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Differential Effects of Acute and Chronic Ethanol Treatment on Particular Opioid Peptide Systems in Discrete Regions of Rat Brain and Pituitary

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SEIZINGER, B. R., K. BOVERMANN, D. MAYSINGER, V. HÖLLT AND A. HERZ. *Differential effects of acute and chronic ethanol treatment on particular opioid peptide systems in discrete regions of rat brain and pituitary.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 361–369, 1983.—Acute ethanol treatment induced a significant increase in the tissue levels of immunoreactive (ir-) Met-enkephalin in hypothalamus, striatum and midbrain, but not in hippocampus. Levels of ir-dynorphin, ir-α-neo-endorphin and ir-β-endorphin were not found to be significantly altered in brain and pituitary. Chronic ethanol treatment (by the use of ethanol liquid diet) resulted in a more than 50% decrease of the tissue levels of ir-dynorphin and ir-α-neo-endorphin in hypothalamus and hippocampus, while both peptides remained unchanged in midbrain, striatum, adenohypophysis and neurointermediate pituitary. In contrast, ir-met-enkephalin was decreased in striatum and hypothalamus, but unaffected in midbrain and hippocampus. Levels or ir-β-endorphin remained unchanged in the brain and in the pituitary. However, the de novo biosynthesis of β-endorphin and its prohormones β-lipotropin and pro-opiomelanocortin was increased in the intermediate pituitary and to an even more pronounced degree, in the adenohypophysis, after chronic treatment of rats with ethanol liquid diet. nevertheless, the amounts of opiate-active β-endorphin were found to be reduced in both lobes of the pituitary: In the adenohypophysis, this was due to a retardation of the enzymatic processing of β-endorphin from its precursor β-lipotropin, while in the intermediate pituitary the α-N-acetylation of β-endorphin to opiate-inactive α-N-acetyl-β-endorphin was stimulated. In conclusion, acute and chronic ethanol treatment caused selective alterations on different opioid peptide systems within distinct areas of the rat brain and pituitary.

Acute and chronic ethanol treatment Endogenous opioid peptides Brain Pituitary
Biosynthesis and processing

IT is well-known that effects of ethanol and opiate administration are similar in certain aspects, e.g., the production of euphoria, the development of tolerance and dependence, including certain aspects of withdrawal reactions subsequent to discontinuation of prolonged administration of the drug (for review: [31]). Moreover, morphine has been found to significantly attenuate the alcohol withdrawal syndrome [6]. Several common biochemical effects of ethanol and morphine have been documented, e.g., upon β-adrenergic receptor binding [1,35], calcium depletion [54] or biogenic amine metabolism (for review: [6]).

The evident similarities between a variety of the effects of ethanol and morphine raise the question as to possible common biochemical mechanisms of action for these drugs and lead to the hypothesis that ethanol may act indirectly via an activation of opiate receptors.

This hypothesis is supported by investigations showing that the specific opiate receptor antagonist naloxone is able to modify several behavioral and biochemical effects of ethanol [5, 28, 29, 54]. Moreover, we have recently demonstrated that chronic ethanol imbibition selectively interferes with δ-opiate receptors [42].

Two probably alternative mechanisms have been postulated to account for such an indirect action of ethanol at the opiate receptors: (a) The ethanol metabolite, acetaldehyde, may condensate with dopamine to form isoquinolines, alkaloid-like substances which are capable of binding to the opiate receptor [10]. (b) Ethanol may influence the synthesis and release of opioid peptides, the endogenous ligands of the opiate receptors. While the question of the putative relationship of isoquinolines to the link between alcohol and opiates is still controversal [3], there is increasing evidence that ethanol influences the activity of endogenous opioid peptide systems.

For example, Naber *et al.* [39] reported a 4-fold increase in levels of opioid activity in the plasma of normal volunteers upon acute ethanol administration. A recent investigation in our laboratory [45] demonstrated differential effects of acute

and chronic ethanol treatment on β-endorphin (β-E) and met-enkephalin (MET-E) levels in the rat brain and pituitary. Moreover, Blum *et al.* [4] recently documented a reduction in leu-enkephalin-like immunoreactivity in hamster basal ganglia after long-term ethanol exposure.

In addition to these well-known peptides, novel opioid peptides have recently been isolated and characterized, e.g., dynorphin (DYN) [16, 17, 18, 24, 26, 48, 49, 50, 57] and α-neo-endorphin (α-NEO-E) [32, 37, 38, 57].

Very recently, the complete primary sequences of two new opioid peptide precursors were determined: (a) pre-proenkephalin, which contains 6 sequences of MET-E and one sequence of leu-enkephalin [8, 20, 41], and (b) a precursor molecule containing the sequences of DYN, α-NEO-E and a third opioid peptide sequence [30].

Thus, three different opioid peptide systems are, at this time, known: (a) the pre-proenkephalin system (from which is derived MET-E), (b) the pro-DYN/α-NEO-E system, and (c) the pro-opiomelanocortin (POMC-) system (which contains β-E and adrenocorticotropic hormone (ACTH) [40].

The present study examines the effects of acute and chronic ethanol treatment upon each of the three opioid peptide systems (as represented by tissue levels of MET-E, DYN/α-NEO-E and β-E) in several areas of the rat brain and pituitary.

Moreover, in order to enhance our understanding of the biochemical mechanisms underlying ethanol-induced alterations in tissue levels of opioids, the effects of chronic ethanol treatment upon the biosynthesis, post-translational processing and modification of β-E-related peptides in the rat adenohypophysis and intermediate pituitary were investigated.

METHOD

Animal Treatment

Male Sprague-Dawley rats initially weighing 200–220 g were treated with ethanol either acutely or chronically.

Acute Ethanol. In the acute experiments, ethanol was injected IP (2.5 g/kg body weight; 25% v/v ethanol in 0.9% saline). Controls received only saline and both groups were maintained on food and water ad lib. Animals were sacrificed 1 hr following the ethanol injection.

Chronic ethanol. Chronic treatment was performed using the liquid diet method (7% v/v ethanol) as described by Lieber and De Carli [34]. Briefly, after an initial fasting period of 2 days, which reduced animal body weight to ca. 80% of initial weight, rats were pair-fed for 14 days. Controls received exactly the same amounts of diet as the ethanol-treated group, but, rather than ethanol, an isocaloric amount of carbohydrates was provided. Body weight was measured every second day, at the time of refilling feeding tubes (6 p.m.), over the two-week period.

Tissue Extraction

After the treatment period, rats were killed by decapitation, trunk blood was collected, and the brains immediately removed and dissected according to a procedure described by Glowinsky and Iversen [15]. Pituitaries were divided *in situ* into anterior lobes (ALs) and neurointermediate lobes (NILs). Tissues were boiled in 0.1 M HCl for 15 min, homogenized, centrifuged and the supernatants frozen prior to radioimmunological detection of opioid peptides as described [24].

Radioimmunoassay (RIA-) Procedures

All RIAs were performed according to the protocol previously described [24].

Antibodies used: (a) For α-NEO-E-RIA: antiserum "Agathe" [37]; (b) for DYN-RIA: antiserum "Lucia" (kindly provided by Dr. Avram Goldstein, Stanford, CA [13]; (c) for total β-E-immunoreactivity: antiserum "Horace" [23]; (d) for α-N-acetylated β-endorphins: a highly specific antiserum, directed against α-N-acetyl-camel-β-E_{1-31}, which only recognizes α-N-acetylated opioid peptides (kindly provided by Dr. E. Weber, Stanford, CA) [56]; (e) for opiate-active non-acetylated β-endorphins: a monoclonal β-E-antiserum, highly specific for the sequence Tyr-Gly-Gly-Phe, which does not recognize α-N-acetylated opioid peptides [21]; Gramsch *et al.*, submitted); (f) for MET-E-RIA: antiserum "Bübchen I." "Bübchen I," directed against synthetic MET-E, was generated in rabbits as described for β-E antibodies [23].

"Bübchen I," used at a final dilution of 1:1000, has a detection limit of ~30 fmoles/tube. Leu-Enkephalin has an ~15% cross-reactivity to this antiserum. Camel-β-$E_{1-31,1-27}$, α-N-acetyl-camel-β-$E_{1-31,1-27}$ and $_{1-26}$, DYN_{1-17}, DYN_{1-8}, α-NEO-E, β-neo-endorphin, BAM-12P, BAM-22P, peptide E and Tyr-Gly-Gly-Phe are not recognized.

Determination of Blood Ethanol Levels

Blood, collected after decapitation, was de-proteinized with TCA, and blood ethanol levels were determined by use of the enzymatic method with ADH-NAD-NADH [7].

Biosynthesis Studies

Freshly dissected NILs and ALs from rats chronically treated with ethanol- or control-liquid diet were incubated in 1 ml Krebs-Ringer-bicarbonate solution (2 NILs, or 2 ALs per incubation tube). ^{3}H-Tyr (0.3 mCi/tube) was incorporated into peptides of the NIL for 2 hrs, and into those of the AL for 4 hrs (for exact incubation conditions see [46]). After the incubation periods, tissues were homogenized, and ^{3}H-Tyr-labelled β-E-related peptides were immunoprecipitated with β-E antiserum "Horace" and analyzed on sodium-dodecyl-sulfate (SDS)-polyacrylamide gel electrophoresis as previously described [25,46].

Statistical Analyses

Data represent means±S.E.M. Significance of changes detected was evaluated by use of Student's two-tailed-*t*-test.

Materials

Ethanol (Merck, Darmstadt, FRG); liquid diet (No. 711 by BIO-SERVE Inc., Frenchtown, NJ); enzymatic alcohol determination Kit No. 331-UV (Sigma, Taufkirchen, FRG); ^{125}-Iodine (code JMS 30, Amersham, Braunschweig, FRG); ^{125}I-Met-E and ^{3}H-Tyr (52.5 mCi/mmole=1.94 TB/mmole) (NEN, Dreieich, FRG); SDS-polyacrylamide-gels (Bio-Phore 12% gels, BIO-RAD, Richmond, VA). All peptides were from Peninsula Labs, San Carlos, CA.

TABLE 1

LEVELS OF OPIOIDS IN THE RAT BRAIN AND PITUITARY AFTER ACUTE ALCOHOL TREATMENT

	Peptide (pmoles/g)							
	ir-dynorphin		ir-α-neo-endorphin		met-enkephalin		β-endorphin	
Structure	Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Ethanol
Hypothalamus	41.0±6.3	52.8±7.2	83.25±5.5	91.1±4.8	620.3±10.2	782.1±12.8†	35.1 ±1.7	40.3 ±2.5
Striatum	12.3±1.9	15.0±1.4	47.5 ±2.8	49.1±2.5	901.8±20.2	1168.5±29.7†	—	—
Midbrain	9.1±0.9	9.0±0.7	26.6 ±2.7	21.8±2.3	426.4±25.4	534.4±34.8*	4.35±1.1	4.53±0.8
Hippocampus	10.3±1.1	11.9±1.6	20.9 ±1.7	24.0±1.9	368.3±15.9	316.8±28.5	—	—
Pituitary								
Neurointerm.	1557.2 ±50.6	1569.5 ±63.7	2806.34 ±105.1	28.46.1 ±111.1	—	—	660000 ±11000	620000 ±22101
Anterior	101.3 ±13.3	124.8 ±12.3	93.7 ±8.8	96.1 ±10.8	—	—	45333 ±2570	38194 ±2155

2.5 g/kg of alcohol (as a 25% solution in saline) was injected IP 1 hour before decapitation.
Each value represents the mean±S.E.M.; n=10.
*Significant at $p<0.05$.
†Significant at $p<0.01$.

RESULTS

Effects of Acute Ethanol Treatment on Levels of Particular Opioid Peptides in Rat Brain and Pituitary

In order to study acute effects of alcohol on opioid peptides, rats received 2.5 g ethanol (IP) per kg body weight—which is considered to be a moderate dose in rats [58]—causing sedation of the animals.

Table 1 and the hatched bars in Fig. 1 and Fig. 2 show the effects of acute ethanol treatment on tissue levels of immunoreactive (ir-) DYN, ir-α-NEO-E, ir-MET-E and ir-β-E: (a) There was a tendency for an ethanol-induced increase in the levels of ir-DYN and ir-α-NEO-E in hypothalamus, striatum, hippocampus, NIL and AL, which did not attain statistical significance. The midbrain showed a slight non-significant decrease in the tissue levels of these opioid peptides. (b) In contrast to ir-DYN and ir-α-NEO-E, tissue levels of ir-MET-E were elevated by almost 30% in hypothalamus, striatum ($p<0.01$) and midbrain ($p<0.05$), but slightly (non-significantly) decreased in hippocampus. (Ir-MET-E was not measured in the pituitary, since concentrations of ir-MET-E were ~100–1000 times lower than those of β-E-related peptides in this tissue; thus, artifactual formation of MET-E from β-E cannot be completely excluded.) (c) Acute ethanol treatment did not significantly alter tissue levels or ir-β-E, although there was a slight tendency for increased levels in hypothalamus and hippocampus, and for a decrease in levels in the AL and NIL.

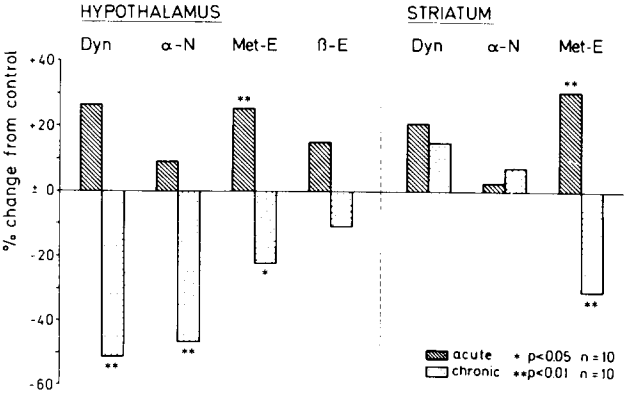

FIG. 1. Effects of acute (hatched bars) and chronic (spotted bars) ethanol treatment on tissue levels of immunoreactive dynorphin, α-neo-endorphin, met-enkephalin and β-endorphin in rat hypothalamus and striatum. Tissue levels of immunoreactive peptides after ethanol treatment are expressed as % of controls. For absolute tissue levels see Table 1 and 2. (For details see: Methods and Results sections.)

Effects of Chronic Ethanol Tretment on Levels of Particular Opioid Peptides in Rat Brain and Pituitary

In order to study chronic effects of alcohol, an ethanol liquid diet (7% (v/v) ethanol) was used in combination with an isocaloric control liquid diet, according to Lieber and De Carli [34].

Ethanol-treated rats had a high ethanol intake of about 15 g/kg/day. In trunk blood, ethanol levels were found to be ~220±25 mg% (mean±S.E.M., n=10). There were no significant differences in body weights between ethanol-treated rats and pair-fed control rats (about 40% increase over two weeks in each case).

Table 2 and Figs. 1 and 2 show the effects of chronic ethanol treatment on tissue levels or ir-DYN, ir-α-NEO-E, ir-MET-E and ir-β-E: (a) There was a pronounced decrease in tissue levels of ir-DYN and ir-α-NEO-E in hypothalamus (by, respectively, 52% and 45%; $p<0.01$) and in hippocampus (by, respectively, 61% and 67%; $p<0.01$), whereas levels of both opioid peptides remained unchanged in the other tissues investigated (with a

TABLE 2

LEVELS OF OPIOIDS IN THE RAT BRAIN AND PITUITARY AFTER CHRONIC ALCOHOL TREATMENT

	Peptide (pmoles/g)							
	ir-dynorphin		ir-α-neo-endorphin		met-enkephalin		β-endorphin	
Structure	Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Ethanol
Hypothalamus	46.1±1.3	23.3±0.4†	98.3±2.7	52.4±1.0†	684.2±17.1	533.1±17.1*	44.1±3.0	39.3±2.0
Striatum	11.6±0.4	13.3±0.6	33.3±1.8	35.8±1.5	1055.2±31.2	728.1±25.3†	—	—
Midbrain	7.7±0.3	7.1±0.3	20.3±1.2	18.8±0.7	403.5±23.4	345.6±21.7	6.8±0.4	6.3±0.4
Hippocampus	10.4±0.5	4.1±0.2†	17.1±0.7	5.5±0.3†	336.1±14.1	331.3±22.7	—	—
Pituitary								
Neurointerm.	1010.7	1125.3	2650.2	2800.1	—	—	640000	595000
	±38.0	±42.4	±99.8	±95.0	—	—	±25.300	±6300
Anterior	140.7	125.6	112.3	95.9	—	—	33250	30820
	±6.3	±5.5	±5.6	±3.7			±1260	±1170

Liquid diet method [34].
Each value represents the mean±S.E.M.; n=10.
*Significant at $p<0.05$.
†Significant at $p<0.01$.

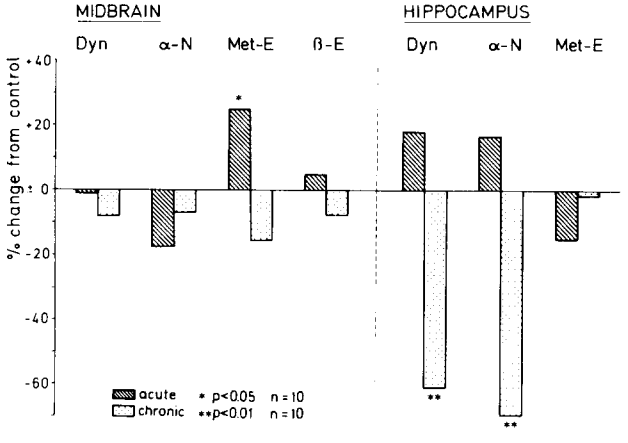

FIG. 2. Effects of acute (hatched bars) and chronic (spotted bars) ethanol treatment on tissue levels of immunoreactive dynorphin, α-neo-endorphin, met-enkephalin and β-endorphin in rat midbrain and hippocampus. Tissue levels of immunoreactive peptides after ethanol treatment are expressed as % of controls. For absolute tissue levels see Table 1 and 2. (For details see: Methods and Results sections.)

tendency to decrease in midbrain and AL and a tendency to increase in striatum and NIL). (b) Ir-MET-E was significantly decreased by chronic ethanol treatment in hypothalamus (by 22%; $p<0.05$) and, to a more pronounced degree, in striatum (by 30%; $p<0.01$). In contrast, ir-MET-E in midbrain and hippocampus remained unaffected. (c) Tissue levels of ir-β-E remained unchanged by chronic ethanol treatment in all tissues investigated (hypothalamus, midbrain, NIL and AL), although there was a slight tendency to a decrease in each area.

Alterations in the Biosynthesis and Post-Translational Processing of β-Endorphin-Related Peptides in the Rat Adenohypophysis After Chronic Ethanol Treatment

The tissue level of a peptide represents an equilibrium between (a) de novo synthesis of the peptide (i.e., the biosynthesis of the pro-hormone and its processing to the respective peptide), and (b) the release and/or enzymatic degradation of the peptide. Thus, changes in tissue levels may reflect alterations in biosynthesis and/or the release and degradation. Moreover, there could be alterations in the biosynthesis, processing and release of a peptide, although tissue levels remain unchanged.

Figure 3 right column shows the effects of chronic treatment of rats with ethanol liquid diet on ^{3}H-Tyr incorporation in vitro into β-E-related peptides (POMC + β-lipotropin (β-LPH) + β-E) in the AL. The biosynthesis of β-related peptides was found to be enhanced by ~150% in the AL ($p<0.02$). chronic ethanol treatment also increased the biosynthesis of the precursor POMC (not shown).

Within the AL, POMC is enzymatically processed into two opioid-related endproducts, β-LPH and β-E [11]. In order to investigate putative alterations in the post-translational processing of POMC into β-LPH and β-E, a monoclonal β-E antibody was used, which is selective for the N-terminal sequence of opioid peptides ((Tyr-Gly-Gly-Phe) and has no avidity for β-LPH ([21]; Gramsch *et al.*, submitted). (Since β-E is ~100 times more concentrated than all other opioid peptides as yet known in the AL, this monoclonal β-E antibody can be considered to recognize almost exclusively opiate-active β-E in the AL.) By the use of this monoclonal β-E antiserum in combination with a β-E antiserum which recognizes both β-E + β-LPH, ethanol-induced changes in the ratio β-LPH/β-E within the AL were investigated.

Figure 4 shows that chronic ethanol treatment strongly decreased the amount of opiate-active β-E by ~40% ($p<0.005$), whereas total β-E + β-LPH immunoreactivity remained unchanged. This is most probably explained by an increase in the quantity of β-LPH upon chronic ethanol treatment. Preliminary studies with an antiserum selective

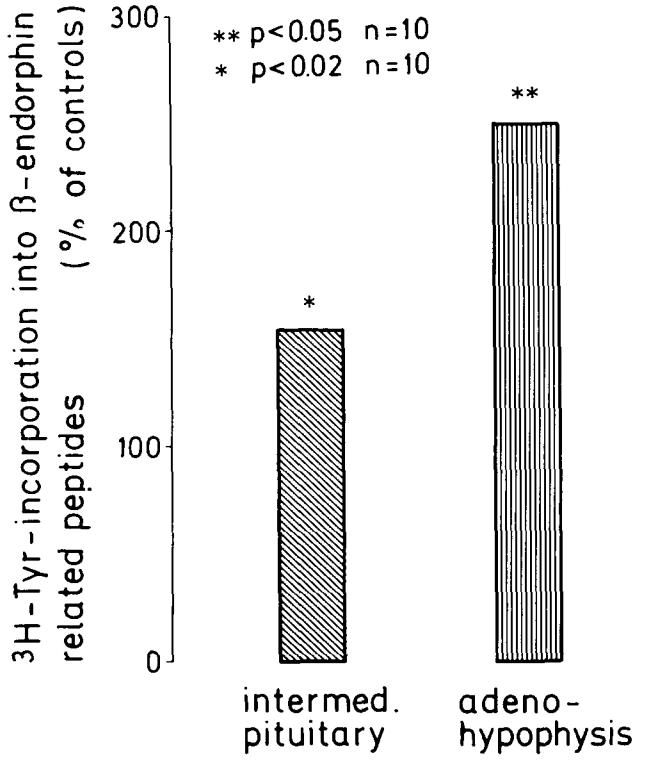

FIG. 3. Effects of chronic ethanol treatment on the biosynthesis of β-endorphin-related peptides in the rat intermediate lobe and adenohypophysis. Intermediate lobes of pituitaries and adenohypophyses were incubated *in vitro* with 0.3 mCi of ^{3}H-Tyr for 2 hrs or 4 hrs, respectively. After the incubation periods, tissues were extracted and ^{3}H-Tyr-labelled β-endorphin-related peptides purified from the extracts by immunoprecipitation with β-endorphin antibodies and SDS-gel electrophoresis (for details see: Methods section). ^{3}H-Tyr-radioactivity, incorporated into β-endorphin + β-lipotropin + pro-opiomelanocortin of rats chronically treated with ethanol is expressed as % of ^{3}H-Tyr incorporated into these peptides of control rats. Absolute values of controls (^{3}H-Tyr-radioactivity): Intermediate lobe of pituitary: 3600 cpm; adenohypophysis: 600 cpm.

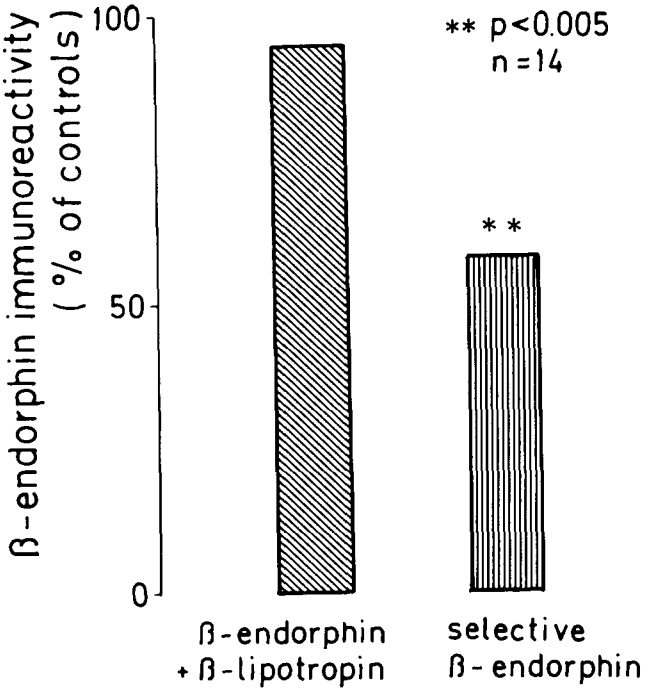

FIG. 4 Changes in the ratio β-lipotropin/β-endorphin in the rat adenohypophysis after chronic ethanol treatment. Tissue levels of immunoreactive β-endorphin after chronic ethanol treatment are expressed as % of controls. Left bar: β-Endorphin immunoreactivity, determined with a β-endorphin antiserum ("Horace"), which has the same avidity for β-endorphin and β-lipotropin (absolute value of the control (≙100%): 33 pmoles/mg tissue). Right bar: β-Endorphin immunoreactivity, determined with a monoclonal β-endorphin antiserum, which is highly selective for opiate-active β-endorphin in the adenohypophysis and does not cross-react with β-lipotropin (absolute value of the control (≙100%): 18 pmoles/mg tissue). (For details see: Methods and Results sections.)

for β-LPH support this hypothesis (Seizinger *et al.*, in preparation). Thus, chronic ethanol treatment appears to inhibit the enzymatic processing of β-LPH into β-E in the AL.

Alterations in the Biosynthesis of β-E and in Its Subsequent Opiate Inactivation by α-N-Acetylation, in the Rat Intermediate Pituitary After Chronic Ethanol Treatment

Figure 3, left column exhibits the effects of chronic treatment of rats with ethanol liquid diet on ^{3}H-Tyr-incorporation in vitro into β-E-related peptides (POMC + β-LPH + β-E) in the NIL. Similarly to the AL, but to a less pronounced degree, the biosynthesis of β-E-related peptides was found to be increased in the NIL by ~55% ($p<0.05$). Again, chronic ethanol treatment increased the biosynthesis of POMC (not shown).

In contrast to POMC in the AL, POMC within the NIL is enzymatically processed into the transient intermediate β-LPH, and, subsequently, into β-E [9,36]; the majority of the newly synthesized β-E in the NIL is, finally, α-N-acetylated in a process of post-translational modification, resulting in a complete loss of opiate-like activity [12, 46, 47, 51].

The influence of chronic ethanol treatment on the α-N-acetylation of β-E in the NIL was studied by the combined use of the monoclonal β-E antiserum, which is specific for opiate-active peptides (and does not recognize opiate-inactive, α-N-acetylated derivatives) and an antiserum which is highly specific for α-N-acetylated opioids (and does not recognize opiate active non-acetylated opioids). (Since β-E-related peptides are present at an at least 100–1000 times higher concentration than all other opioid peptides in the NIL, both antisera can be considered to almost exclusively recognize peptides from the β-E system in this tissue.

As shown in Fig. 5, the amount of α-N-acetylated derivatives of β-E was increased by more than 50% ($p<0.001$) after chronic ethanol treatment, while the amount of opiate-active, non-acetylated β-E was decreased by more than 60% ($p<0.02$).

These findings suggest that chronic ethanol treatment increases the α-N-acetylation of β-E in the NIL, most probably via a stimulation of the activity of the appropriate N-acetyl-transferases.

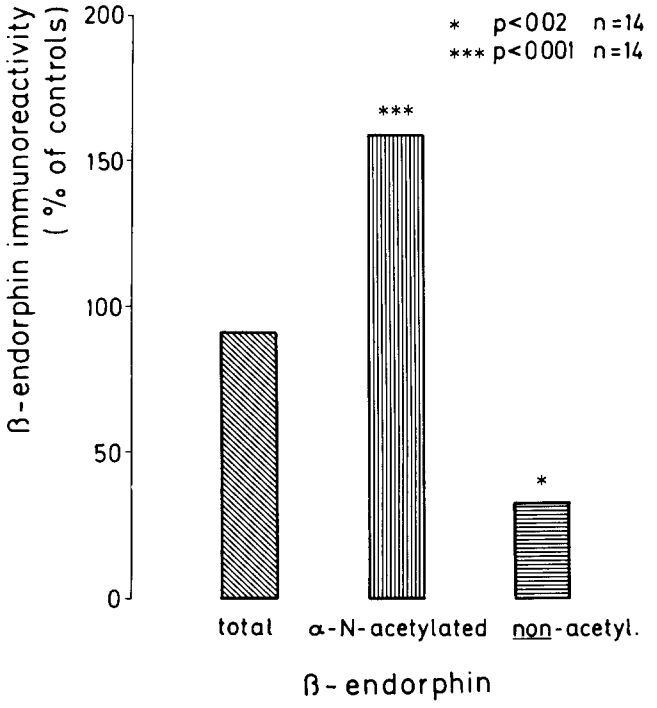

FIG. 5. Changes in the ratio of α-N-acetylated and non-acetylated, opiate-active forms of β-endorphin in the rat intermediate pituitary after chronic ethanol treatment. Tissue levels of immunoreactive β-endorphin after chronic ethanol treatment are expressed as % of controls. Left bar: Total β-endorphin immunoreactivity (antiserum "Horace") (absolute value of the control: 640 pmoles/mg tissue). Middle bar: β-Endorphin immunoreactivity, determined with a β-endorphin antiserum which is highly specific for opiate-inactive α-N-acetylated derivatives of β-endorphin in the intermediate pituitary and does not recognize non-acetylated forms (absolute value of the control: 430 pmoles/mg tissue). Right bar: β-Endorphin immunoreactivity, determined with a monoclonal β-endorphin antiserum which is highly specific for opiate-active β-endorphin in the intermediate pituitary and does not recognize opiate-inactive α-N-acetylated forms (absolute value of the control: 260 pmoles/mg tissue). It should be noted that the total β-endorphin immunoreactivity, measured with antiserum "Horace" (left bar), cannot be considered as the simple addition of α-N-acetylated forms of β-endorphin (middle bar) + non-acetylated opiate-active forms (right bar), since antiserum "Horace" exhibits different cross-reactivities to different forms of β-endorphin.

DISCUSSION

Acute Ethanol

Acute ethanol treatment resulted in a significant increase in the tissue levels or ir-MET-E in hypothalamus, striatum and midbrain, but not in hippocampus. However, levels of ir-DYN, ir-α-NEO-E and ir-β-E were found not to be significantly altered in brain and pituitary. These results are in agreement with a recent study from our laboratory concerning the effects of acute ethanol treatment on MET-E and β-E [45]. These effects of acute ethanol treatment were specific for the respective peptides and tissues, since (a) particular opioid peptides were affected differently within the same tissue, and (b) particular opioid peptides were distinctively modulated in various tissues.

Whether the increased tissue levels or ir-MET-E in response to acute ethanol treatment reflect a decrease in release, and/or an increase in biosynthesis of the opioid peptide needs to be clarified. Both an increase in synthesis and/or a decrease in release in response to acute ethanol administration has been described for other neurotransmitters (for review see [52]).

Chronic Ethanol

Chronic ethanol treatment resulted in a dramatic decrease of tissue levels of ir-DYN and ir-α-NEO-E in hypothalamus and hippocampus, while each peptide remained unchanged in midbrain, striatum, AL and NIL. In contrast, ir-MET-E was decreased in striatum and hypothalamus, but unaffected in midbrain and hippocampus. Levels of ir-β-E remained unchanged in response to chronic ethanol treatment in all tissues investigated (midbrain, hypothalamus, NIL and AL). Thus, chronic ethanol treatment also caused selective alterations in different opioid peptides within distinct areas, which were in several cases opposite to the effects of acute ethanol administration.

However, levels of ir-DYN and ir-α-NEO-E were always concomitantly altered in response to acute and chronic ethanol treatment, while ir-MET-E and ir-β-E were differently and independently regulated. This is in agreement with recent findings showing that DYN and α-NEO-E possess a common precursor peptide [30], while MET-E and β-E are derived from their own distinctive pro-hormones, respectively, pre-proenkephalin [8, 20, 41], and proopiomelanocortin [40].

It should be noted that within the striatum, chronic ethanol treatment induced contrasting effects on the preproenkephalin- and DYN/α-NEO-E-systems: while levels of ir-MET-E were greatly decreased, those of ir-DYN/ir-α-NEO-E tended to increase. There is some indication (Weber, personal communication) that peptides of the preproenkephalin system and those of the DYN/α-NEO-E system are co-localized in the same neurones of the rat striatum. This suggests that different opioid peptide systems (with different precursors) may be selectively regulated even within the same neurone.

It is well-known that opioid peptides interact with classical neurotransmitter systems, e.g., the dopamine system [2,54]. MET-E synthesis in the striatum was shown to be subject to dopaminergic inhibition [27]. Moreover, there is some evidence that chronic ethanol treatment increases the activity of the nigro-striatal dopamine system [19, 33, 55]. Thus, an increase in dopaminergic activity may result in suppression of the biosynthesis of MET-E in the striatum, leading to decreased tissue levels of this peptide, as found in the present investigation. However, we have recently provided evidence that not only MET-E synthesis, but also synthesis of striatal DYN and α-NEO-E are subject to dopaminergic inhibition, since chronic treatment of rats with the dopamine antagonist haloperidol not only increased levels of ir-MET-E, but also those of ir-DYN and ir-α-NEO-E in the striatum (Bovermann et al., in preparation). Therefore, changes in striatal tissue levels of the opioid peptides after chronic ethanol treatment can not only be due to alterations in the activity of the striatal dopaminergic system.

In the present investigation, hypophyseal and hypothalamic levels of ir-β-E remained unchanged in response to chronic ethanol treatment, while Schulz *et al.* [45] recently found large decreases in β-E levels. This apparent discrep-

ancy may be explained by different methods of alcohol administration: while in the former study, rats were forced to drink ethanol in tap water, in the present study, rats were treated with a special liquid diet (according to [34]), which offered two main advantages: (a) By incorporating ethanol into a sweet liquid diet, the natural aversion of rats to ethanol was circumvented. Therefore, ethanol-treated rats had a high intake and high blood levels of ethanol. Application of such an ethanol-containing diet for 2 weeks has been shown to produce rats, tolerant to and physically dependent on ethanol [34]. (b) Since ethanol-treated and control rats were pair-fed with an isocaloric amount of food, the change in their body weights was almost identical. Thus, the problem of undernutrition in ethanol-treated rats in comparison to controls could be avoided.

We have recently compared three different methods of ethanol administration with regard to their effects on opioid peptides: forced drinking of ethanol in tap water as the only source of liquid, intragastric intubation of ethanol, and ethanol in liquid diet (Seizinger *et al.*, in preparation; Bovermann *et al.*, in preparation). The different results obtained clearly demonstrate that the method of administration of alcohol is of critical importance. Different methods of ethanol administration may, thus, also contribute to many discrepancies in alcohol research.

In order to acquire more information concerning the biochemical mechanisms underlying effects of chronic alcohol on opioids, the biosynthesis, processing and modification of β-E-related peptides was investigated. (The present stage of knowledge and the techniques available would render it very difficult to investigate this for other opioid peptides.)

The de novo biosynthesis of β-E, β-LPH and POMC was increased in the NIL, and to an even more pronounced degree, in the AL, after chronic treatment of rats with ethanol liquid diet. This is in agreement with recent investigations by Gianoulakis *et al.* [14] showing an increased biosynthesis of β-E-related peptides in the NIL after chronic treatment of rats with ethanol by intragastric intubation. However, it should be mentioned that just the opposite effect, a large decrease in biosynthesis, could be achieved, when rats were forced to drink ethanol in tap water for 2 weeks (Seizinger *et al.*, in preparation). This finding similarly exemplifies the critical importance of the administration procedure for ethanol.

Although tissue levels of total β-E immunoreactivity remained unchanged in the NIL and AL after chronic ethanol treatment with liquid diet, the amounts of opiate-active β-E were reduced in both lobes. This was found to be due to completely different enzymatic mechanisms: (a) In the AL by retarding of the enzymatic processing of β-E from its precursor β-LPH, and (b) in the NIL by stimulation of the α-N-acetylation of opiate-active β-E to opiate-inactive α-N-acetyl-β-E.

Thus, ethanol exhibited relatively selective effects on particular enzymatic steps in the biosynthesis and processing of β-E in different lobes of the pituitary.

It is noteworthy that although chronic ethanol treatment results in an acceleration of de novo synthesis of the precursor POMC, this is, in fact, associated with a suppression in generation from POMC of the biologically active opioid peptide β-E. Elevated corticosterone levels have been found in mice chronically treated with an ethanol liquid diet, indicating that the release of ACTH from the AL was enhanced [53]. Consideration of these data, in combination with our results, suggests that chronic ethanol administration selectively increases the biosynthesis (and release) of ACTH in the AL, while that of β-E is suppressed, although both peptides are derived from the same precursor, POMC. This is not contradictory, since ethanol may selectively suppress the post-translational processing of POMC to opiate-active β-E (via β-LPH) without affecting its enzymatic processing to ACTH.

It is of interest to compare the effects of chronic ethanol treatment on opioids with those induced by chronic morphine treatment, in order to address the hypothesis that alcohol and morphine exert similar effects on endogenous opioid peptides.

Chronic morphine treatment was recently shown to reduce MET-E levels in rat striatum [43]. The present investigation shows a similar effect of chronic ethanol treatment on rat striatal MET-E. However, we have recently demonstrated that chronic morphine treatment greatly reduced the tissue levels, as well as the biosynthesis, of β-E in the rat NIL [25]. In contrast, chronic ethanol treatment even increased the biosynthesis of β-E in the rat NIL (present study and [14]). Moreover, chronic morphine treatment enhanced the levels of ir-DYN in the rat posterior pituitary [22], while ethanol did not affect levels of ir-DYN in this tissue (present study).

In conclusion, although both ethanol and morphine influence endogenous opioid peptides, their effects on opioids can be different in nature or even opposite, and depend upon the particular opioid peptide system and respective tissue examined, in which opioids may have completely different functions as neurotransmitters, neuromodulators, neurohormones or hormones.

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