in vivo and in vitro on the endogenous opioid peptides

Proopiomelanocortin system

β-Endorphin is synthesized and released in the peripheral circulation by the pituitary gland. In addition, β-endorphin-related peptides are synthesized in neurons of the arcuate nucleus and the nucleus tractus solitarius and are carried by the nerve fibers to distinct regions of the brain, where they are released by the appropriate stimuli. Alcohol interacts with both the pituitary and the brain β-EP systems. Rats injected i.p. with ethanol (2.5 g ethanol per kg b. wt) exhibited an increase in the content of irβ-EP in the hypothalamus at 20 min post-injection. This increase was still present 60 min after ethanol administration. However, in this study no significant change was noticed in the pituitary content of irβ-EP[64]. In another study a similar dose of ethanol had no effect on either pituitary or hypothalamic content of irβ-EP at 60 min post-ethanol[65]. On the other hand, at 45 min after i.p. injection of 3.5 g ethanol per kg b. wt the content of irβ-EP was increased in the plasma and decreased in the AL of the pituitary gland. Furthermore, a small not statistically significant decrease was observed in the irβ-EP content in the NIL and hypothalamus[25]. Thus it seems that there is no agreement on the effects of acute ethanol treatment on the β-EP system.

Since the tissue content of a substance is usually the net balance of its rate of biosynthesis, release and degradation, ethanol treatment could induce changes in these processes which might balance each other, so there would be no apparent change in the total tissue content. The release of β-EP is controlled mainly by CRF in the AL and by an inhibitory dopaminergic and a stimulatory adrenergic system in the NIL[3,30,49,57,58,71]. A dopaminergic control on the β-EP release by the hypothalamus has been reported[72]; however, a recent report demonstrated that neither dopamine nor dopamine antagonists alter the hypothalamic content of β-EP[18]. Ethanol has been shown to modify the activity of the brain dopaminergic system, and the specific effect depends on the dose of ethanol administered and on how long after ethanol administration the investigations were carried out[9,44]. Ethanol at low doses decreases the release of dopamine, while ethanol at high doses increases the release of dopamine[44]. Furthermore, both low and high doses of ethanol increase the synthesis of dopamine[44]. Thus the effect of ethanol on the pituitary and brain β-EP systems may vary depending on the dose of ethanol used and how long after ethanol administration the investigations are carried out.

ACTH, a hormone which has the same precursor as β-EP, is co-released with β-EP from the pituitary gland under a number of physiological conditions such as stress[1,6,32,47]. Acute ethanol administration at 1.0 and 3.0 g ethanol per kg b. wt increased the release of ACTH and corticosterone[60]. Furthermore, i.v. injection of antiserum to corticotropin-releasing factor, together with the i.p. injection of ethanol, abolished the increased release of ACTH and corticosterone observed when ethanol was administered alone[60]. Thus there is evidence that ethanol exerts its effect on AL and NIL by its effects on hypothalamic neurotransmitters like CRF. However, ethanol could also have a direct effect on the AL and NIL of the pituitary gland. To investigate the direct effect of ethanol on AL and NIL a number of in vitro studies have been performed. Exposure of cultured pituitary cells to 0.2% ethanol did not alter the basal release of ACTH[60]. However, in a different study, exposure of dispersed mouse adenohypophyseal cells to 17 mM ethanol induced a threefold increase in the release of both ACTH and β-EP[43]. This ethanol-induced increase was transient, lasting for 10–15 min, and required extracellular calcium. Furthermore, a second ethanol exposure less than one hour after the first exposure did not stimulate the release of β-endorphin, which suggested that the adenohypophyseal cells become insensitive to ethanol[43]. In another study superfused fragments of rat anterior lobes were exposed to various concentrations of ethanol from 4 to 44 mM (20–200 mg/dl). Results indicated a dose-related increase in ACTH release, which was maximum at 8.7 mM ethanol (40 mg/dl)[59]. In fact a higher concentration of ethanol, 43.5 mM (200 mg/dl), led to a decrease in ACTH release. The response to each dose of ethanol was multiphasic, consisting of three peaks of ACTH release within the first 30 min after ethanol addition in the incubation medium[59]. In these studies the release of β-EP was not measured, but since usually ACTH and β-EP are co-released, it is reasonable to anticipate a similar pattern

of β-EP release from the AL following ethanol exposure. Such a multiphasic pattern of release may partially account for the conflicting results obtained by various laboratories.

A limited number of studies have been performed investigating the direct effect of ethanol on the NIL of the pituitary gland. In one study, NILs were exposed to 60 mM ethanol (≈ 300 mg/dl) for 3 h [22]. Results indicated that ethanol had no direct effect on either the biosynthesis or release of β-EP related peptides by the rat NIL. However, additional studies should be performed using lower concentrations of ethanol and shorter incubation periods. Such studies may show a direct effect of ethanol on the NIL β-EP system as was shown for the AL.

Proenkephalin and prodynorphin systems

There are a limited number of studies investigating the effect of acute ethanol treatment on the brain enkephalin and dynorphin related peptides. 2.5 g ethanol per kg b. wt (i.p.) induced no significant change in the content of met-enkephalin in the hypothalamus, medulla/pons and midbrain measured at 20 and 60 min post-ethanol treatment, while a significant increase was noticed in the striatum at 60 but not at 20 min post-ethanol [64]. In a different study 2.5 g ethanol per kg b. wt i.p. induced a significant increase in the met-enkephalin content in the hypothalamus, striatum and midbrain but not in the hippocampus at 60 min after ethanol administration [65]. The levels of immunoreactive dynorphin and α-neo-endorphin in the hypothalamus, striatum midbrain and hippocampus were not significantly altered at 60 min after i.p. injection of 2.5 g ethanol per kg b. wt [65].

Thus a) the effect of ethanol is different for the various types of opioid peptides even when they are tested in the same tissue e.g. hypothalamus, and b) the effect of ethanol on the same opioid peptide may be different in different tissues, e.g. the effects on β-endorphin in the AL, hypothalamus and midbrain. Furthermore, it seems that the effect of ethanol on the endogenous opioid peptides is a specific effect involving interactions of various neurotransmitters and neurohormones (such as dopamine and CRF) with the endogenous opiate system. As a result of these interactions the effect of ethanol may be different for the different classes of opioid peptides or for the same opioid peptide in different tissues. In addition, the dose of ethanol used is important in determining its effect since low doses may stimulate and high doses may inhibit the release of endogenous opioid peptides by particular tissues.

It appears then that when an individual drinks a certain quantity of alcohol, the endogenous opioid system may exhibit a number of responses. Within a short time after ethanol ingestion, when the blood alcohol concentration is low, ethanol may induce a stimulatory effect on the release of pituitary and hypothalamic β-endorphin, leading to decreased β-EP content in the tissue. However, at longer time intervals after ethanol ingestion, when the blood alcohol content reaches a higher level, depending on the quantity consumed, ethanol may exert an inhibitory effect on the release of pituitary and hypothalamic β-EP. In fact such a pattern of release of endogenous opioid peptides may partially explain the initial mild euphoric and anxiolytic effects of ethanol, and the depressive and anxiogenic effects which have also been observed after ethanol consumption.

Effect of chronic ethanol treatment on the endogenous opiate system

Proopiomelanocortin system

It has been clearly demonstrated that chronic ethanol treatment influences the activity of the endogenous opiate system. Furthermore, as with acute ethanol treatment, there is variation in the results obtained by different laboratories. Thus increase, decrease or no change in the activity of the endogenous opioid systems following chronic ethanol treatment have been reported. This variability may be partially due to differences in the route of ethanol administration, the quantity of alcohol consumed, the length of ethanol treatment, and the species and strains used for the studies.

Ethanol administered as a 5% or 20% solution in the drinking water induced a decrease in the β-EP content in the rat AL and NIL of the pituitary gland [64]. This decrease was evident as early as 6 days after initiation of the ethanol treatment. A maximum decrease of about 20% compared with the controls was observed after 14 days of ethanol treatment [64]. Following ethanol withdrawal the levels of immunoreactive β-EP were restored to normal values within 6 days in the AL and 14 days in the NIL [64]. In contrast to acute ethanol treatment, which induced an increase in the hypothalamic content of β-EP, chronic ethanol treatment had no effect [64, 65]. Using guinea pigs as the experimental animal, chronic ethanol treatment (5–15% ethanol in the drinking water) for 30 days induced a decrease in the AL, NIL and hypothalamic content of irβ-EP [64]. Golden hamsters given a 10% ethanol solution for two weeks presented no significant difference in brain β-EP values from untreated controls [13].

The administration of 15% ethanol in the drinking water to Sprague-Dawley rats was not associated with any alteration in hypothalamic β-EP content unless a weight loss had occurred as a result of the ethanol treatment [28]. Chronic ethanol treatment using an ethanol-containing liquid diet had no effect on the immunoreactive β-EP content in the hypothalamus, midbrain, AL and NIL of the rat, though small non-significant decreases were observed [65]. Administration of ethanol in a liquid diet, which allowed animals to maintain stable growth at the same rate as pair-fed non-alcohol control rats [41], induced a significant decrease in the AL content of β-EP but not in the NIL [23, 36]. Following ethanol withdrawal the content of immunoreactive β-EP was decreased in both the AL and NIL and returned to control values within 8 days

of ethanol withdrawal[26, 36]. No significant change was noticed in the β-EP content of distinct areas of the brain after 21 days of ethanol administration in a liquid diet[36]. However, a decrease in the β-EP content was observed in the arcuate nucleus, amygdala, septum, periventricular thalamus and preoptic periventricular hypothalamus, on days 1 and 3 of ethanol withdrawal, with complete recovery to control levels by days 8 and 15 of withdrawal[36]. Thus it appears that ethanol withdrawal induces an initial depletion of β-EP related peptides in the AL, NIL and distinct brain regions[26, 36]. Most likely this depletion is not due to a direct effect of ethanol treatment, but to the increased release of β-EP peptides following the stress of the withdrawal reaction[26, 36]. Administration of ethanol to rats via a different route, chronic exposure to ethanol vapors in a vapor inhalation chamber, induced a decrease in the content of immunoreactive β-EP in the pituitary gland and the plasma, associated with decreases in the CRF binding and adenylate cyclase activity in the cell membranes of AL and NIL[16].

Thus, there are a number of conflicting reports on the effect of chronic ethanol treatment on the content of β-EP in the pituitary gland and distinct areas of the brain. However, tissue levels of β-EP reflect the net balance between the de novo synthesis of the β-EP/ACTH precursor, proopiomelanocortin, its maturation to β-EP and the release and degradation of β-EP. It is possible that there might be changes in synthesis, release and degradation of β-EP which would balance each other and therefore not give rise to changes in the tissue content. To investigate this possibility a number of laboratories studied the effect of alcohol treatment on the in vitro incorporation of radioactive amino acids into β-EP and its precursor peptides POMC and β-LPH, and also the content of mRNA coding specifically for POMC. Thus chronic treatment of rats with 15% (vol/vol) ethanol in tap water for 3 weeks induced a decrease in the NIL content of β-EP and its release into the incubation medium, as well as in the in vitro incorporation of ^{3}H-phenylalanine into β-EP related peptides[67]. In fact, ethanol treatment induced a decrease in the biosynthesis of POMC as well as in the rate of the post-translational processing of POMC to β-EP[67], which suggested that the ethanol treatment influenced the activity of the enzymes processing the POMC to β-EP[71].

In contrast to these findings, chronic treatment of rats with ethanol by intragastric intubation[27] or by an ethanol-containing liquid diet[23, 26, 66] resulted in a pronounced increase in the in vitro incorporation of ^{3}H-phenylalanine and ^{3}H-tyrosine into POMC, β-LPH and β-EP by the NIL and AL of the pituitary gland. Furthermore, the time course of the post-translational processing of POMC to β-LPH and β-EP showed that this was accelerated[66]. Since the in vitro biosynthesis of total proteins by the AL and NIL was not significantly altered by the ethanol treatment, the ethanol-induced stimulatory effects on the β-EP biosynthesis were not due to a non-specific increase in protein synthesis[13, 67, 69]. This increase in the biosynthesis of β-EP was associated with an increase in the in vitro release of β-EP related peptides[23, 26, 66]. The increased in vitro release is consistent with the increased plasma content of irβ-EP after 15 and 21 days of ethanol treatment[67], and with the elevated corticosterone levels in the plasma of mice treated chronically with a liquid ethanol diet, suggesting an increased ACTH release by the AL[70].

Exposure of rats to ethanol vapors for 1, 7 and 14 days produced a time-related decrease in the POMC mRNA levels relative to total RNA levels in both AL and NIL[16]. The decrease was more pronounced in the NIL[16]. This decrease in POMC mRNA was associated with a decreased content of β-EP peptides in the plasma, suggesting a decreased release of β-EP by the pituitary gland[16]. Furthermore, the pituitaries of animals treated chronically with inhalation of ethanol vapors exhibited a decreased binding of CRF and a decreased adenylate cyclase activity[16] which may be responsible for the decreased release of β-EP by the pituitary gland.

One of the important post-translational modifications of β-EP is α-N-acetylation, which renders the molecule opiate-inactive[75]. Thus it is noteworthy that chronic ethanol treatment increases the rate of α-N-acetylation of β-EP by the NIL. In the NILs from control animals, 70% of the radio-labelled β-EP underwent α-N-acetylation[67]. In the NILs from ethanol-treated animals the relative proportions of the acetylated forms of β-EP were significantly increased[67]. An increased content of the acetylated forms of β-EP was also observed in the NIL extracts following 15 days of ethanol withdrawal[26]. Therefore ethanol increases the activity of the enzyme acetyltransferase, leading to the synthesis of a large proportion of acetylated opiate-inactive forms of β-EP.

There are thus a number of conflicting reports on the effect of ethanol treatment on the pituitary β-EP system. It seems that the route of ethanol administration, and the effect of the treatment on the nutritional state of the animal, may be important factors determining whether an increase or a decrease in the activity of the pituitary β-EP system will occur. For example, continuous exposure of rats to ethanol vapor in a vapor inhalation chamber, under conditions which also sustained normal body growth, led to decreased content of β-EP and mRNA coding specifically for POMC in the AL and NIL. The vapor inhalation technique results in constant high blood ethanol concentrations[41], so that the animal does not experience withdrawal until it is removed from the vapor chamber.

In contrast, animals consuming ethanol in a liquid diet show marked diurnal variations in blood alcohol concentration. During nocturnal feeding, the blood alcohol content can exceed 200 mg/dl. By 09.00 h (3 h after the light cycle) the blood alcohol content has fallen to 43–120 mg/dl. By mid-afternoon, when the animals are mainly sleeping and have not eaten for many hours, the blood alcohol

content can fall to undetectable levels. Thus, the animals experience frequent periods of relative or actual withdrawal during the course of chronic ethanol treatment. There are at least two ways in which this could conceivably affect the function of the pituitary β-EP system. The first way might be the intermittent normalization of the CRF response. Ethanol has been shown to reduce the hypothalamic content of CRF[60] and the binding of CRF to pituitary membranes[16]. Therefore, the intermittent periods of low or zero blood alcohol content might permit replenishment of hypothalamic CRF and restoration of normal CRF binding ability of the pituitary receptors. Such replenishment is not possible when ethanol is administered by the constant presence of ethanol vapor. The second way might be that repeated short withdrawal periods may intensify the withdrawal reaction[10]. Thus the activity of the pituitary β-EP system may become magnified by repeated experiences of mini-withdrawals during the period of chronic ethanol ingestion.

Proenkephalin and prodynorphin systems

Though the effect of ethanol on the pituitary β-EP systems has been investigated more extensively, there are a number of studies on the effect of chronic ethanol treatment on the other two classes of opioid peptides, the enkephalins and the dynorphins. Chronic administration of ethanol to rats (as a 20% solution in the drinking water) for 30 days induced a significant decrease in the met-enkephalin content in the striatum, medulla/pons and midbrain but not in the hypothalamus[64]. A complete recovery to control levels was achieved within 6 days after withdrawal of the ethanol treatment[64]. Guinea pigs given 15% ethanol in their drinking water for 30 days exhibited a significant decrease in the met-enkephalin content in the striatum, medulla/pons and midbrain and a non-significant decrease in the hypothalamus[64]. Chronic ethanol treatment of rats using an ethanol-containing liquid diet induced a significant decrease in the content of met-enkephalin in the hypothalamus and in the striatum but not in the midbrain and hippocampus[65]. In contrast the content of immunoreactive dynorphin and α-neoendorphin was significantly decreased in the hypothalamus and hippocampus but not in the striatum, the midbrain, or the anterior and neurointermediate lobes of the pituitary gland[65]. A decrease in the immunoreactive leu-enkephalin content was noticed in hamster basal ganglia after long-term treatment with ethanol[8].

It is apparent that both acute and chronic ethanol treatments influence the activity of the endogenous opioid peptides. However, there is considerable conflict about the precise effects of both acute and chronic ethanol treatment. This conflict could be partially due to the fact that various routes of ethanol administration have been used, various doses of ethanol have been administered, and the duration of ethanol treatment, as well as the species and strains of experimental animals, has varied.

Effect of ethanol on the endogenous opiate system in humans

It is generally accepted that ethanol stimulates the release of CRF and ACTH, which in turn stimulates the release of cortisol[46, 60]. A number of studies with normal human volunteers have indicated that acute ethanol treatment could induce either an increase or a decrease in the blood cortisol content[37, 53, 68]. An increase in the release of cortisol would indicate an increase in the ACTH release by the pituitary gland. ACTH and β-EP share the same precursor[15, 20] and are co-released from the pituitary gland under a number of physiological conditions such as stress[1, 32, 47]. Studies using chronic alcoholics undergoing rehabilitation treatment indicated a decrease in the CSF content of ACTH[29, 62], but no effect on plasma β-EP and ACTH levels[29, 62]. However, since at the time of testing these subjects were alcoholics under rehabilitation treatment, the differences noticed among the alcoholics and controls could be due to the ethanol withdrawal reaction and not to the ethanol treatment. Thus a depletion of the brain β-EP content due to increased release of β-EP by the stress of the ethanol withdrawal reaction may partially explain the low CSF levels of β-EP. Furthermore, degeneration of the β-EP producing neurons as a result of the chronic ethanol treatment cannot be excluded.

Recent studies in our laboratory using individuals at high and low risk for the future development of alcoholism (as determined from the history of alcoholism in their families for the last 3 generations) indicated that individuals at high risk for the future development of alcoholism had lower plasma levels of β-EP and cortisol at 09.00 h. Furthermore, previous alcoholics who had been abstinent for at least six months prior to testing also had lower plasma levels of β-EP and cortisol at 09.00 h (unpublished data). Administration of a low dose of ethanol (0.5 g ethanol per kg b. wt) to healthy non-alcoholic individuals with a high and low risk for future development of alcoholism induced a small increase in the plasma content of β-EP and cortisol of the high risk individuals but not of the low risk individuals, suggesting the implication of genetic factors on the response of the hypothalamic-pituitary-adrenal axis to ethanol. Support for a genetic control of the activity of the β-endorphin system is provided by basic studies using inbred strains of mice with varying sensitivity to alcohol[9, 25].

However, other investigators using different selection criteria for the individuals at high risk for the future development of alcoholism did not observe an increase in the plasma cortisol levels[68]. Estimations of β-EP and/or ACTH were not performed[68]. In different studies, using healthy human volunteers without consideration of family history for alcoholism, it was demonstrated that ingestion of low to moderate quantities of ethanol induced a decrease in plasma cortisol content[54] while high doses of ethanol induced an increase in plasma cortisol, ACTH and β-EP[54]. Thus, studies with human subjects also indi-

cate the importance of the ethanol dose used, the time interval after ethanol treatment and the implication of genetic factors in the response of the β-endorphin system to ethanol.

Abbreviations used. POMC = pro-opiomelanocortin; β-EP = β-endorphin; β-EPLPS = β-endorphin like peptides; β-LPH = β-lipotropin; NIL = neurointermediate lobe of the pituitary gland; AL = anterior lobe of the pituitary gland; irβ-EP = immunoreactive β-endorphin; ACTH = adrenal corticotropin; CRF = corticotropin releasing factor.

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