*Alcohol*, Vol. 4, pp. 449–456. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0741-8329/87 $3.00 + .00

# Enkephalinase Inhibition: Regulation of Ethanol Intake in Genetically Predisposed Mice

KENNETH BLUM,[1] ARTHUR H. BRIGGS, MICHAEL C. TRACHTENBERG,[2] LEO DELALLO AND JACK E. WALLACE

*Division of Substance and Alcohol Misuse, Departments of Pharmacology and Pathology The University of Texas Health Science Center, San Antonio, TX 78234*

Received 23 February 1987; Accepted 12 May 1987

BLUM, K., A. H. BRIGGS, M. C. TRACHTENBERG, L. DELALLO AND J. E. WALLACE. *Enkephalinase inhibition: Regulation of ethanol intake in genetically predisposed mice.* ALCOHOL 4(6) 449–456, 1987.—This is the first report of alteration in alcohol intake in mice with a genetic predisposition to alcohol preference and known to have innate brain enkephalin deficiencies. We have been able to significantly attenuate both volitional and forced ethanol intake respectively by acute and chronic treatment with hydrocinnamic acid and D-phenylalanine, known carboxypeptidase (enkephalinase) inhibitors. Since these agents, through their enkephalinase inhibitory activity, raise brain enkephalin levels, we propose that excessive alcohol intake can be regulated by alteration of endogenous brain opioid peptides.

Enkephalinase Alcohol Alcohol consumption Genetics D-phenylalanine Amino acids

A growing body of evidence indicates two distinct relationships which correlate brain chemistry and alcohol consumption. The first, a neurochemical interaction, between the CNS actions of endorphins and enkephalins and the effects of alcohol and alcohol metabolites [19, 20, 28, 29, 32, 60, 65, 67]. The second, a chemical-behavioral interaction, between this neurochemistry and volitional consumption of alcohol, i.e. "drug hunger" [14]. The relationship between alcohol and opiate peptides and endogenous receptor systems are depicted in Fig. 1.

Collectively, the more than 100 publications generated in this area point to an interaction between opiates and ethanol in terms of their behavioral and pharmacological actions. These interactions have been characterized as the "link" hypothesis [2, 31, 66, 78, 81]: (1) opiates and ethanol act through the opiate receptors [81]; (2) TIQs directly or indirectly interact at delta and mu opiate receptor and/or allosteric sites [47, 61, 72]; (3) narcotic antagonists significantly reduce TIQ-induced abnormal ethanol drinking behavior in animals [30]; (4) in animals and humans reduction in brain endorphins/enkephalins is correlated with pronounced alcohol consumption. Reduction in brain endorphins/enkephalins is evident in three model systems—genetic predisposition to alcohol, stress-induced alcohol intake and alcohol toxicity due to chronic alcohol intake [11].

## *Animal Evidence*

Ethanol, as opiates, alters brain concentrations of B-endorphin, enkephalin and other opioid peptides [23, 69, 74]. Additionally, ethanol interferes with the synthesis of brain peptides [76]. Ethanol, acetaldehyde and TIQs preferentially bind to one or more multiple opiate receptors [46, 53, 80]. While much of the literature indicates preferential binding to the delta opioid site (endogenous enkephalin receptor) [27, 44, 57] this does not negate the possibility that other receptor sites may be occupied [51]. In fact there is evidence that under certain conditions salsolinol may act as an antagonist at delta sites and an agonist at mu sites [1].

Our laboratory has suggested the possible involvement of opioid peptides in the actions of alcohol. It has been shown that certain opioid peptides significantly reduce alcohol consumption in rodents [52]. Initially, ethanol consumption is increased after ethanol withdrawal with a concomitant decrease in brain methionine-enkephalin [56]. Morphine itself may even be involved as a mediator in alcohol seeking behavior. In fact, a nonpeptide opioid recently found in mammalian and other vertebrate tissues has been identified as morphine [34].

It is well established that isoquinolines can be formed in mammalian tissue from ingestion of ethanol [32, 64, 79].

[1]Requests for reprints should be addressed to Dr. Kenneth Blum, Ph.D., Division of Substance and Alcohol Misuse, Department of Pharmacology, The University of Texas Health Science Center, San Antonio, TX 78234.

[2]Matrix Technologies, Inc., Houston, TX 77058.

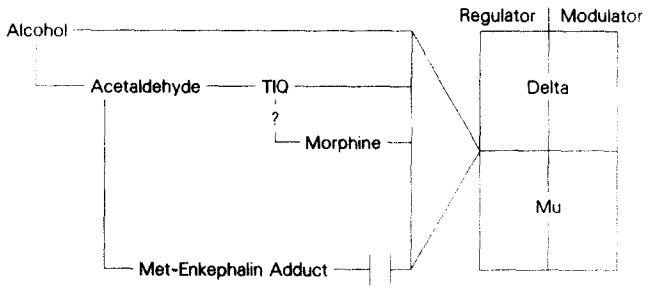


FIG. 1. Schematic representation of the interrelationship of ethanol, TIQs, opioid-peptides and opioid receptor sites.

Administration of isoquinolines to mammals results in the in vivo formation of morphine [34]. It is possible then, that ethanol has part of its effect by virtue of either TIQs or morphine or both. In fact, TIQs can induce alcohol drinking behavior in alcohol avoiding rodents [35]. TIQ-induced alcohol drinking behavior may be the result of a compensatory mechanism whereby TIQs produce an enkephalin deficit [21]. Alcohol compensates for the deficiency of natural enkephalin. The initial acute consumption elicits a restoration of these functions, whereas in chronic consumption, a genetic deficiency is aggravated. This results in insufficient transmitter regulation, inducing formation of aberrant products with a potential for production of endogenous neurotoxins.

To demonstrate the existence of a neurochemical relationship between ethanol seeking behavior and the endorphin system, such that both ethanol and narcotic drugs share similar reward properties [9,82], requires clear differences in brain endorphin chemistry and alcohol consumption in mice with a predilection towards or away from alcohol intake. Using a 14-day preference test, our laboratory found an estimated correlation of 0.909 between mouse whole brain methionine-enkephalin ([Met]-enk) levels in alcohol preferring (C57BL/6J, C58/J) and alcohol avoiding strains (DBA/2J) [8, 10, 12, 40].

Our laboratory further observed that one of the C57BL strains, C57BL/6N from the Simonsen Laboratories, had reverted to the more normal condition and avoids alcohol while the C57BL/6J, from the Jackson Laboratories, maintains its alcohol preference [13]. There is a statistically significant difference in brain levels of [Met]-enk in these two substrains [15].

Correlating the amounts of alcohol consumed by mouse strains revealed a significant relationship ($p<0.5$) such that the C57BL/6J mice, significantly deficient in [Met]-enk, drink more ethanol than do DBA/2J, [Met]-enk normal, mice [18]. Similarly, long-term ethanol consumption in hamsters significantly reduces the concentration of a leucine-enkephalin-like immunoreactive substance in the basal ganglia [16,26]. Long-term alcohol administration to rats and guinea pigs decreases the synthesis of B-endorphin by lowering the activity of mRNA for proopiomelanocortin, the precursor for B-endorphin [54,76].

Additional data suggest that stressful situations may exacerbate a tendency to alcohol craving [14,62]. McGivern and associates (unpublished), using a Casey-Stress paradigm, showed that following stress rodents had significantly lowered whole brain enkephalin levels. Animals subjected to this stress paradigm consumed increased amounts of alcohol in their home cages [62].

Recent findings point to the formation of adduct products following ethanol intake which are biologically inactive and/or sequestor viable neuropeptide transmitters [74]. For example, in the pituitary an N-acetylation of B-endorphin, observed following ethanol administration, rendered this endorphin devoid of opiate activity [75]. In vitro experiments by Summers [80] provide another mechanism by which aberrant adducts between acetaldehyde and [Met]-enk could interfere with opioid peptide mediation of alcohol intake in mammals.

*Human Evidence*

Genazzani *et al.* [43] recently reported a central deficiency of B-endorphin in alcohol addicts. B-endorphin levels, measured in CSF, were reduced by over 65% in the 29 chronic alcoholics. Other indications of brain endorphinergic abnormality in alcoholics come from Fachinetti *et al.* [41]. Additionally, P3 waves are lower in 80% of alcoholics as compared with normals [7]. Further, speaking to the genetic character of this illness, 35% of the sons of these alcoholics also exhibited abnormal P3 activity. Finally, extensive longitudinal studies with identical and fraternal twins raised apart show the off-spring from an alcoholic parent to be far more prone to alcoholism even though reared in a nonalcohol associated environment [45, 63, 73].

These animal and human data allow the hypothesis that craving for alcohol correlates with a decreased [Met]-enk level [11].

*Rationale for the Development of Enkephalinase Inhibitors as Anti-Alcohol Craving Agents*

In view of the inverse correlation between enkephalin levels and alcohol consumption it became of interest to raise brain enkephalin levels and to examine the effects on alcohol consumption. Direct endorphin replacement therapy does not appear to be a viable alternative for several reasons. First, the replacement drug will be addictive, e.g., Pert *et al.* [68]. Second, oral administration of endogenous opiates, for therapeutic purposes, is limited because of the extremely labile nature of the substances and poor penetration in the brain, resulting in short duration of action. An alternative strategy would be to use an antagonist such as naltrexone (Trexan) to occupy opiate receptors. However, narcotic antagonists are not clinically useful antidotes for acute alcohol intoxication. While naltrexone and some delta and mu receptor blockers can reduce certain actions of alcohol, they probably do not affect alcohol craving per se and only weakly attenuate intoxication [50,58]. This fact is borne out in both animal [2] and human investigations [58].

An alternate strategy to increase enkephalin levels directly would be to reduce destruction. Endogenous opiates are rapidly destroyed by endogenous enzymes that cleave amino acid peptides such as enkephalins and endorphins. At least three enzymes act by this mechanism—carboxypeptidase A and B and leucine aminopeptidase [33].

Ehrenpreis and coworkers [39] developed the concept of using inhibitors of enzymes which degrade opiate peptides as possible therapeutic agents to avoid the disadvantages of exogenous administration of an endorphin or endorphin surrogate. Further, there appears to be a correlation between the level of opioid receptors and responsiveness to D-phenylalanine (DPA), a carboxypeptidase A inhibitor, in inbred mice [25].

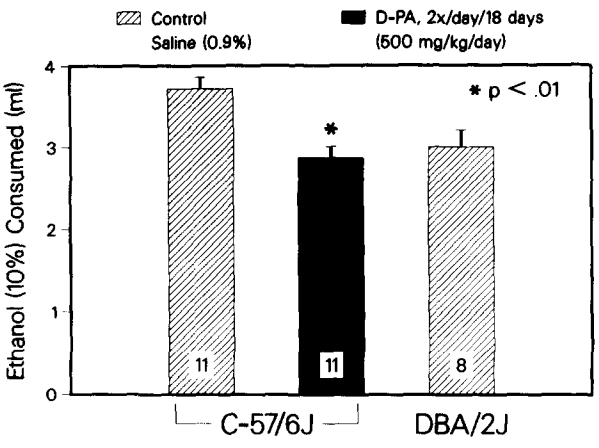


FIG. 2. Effect of DPA (D-phenylalanine) administered at 500 mg/kg twice daily for 18 days by intraperitoneal injection to C57BL/6J mice on a one day ethanol acceptance test (volume in milliliters of 10% ethanol consumed). Number of mice tested shown in the columns. *Indicates significance at $p<0.01$ as determined by the paired Student's $t$-test compared to saline (0.9% sodium chloride) as a control. Striped column: control; solid column: DPA.

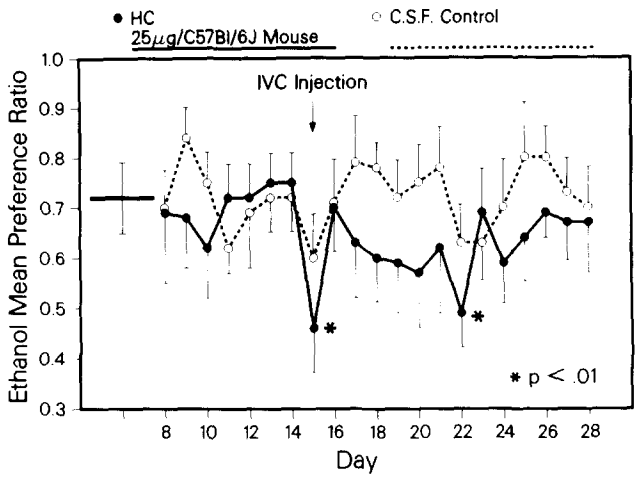


FIG. 3. Effect of HC (hydrocinnamic acid) administered by intracerebral ventricular injection (ICV) of 25 micrograms to C57BL/6J mice on a 14-day three choice 2-bottle preference test. Bar at right indicates mean ethanol preference ratio (10% ethanol/tap water) over the initial 7-day test period (n=36). Open circle denotes cerebrospinal fluid vehicle control group (n=18). Filled circle indicates HC experimental group. *Significance determined by ANOVA and the Duncan Multiple Range test ($p<0.015$). All data points are compared to first week mean control value.

The effects of these inhibitors (termed "enkephalinase" inhibitors) on morphine tolerance withdrawal [4, 24, 36] coupled with the interaction between morphine, endorphins, and alcohol consumption, stimulated our laboratory to investigate the possibility that enkephalinase inhibitors would alter alcohol seeking behavior and thereby act as potential anti-alcohol craving agents.

Two different enkephalinase inhibitors were examined: hydrocinnamic acid (HC), which crosses the blood-brain-barrier (BBB) poorly, and D-phenylalanine (DPA), which crosses the BBB more readily. These were chosen because preliminary data by Ehrenpreis and others indicated that they were relatively nontoxic and alter tissue enkephalin levels [33, 38, 49, 71].

DPA has been administered to mice at a variety of doses and for different durations without untoward effect. The LD 50 for DPA is 5,452 mg/kg, a value slightly greater than that for the comparable L-form. For a standard human male this toxicity level translates (if a one to one relationship is assumed) to an LD 50 dose of 436,160 mg.

No toxic effects were seen following acute administration to monkeys of 3000 mg/kg or chronic administration of 1000 mg/kg/day for 30 days [38]. Ehrenpreis (personal communication) has carried out 2-month and 6-month oral toxicity studies of DPA in mice. No deaths occurred with acute doses of 10,000 mg/kg. No toxic effects were seen, in a 2-month study, at a dose range of 1 g/kg/day. Using this same dose, and examining 35 tissues for pathology, mice showed no observable toxic effects after 6-months of chronic oral administration. In addition, no behavioral changes were seen in mice over this time period. Heller has comparable results after 2 continuous years administration at 10 times the equivalent human dose. His study, using several dose levels and examination of 8 tissues, focuses on mortality, teratology, carcinogenicity and pathology. No negative findings were reported [49] and personal comunications.

Although alternate possibilities exist which might be inferred from the available literature, several conclusions can be stated. First, genetically alcohol preferring mice have alterations in brain enkephalin levels. Second, enkephalinase inhibitors produce an elevation in brain enkephalins. Third, as a direct or indirect consequence of enkephalinase inhibition alcohol consumption may be reduced.

## METHOD

### *Animals, Housing and Acclimation*

In this experiment a total of 102 inbred mice were used. Male C57BL/6J and DBA/2J mice were obtained from Jackson Laboratories. All animals were maintained on a 12L/12D lighting schedule, under conditions of constant temperature (25±2°C). Acclimation required 7 days. Animals were weighed daily. The animals were tested at 14 weeks of age. Fluids were provided in sealed containers to prevent evaporation (inverted 12 ml syringes with a standard laboratory angled drinking spout). Animals were housed in individual cages to measure baseline water consumption and thereby allow division into matched experimental and control groups for each of the C57BL/6J and DBA/2J mouse groups.

### *1-Day Ethanol Acceptance Test*

C57BL/6J mice were maintained as naive animals for 6 weeks as described and then divided into matched experimental and control groups. The experimental group received IP injections of DPA (500 mg/kg/day in saline as two divided doses) for 18 days. Control groups were treated similarly save that the injectate was saline alone. Following the last injection each group was deprived of food and water for 24 hr before being challenged by 10% ethanol in tap water. Total fluid volume consumed was measured to provide a baseline

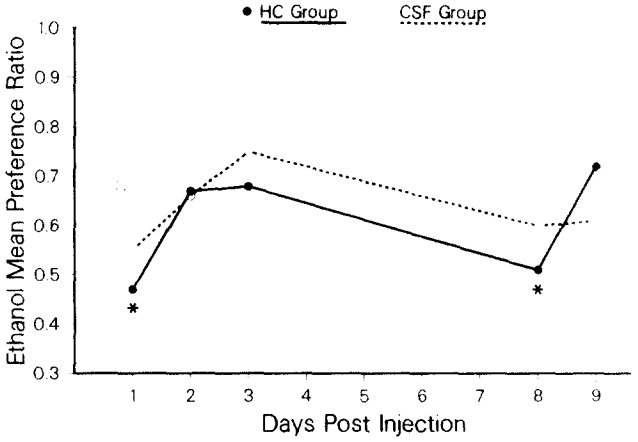


FIG. 4. Effect of HC (hydrocinnamic acid) administered by intracerebral ventricular injection (ICV) at a dose of 25 micrograms to C57BL/6J mice in a 14-day three choice 2-bottle preference test. The inflection points evident in Fig. 3 were extracted for analysis using the Duncan Multiple Range test. The square represents the mean alcohol consumption for 7 days prior to injection. All data are compared to this mean. Open circle denotes CSF group. Filled circle denotes HC group. *Indicates significance at the $p<0.05$ level.

of ethanol acceptance so that individual variation could be compensated.

The 1-day acceptance test data were evaluated (n=30) using ANOVA procedures. Paired *t*-tests were applied to pre- and post-drug ethanol challenge data to evaluate proband specific changes. These are necessary because even among C57BL/6J animals there is a distribution of ethanol preference which we have previously shown to covary with brain [Met]-enk levels [18].

*14-Day Preference Test*

Each group of C57BL/6J mice received a single injection into the cerebral ventricle (ICV) before ethanol challenge. The experimental group injectate was 25 $\mu$g of HC in artificial CSF, the control group, CSF alone.

The standard 14-day, 3 bottle, 2-choice preference method of Holman and Myers was used to determine the effects of HC on alcohol preference [55]. This test required that 3 drinking spouts be provided—one leading to tap water, a second to the 10% ethanol supply and the third to an empty container. The bottles were rotated daily to avoid positional cues. Consumption of ethanol (10% in tap water) and tap water was measured daily over a 14-day baseline period. Readings were taken at 9:00 a.m. daily. Calculations of total fluid, total water and water/ethanol ratio were performed. All calculations were corrected for body weight. A mean of the daily preference ratios, corrected for weight, for the pretest 14-day period was calculated for each animal. Substantial changes in weight or total fluid volume consumption warranted exclusion from the study. Food was available ad lib.

Three statistical analyses were performed—ANOVA, Duncan Multiple Range test, and paired *t*-tests. ANOVA—drug and group effects were analyzed in a 2 factor repeated measures analysis of covariance. Repeated measures of pretreatment (as a mean) for the two independent groups and the group by time interaction was tested with animal weight, ethanol and water intake as covariates. Duncan test—within group means adjusted for the covariates, water and ethanol intake, were compared. The error mean square and degrees of freedom from the main effect for time was used as the error term.

*Whole Brain Methionine-Enkephalin Analysis*

Eight week old C57BL/6J mice, weighing 18–22 g, were utilized to analyze the effect of HC on whole brain [Met]-enk levels. The mice were divided into four groups of 5–7 mice per group.

The animals were decapitated, the brains removed and placed on dry ice. The samples were weighed and homogenized at 95°C in a solution of 2 M acetic acid for 5 min. Samples were then chilled to 4°C and centrifuged at 14,000×g for 15 min. The supernatant was removed, shell frozen in borosilicate tubes and lyophilized overnight. The next day the residue was resuspended in 0.1% BSA in 0.1 M phosphate buffer, pH 6.8 and centrifuged at 1,000×g for 15 min. The supernatant fractions were assayed for [Met]-enk (Immunonuclear Kits, Stillwater, MN). Duplicate samples were run and a log/logit Y/1-Y graph was used to determine binding. A complete description of our procedure as well as the validation tests performed has been described [18].

Four dilutions of three different samples were tested to determine the reliability of values at different areas on the standard curve. An analysis of variance revealed no significant difference between columns ($p<0.1$).

## RESULTS

*D-Phenylalanine Effect on 1-Day Acceptance Test*

Figure 2 illustrates that alcohol-preferring mice exhibited a statistically significant (Student's *t*-test, $p<0.01$) 21% decline in alcohol consumption consequent to DPA injection. The alcohol consumption of the DPA-treated mice is now comparable to and not significantly different from that of the alcohol-aversive saline treated DBA/2J controls.

C57BL/6J mice treated with DPA drank significantly less 10% ethanol (2.87±0.14 ml; n=11) than did the saline-treated group (3.72±0.14 ml; n=11). This decline is statistically significant at the $p<0.01$ level. The ethanol-aversive DBA/2J mouse strain drank comparable amounts of alcohol (3.0±0.21 ml; n=8). In other studies we have determined that reducing the course of injections to 10 days resulted in a decreased ethanol consumption which was not significant ($p<0.1$) relative to saline treated controls.

*Hydrocinnamic Acid (HC) Effect on 14-Day Preference Test*

Figure 3 shows that on the first (day 14) and eighth (day 21) days, following ICV injection of HC, the experimental group exhibited a significant decrease in ethanol consumption. Data of this sort are normally highly variable [57]. To compensate, in part, for this variability scores were adjusted by use of the regression coefficient. HC itself had no effect on total fluid volume consumed on the day of injection or throughout the experiment.

Drug and group effects were analyzed in a 2 factor repeated measures analysis of covariance. Repeated measures from pretreatment, at inflection points, days 1, 2, 3, 8, 9 post-injection were used for the two independent groups. The group by time interactions were tested with animal weight, ethanol and water intake as covariates. Water and ethanol intake covariates were significant with a negative regression for water; weight was not a significant con-

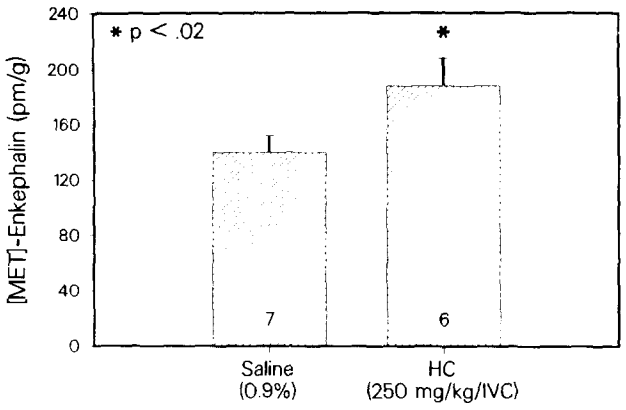


FIG. 5. Whole brain methionine-enkephalin levels in picomoles per gram following HC (hydrocinnamic acid) at 250 mg/kg intraperitoneally, compared to saline controls. n=number of mice tested. *Indicates significance at $p<0.02$ as determined by Student's $t$-test.

tributor. Within group means adjusted for the two significant covariates of ethanol and water intake were compared for the drug treatment group using a Duncan multiple range test (a=0.05). The error mean square and degrees of freedom from the main effect for time were used as the error term for the Duncan test of means. Significant differences were found at pre-test and post-injection day 1 and day 8, there were no other significant differences from drug group pre-test. Other drug group differences were such that days 1 and 8 differed significantly from days 2, 3 and 9 (Fig. 4).

While the reduced drinking by C57BL mice appears to be at least as great on day 2 pre-injection as on days 1 and 8 post-injection this is a false impression. Paired $t$-test analysis of pre-injection day 2 data for the experimental and control groups shows no significant differences. The animals exhibited no changes in food or water intake, no weight changes, nor did they appear either more agitated or more sluggish than usual, there were no observable toxic effects.

*Enhancement of Whole Brain Methionine-Enkephalin by Hydrocinnamic Acid*

HC was provided to 25, 8 week old, C57BL/6J, alcohol-craving mice, by IP injection 2 hours before sacrifice. IP injection was used to avoid direct and unintended effects on brain chemistry possibly associated with the ICV route; consequently the dose had to be significantly greater than that used with ICV injections. The dose range chosen was based on prior work by Ehrenpreis who showed an optimal analgesic response, measured by tail-flick, of 125 mg/kg [38]. HC was delivered in 2 equally divided injections over an 8 hr period at doses of 100, 150 and 250 mg/kg; control animals received saline injections similarly to control.

Figure 5 shows a significant ($p<0.02$) enhancement of whole brain [Met]-enk levels in C57BL/6J mice treated with HC relative to controls. Although there was no dose-dependent relationship found between 100, 150 and 250 mg/kg all doses resulted in significant increase in [Met]-enk over controls and were 178±8.0 (n=7), 180±8.0 (n=5), and 188±20.0 (n=6) picomoles per gram, respectively, compared to controls at 140±12.0 (n=7).

## DISCUSSION

In terms of ethanol and opiate seeking behavior the "genotype" theory proposed [11] is that individuals prone to such behavior possess a genetic deficiency of the endorphinergic system and both environmental conditions and long-term exposure to ethanol and opiates result in marked reduction of endogenous peptidyl opiates. Animal and human evidence which support opioid peptides a gene defect in the posttranslational processing of endorphins has been forthcoming and includes: (1) ethanol preferring C57BL/6J mice exhibit less brain enkephalin than non-ethanol preferring DBA2/J mice, suggestive of an inverse relationship whereby lowered opioids equates to higher ethanol desire [18]; (2) stress reduces brain endorphins in rodents [62]; (3) long-term exposure consumption of ethanol by hamsters and other rodents results in marked reduction of brain enkephalin and endorphin [16]; at the mRNA level there is a decrease in posttranslation of the pre-enkephalin during chronic ethanol consumption in rodents [76]; and (5) a central deficiency of B-endorphin in human alcoholics [43].

This is the first report of reduced ethanol consumption in genetically bred mice utilizing DPA, a very safe and effective enkephalinase inhibitor [33] and HC, another known enkephalinase inhibitor [36].

The first experiment was designed to evaluate the efficacy of DPA to reduce ethanol intake in inbred mice. Results of this experiment indicate that ethanol consumption in alcohol-preferring mice (C57BL/6J) can be markedly reduced by inhibiting the endogenous metabolism of brain endorphins by utilization of "neuropeptidase" inhibitors. The report illustrates that DPA, by virtue of enkephalinase inhibition [4], significantly reduces ethanol acceptance in C57BL/6J mice so as to be comparable to that of DBA/2J alcohol-aversive animals.

HC was utilized to evaluate the possibility that enkephalinase inhibition would result in a suppression of volitional ethanol consumption. Analysis of these data allows the interpretation that the magnitude of the effect was a function of the severity of the presumed enkephalin deficit as measured by ethanol preference. That is, specific drug dose produced a greater effect in low preference mice as compared to high preference animals. All animals received a single dose of HC; weight was determined not to be a covariate. This observation explains the significant negative regression coefficient for water, and suggests a dose-dependent effect of HC to act as a pharmacologic modulator of ethanol intake.

Furthermore, the data reveal the HC produced a significant ($p<0.015$) reduction of volitional ethanol consumption 24 hr after ICV injection. This finding was all the more significant as it was evident despite the trauma induced by the injection.

Of great interest is the rebound effect observed on post-injection day 2 in both groups (drug and control) toward pre-injection levels (Fig. 4). The rebound effect is currently unexplainable, but suggests a complex behavioral compensation. The finding that a significant difference was observed in the control group only at day 1 and 3 suggests that HC tended to dampen the rebound or overshoot effect possibly due to the stress of the injection. This stress may have caused reduced brain opioid peptide levels which induced the possible increased ethanol preference. Furthermore, there was a day to day trend in the drug group to have lower preference ratio scores from day 3 to day 8 when it reached

significance ($p<0.03$) compared to pre-drug level. On day 9, once again, a significant ($p<0.02$) rebound or overshoot effect occurred for only the drug groups. The 8 day time period is not surprising in that other enzyme inhibitors (e.g., p-chlorophenylalanine) show similar behavioral time effects probably due to regeneration of new enzyme molecules [42].

The data show a reduction in alcohol preference in HC injected mice as compared to controls receiving CSF only. At present we can say with certainty that alcohol preference was reduced while water intake was unaltered. Further studies must enlarge the issue and address questions of butanol as compared to ethanol, of the caloric value of alcohol, as well as the overall effect of enkephalinase inhibitors on volume consumption. We can then examine the specificity of the enzyme inhibitor actions.

With regard to the third experiment, at all doses there was significant increase over control ($p<0.02$) though there were no dose related differences (Fig. 5). These results are not surprising in view of the finding of Ehrenpreis *et al.* [38] who showed that with HC greater analgesia was seen at 125 mg/kg than at 200 mg/kg. This suggests that these concentrations were on the plateau portion of the curve and that dose response data should be obtained. Other reports reveal the enkephalinase inhibitory properties of HC as well as its ability to raise levels of opioid peptides [37].

Although additional intensive systematic research is required to fully characterize this novel finding, it further suggests the possible involvement of the endorphinergic system as a potential critical determinant for both volitional and forced [70] consumption of ethanol.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the expert technical assistance of S. F. A. Elston. A portion of this work was supported by a grant to Drs. W. C. Hall and K. Blum from the Southwest Research Institute of San Antonio, TX.

## REFERENCES

1. Airaksinew, M. M., V. Saano, E. Steidel, H. Juvongn, A. Huhtokangas and J. Gynther. Binding of B-carbolines and tetrahydroisoquinolines by opiate receptors of the Δ-type. *Acta Pharmacol Toxicol (Copenh)* **55:** 380–385, 1984.
2. Altschuler, H. L., E. Appelbaum and T. S. Shippenberg. The effects of opiate antagonists on the discriminative stimulus properties of ethanol. *Pharmacol Biochem Behav* **14:** 97–100, 1981.
3. Altschuler, H. L. and T. S. Shippenberg. Tetrahydroisoquinoline and opioid substrates of alcohol actions. *Prog Clin Biol Res* **90:** 329–344, 1982.
4. Balagot, R. C. and S. Ehrenpreis. Continuing studies of D-phenylalanine induced analgesia in mice and humans. *Anesthesiology* **51:** S231, 1979.
5. Battersby, A. R. Alkaloid biosynthesis II. Studies related to the formation of reticuline and morphine biosynthesis. *J Chem Soc* **2:** 210–216, 1968.
6. Beckman, H., D. Athen, M. Olteanu and R. Zimmer. DL-Phenylalanine versus imipramine: a double-blind controlled study. *Arch Psychiatr Nervenkr* **227:** 49–58, 1979.
7. Begleiter, H., B. Porjesz, B. Bihari and B. Kissin. Event-related brain potentials in boys at risk for alcoholism. *Science* **225:** 1493–1495, 1984.
8. Belknap, J. K. Genetic factors in the effects of alcohol: Neurosensitivity, functional tolerance and physical dependence. In: *Alcohol Tolerance and Dependence*, edited by H. Rigter and J. C. Crabbe. Amsterdam: Elsevier/North Holland Biomedical Press, 1980, pp. 157–173.
9. Belluzzi, J. and L. Stein. Enkephaline may mediate euphoria and drive-reduction reward. *Nature* **266:** 556–558, 1977.
10. Blum, K. Alcohol and central nervous system peptides. *Subst Alcohol Actions Misuse* **4:** 73–87, 1983.
11. Blum, K. Psychogenetics of drug seeking behavior. In: *Central and Peripheral Endorphins*, edited by E. E. Muller and A. R. Genazzani. New York: Raven Press, 1984, pp. 339–355.
12. Blum, K., A. H. Briggs, L. DeLallo and S. F. Elston. Genotype dependent responses to ethanol and normorphine on vas deferens of inbred strains of mice. *Subst Alcohol Actions Misuse* **1:** 459–465, 1980.
13. Blum, K., A. H. Briggs, L. DeLallo, S. F. A. Elston and R. Ochoa. Whole brain methionine-enkephalin in ethanol-avoiding and ethanol-preferring C57BL mice. *Experientia* **38:** 1469–1470, 1983.
14. Blum, K., A. H. Briggs, S. F. Elston and L. DeLallo. Psychogenetics of drug-seeking behavior. *Subst Alcohol Actions Misuse* **3:** 255–257, 1980.
15. Blum, K., A. H. Briggs, S. F. A. Elston and L. DeLallo. Ethanol preference as a function of genotypic levels of whole brain enkephalin in mice. *Toxicol Eur Res* **3:** 261–262, 1981.
16. Blum, K., A. H. Briggs, S. F. Elston, L. DeLallo, P. Sheridan and M. Sar. Reduced leucine-enkephalin-like immunoreactive substance in hamster basal ganglion after long-term ethanol exposure. *Science* **216:** 1425–1427, 1982.
17. Blum, K., A. H. Briggs, F. A. Elston, M. Hirst, M. G. Hamilton and K. Vereby. A common denominator theory on alcohol and opiate dependence: Review of similarities and differences. In: *Alcohol Tolerance and Dependence*, edited by H. Rigter and J. C. Crabbe. Amsterdam: Elsevier/North Holland Biomedical Press, 1980, pp. 371–396.
18. Blum, K., S. F. A. Elston, L. DeLallo, A. H. Briggs and J. E. Wallace. Ethanol acceptance as a function of genotype amounts of brain [Met]-enkephalin. *Proc Natl Acad Sci USA* **80:** 6510–6512, 1983.
19. Blum, K., S. Futterman, J. E. Wallace and H. A. Schwertner. Naloxone-induced inhibitor of ethanol dependence in mice. *Nature* **265:** 49–51, 1977.
20. Blum, K., M. G. Hamilton, M. Hirst and J. E. Wallace. Putative role of isoquinoline alkaloids in alcoholism: A link to opiates. *Alcoholism (NY)* **2:** 113–120, 1978.
21. Blum, K. and H. Topel. Opioid peptides and alcoholism: genetic deficiency and chemical management. *Func Neurol* **1:** 71–83, 1986.
22. Blum, K., J. E. Wallace, A. H. Briggs and M. C. Trachtenberg. Evidence for the "genotype theory" of alcohol seeking behavior. *Alcohol Drug Res* **6:** 455–461, 1986.
23. Borg, S., H. Kvande, U. Rydberg, L. Terenius and A. Wahlstrom. Endorphin levels in human cerebrospinal fluid during alcohol intoxication and withdrawal. *Psychopharmacology (Berlin)* **78:** 101–103, 1978.
24. Budd, K. The use of D-phenylalanine, an enkephalinase inhibitor in the treatment of intractable pain. *Pain* **11:** Suppl 1, S95, 1981.
25. Chang, R. S. and B. Pomeranz. Correlation of genetic differences in endorphin systems with analgesic effects of D-amino acids in mice. *Brain Res* **177:** 583–587, 1979.
26. Chang, R. S. and L. F. Tseng. Chronic administration of ethanol on pituitary and hypothalamic beta-endorphin in rats and golden hamsters. *Pharmacol Res Commun* **14:** 1001–1008, 1982.
27. Charness, M. E. and L. A. Querimit. Opioid binding sites induced by ethanol in NG108-15 cells are functional delta-opioid receptors. *Soc Neurosci Abstr* **11:** 309, 1985.

28. Clow, A., I. P. Stolerman, R. M. Murray and M. Sandler. Ethanol preference in rats: increased consumption after intraventricular administration of tetrahydropapaveroline. *Neuropharmacology* **22:** 563–565, 1983.
29. Cohen, G. and M. A. Collins. Alkaloids from catecholamines in adrenal tissue: Possible role in alcoholism. *Science* **67:** 1749–1751, 1970.
30. Critcher, E. C., C. I. Lin, J. Patel and R. D. Myers. Attenuation of alcohol drinking in tetrahydroisoquinoline-treated rats by morphine and naltrexone. *Pharmacol Biochem Behav* **18:** 225–229, 1983.
31. Davis, V. E. Neuroamine-derived alkaloid: a possible common denominator in alcoholism and related drug dependencies. *Ann NY Acad Sci* **30:** 111–115, 1973.
32. Davis, V. E. and M. D. Walsh. Alcohol, amines and alkaloids: a possible basis for alcohol addiction. *Science* **167:** 1005–1007, 1970.
33. Della Bella, D., A. Carenzi, V. Frigeni and V. Santini. Effect of carboxypeptidase inhibition on in vivo and in vitro pharmacological properties of morphine enkephalins. *Neuropharmacology* **18:** 719–721, 1979.
34. Donnerer, J., K. Oka, A. Brossi, K. Rice and S. Spector. Presence and formation of codeine and morphine in the rat. *Proc Natl Acad Sci USA*, in press, 1986.
35. Duncan, C. and R. A. Deitrich. A critical evaluation of tetrahydroisoquinoline induced ethanol preferance in rats. *Pharmacol Biochem Behav* **13:** 265–281, 1980.
36. Ehrenpreis, S. D-phenylalanine and other enkephalinase inhibitors as pharmacological agents: implications for some important therapeutic application. *Subst Alcohol Actions Misuse* **3:** 231–239, 1982.
37. Ehrenpreis, S., R. C. Balagot, J. E. Comaty and S. B. Myles. Naloxone reversible analgesia in mice prolonged by D-phenylalanine at hydrocinnamic acid, inhibitors on carboxypeptidase A. In: *Advances in Pain Research and Therapy*, vol 3, edited by J. J. Bonica. New York: Raven Press, 1979, pp. 479–488.
38. Ehrenpreis, S., R. C. Balagot, A. D. Mosnaim, P. Szanto, S. Myles, M. Hyodo, C. Okafor, F. E. Ellyin and S. P. Singh. First World Cong Toxicol Environ Health, Am Coll Toxicol, Washington, DC, p. 23, 1982 (abstract).
39. Ehrenpreis, S., R. C. Balagot, S. B. Myles, C. Advocate and J. E. Comaty. Further studies on the analgesic activity of D-phenylalanine (DPA) in mice and humans. In: *Endogenous and Exogenous Opiate Antogonists and Antagonists*, edited by E. L. Way. New York: Pergammon Press, 1980, pp. 370–382.
40. Elston, S. F., K. Blum, L. DeLallo and A. H. Briggs. Ethanol intoxication as a function of genotype dependent responses in three inbred mice strains. *Pharmacol Biochem Behav* **16:** 13–15, 1982.
41. Facchinetti, F., F. Petraglia, G. Nappi, E. Martignoni, E. Sinforiani, G. Bono and A. R. Genazzani. Functional opioid activity variates according to the different fashion of alcohol abuse. *Subst Alcohol Actions Misuse* **5:** 281–291, 1985.
42. Geller, I. and K. Blum. The effects of 5-HTP on parachlorphenylalanine (c-CPA) attenuation of "conflict" behavior. *Eur J Pharmacol* **9:** 319–324, 1970.
43. Genazzani, A. R., G. Nappi, F. Facchinetti, G. C. Mazzella, P. Parrini, E. Sintoriani, F. Petraglia and F. Savoldi. Central deficiency of B-endorphin in alcoholics. *J Clin Endocrinol Metab* **55:** 485–488, 1982.
44. Gianoulakis, C. Long-term ethanol alters the binding of 3H-opiates to brain membranes. *Life Sci* **33:** 725–733, 1983.
45. Goodwin, D. W., F. Schulsinger, L. Hermansen, S. B. Guze and G. Winokur. Alcohol problems in adoptees raised apart from alcoholic biological parents. *Arch Gen Psychiatry* **28:** 238–243, 1973.
46. Greenwald, J. E., R. H. Fertel, L. K. Wong, R. D. Schwarz and J. R. Bianchine. Salsolinol and tetrahydropapaveroline bind opiate receptors in the rat brain. *Fed Proc* **38:** 379, 1979.
47. Hamilton, M. G., M. Hirst and K. Blum. Opiate-like activity of salsolinol on the electrically stimulated guinea pig ileum. *Life Sci* **25:** 2205–2210, 1979.
48. Harris, R. A. and L. K. Erickson. Alteration of ethanol effects by opiate antagonists. In: *Currents in Alcoholism*, vol 5, edited by M. Galanter. New York: Grune & Stratton, 1979, pp. 17–28.
49. Heller, B. Noncatecholic phenylethylamines, Part I. In: *Modern Pharmacology*, edited by A. D. Mosnaim and M. E. Wolf. New York: Marcel Dekker, 1978, p. 397.
50. Hemmingsen, R. and S. C. Sorensen. Absence of an effect of naloxone on ethanol intoxication and withdrawal reactions. *Acta Pharmacol Toxicol (Copenh)* **46:** 62–65, 1980.
51. Hiller, J. M., L. M. Angel and E. J. Simon. Multiple opiate receptors. Alcohol selectively inhibits binding to delta receptors. *Science* **214:** 468–470, 1981.
52. Ho, A. K. and N. Rossi. Suppression of ethanol consumption by met-enkephalin in rats. *J Pharm Pharmacol* **34:** 118–119, 1982.
53. Hoffman, P. L., C. T. Chung and B. Tabakoff. Effects of ethanol, temperature, and endogenous regulatory factors on the characteristics of striatal opiate receptors. *J Neurochem* **43:** 1003–1010, 1984.
54. Holt, V., R. Prezewlocki and A. Herz. Beta-endorphin-like immunoreactivity in plasma, pituitaries and hypothalamus of rats following treatment with opiates. *Life Sci* **23:** 1057–1066, 1978.
55. Holman, R. B. and R. D. Meyers. Ethanol consumption under conditions of psychogenic polydipsia. *Physiol Behav* **3:** 369–371, 1968.
56. Hong, J. S., E. Majchrowitz, W. A. Hunt and J. C. Gillin. Reduction in cerebral methionine-enkephalin content during the ethanol withdrawal syndrome. *Subst Alcohol Actions Misuse* **2:** 233–240, 1981.
57. Hynes, M. D., M. A. Lochner, K. G. Bemis and D. L. Hymson. Chronic ethanol alters the receptor binding characteristics on the delta opioid receptor ligand, D-Ala2-D-Leu5 enkephalin in mouse brain. *Life Sci* **33:** 2331–2337, 1983.
58. Jeffcoate, W. J., A. G. Hastings, M. H. Cullen and M. Herbert. Naloxone and ethanol antagonism. *Lancet* **8228:** 1052, 1981.
59. Kakihana, R. and G. E. McClearn. Development of alcohol preference in BALB/C mice. *Nature* **199:** 511–512, 1963.
60. Lieber, C. S., E. Baraona, E. R. Gordon, P. Jauhonen, A. Lebsack and P. Pikkarainen. Biological approach to alcoholism. Res Monograph 9 DHHS No. (ADM) 83, 1983.
61. Lucchi, L., A. Bosio, P. R. Spano and M. Trabucchi. Action of ethanol and salsolinol on opiate receptor function. *Brain Res* **232:** 506–510, 1982.
62. McGivern, R. F., S. Moussa, D. Couri and G. G. Berntzon. Prolonged intermittent footshock stress decreases met- and leu-enkephalin levels in brain with concomitant decreases in pain threshold. *Life Sci* **33:** 47–54, 1983.
63. Martin, P. The human genetics of alcoholism. *Subst Alcohol Actions Misuse* **2:** 389–406, 1981.
64. Matsubara, K., S. Fukushima, A. Akane, K. Hama and Y. Fukui. Tetrahydro-B-Carbolines in human urine and rat brain—no evidence for formation by alcohol drinking. *Alcohol Alcohol* **21:** 339–345, 1986.
65. Myers, R. D. Multiple metabolite theory, alcohol drinking and the alcogene. In: *Aldehyde Adducts in Alcoholism*, edited by M. A. Collins. New York: Alan R. Liss, Inc., 1985, pp. 201–209.
66. Myers, R. D. and E. C. Critcher. Naloxone alters alcohol drinking induced in the rat by tetrahydropapaveroline (THP) infused ICV. *Pharmacol Biochem Behav* **16:** 827–836, 1982.
67. Myers, R. D., M. L. McCaleb and W. D. Ruwe. Alcohol drinking induced in the monkey in tetrahydropapaveroline (THP) infused into the cerebral ventricle. *Pharmacol Biochem Behav* **16:** 995–1000, 1982.
68. Pert, C. B., A. Pert, J. K. Chang and B. T. Fong. (D-Ala2)-Met-enkephalinamide: a potent, long-lasting synthetic pentapeptide analgesic. *Science* **194:** 330–332, 1976.

69. Prezewlocki, R., V. Hollt, T. Duka, A. Kleber, C. L. Gramsch, I. Harmann and A. Herz. Long-term morphine treatment decreases endorphin levels in rat brain and pituitary. *Brain Res* **174:** 357–361, 1979.
70. Reid, L. D. and G. A. Hunter. Morphine and naloxone modulate intake of alcohol. *Alcohol* **1:** 33–37, 1984.
71. Rose, W. C. D., T. Warner and W. J. Haines. The amino acid requirements of man. IV. The role of leucine and phenylalanine. *J Biol Chem* **193:** 613–620, 1951.
72. Ross, D. H. Molecular aspects of calcium membrane interactions: a model for cellular adaptation to ethanol. In: *Alcohol Tolerance and Dependence*, edited by H. Rigter and J. C. Crabbe. Amsterdam: Elsevier/North Holland Biomedical Press, 1980, pp. 227–233.
73. Schuckit, M. A., D. A. Goodwin and G. Winokur. A study of alcoholism in half siblings. *Am J Psychiatry* **128:** 1132–1136, 1972.
74. Schulz, R., M. Wuster, T. Duka and A. Herz. Acute and chronic ethanol treatment changes endorphin levels in brain and pituitary. *Psychopharmacologia* **68:** 221–227, 1972.
75. Seizinger, B. R., K. Bovermann, V. Holt and A. Herz. Enhanced activity of the B-endorphinergic system in the anterior and neurointermediate lobe of the rat pituitary after chronic treatment with ethanol liquid diet. *J Pharmacol Exp Ther* **230:** 455–461, 1984.
76. Seizinger, B. R., V. Holt and A. Herz. Effects of chronic ethanol treatment on the in vitro biosynthesis of proopiomelanocortin and its posttranslational processing to beta-endorphin in the intermediate lobe of the rat pituitary. *J Neurochem* **43:** 607–613, 1982.
77. Shearman, G. T. and A. Herz. Ethanol and tetrahydroisoquinoline alkaloids do not produce narcotic discriminative stimulus effects. *Psychopharmacology (Berlin)* **81:** 224–227, 1983.
78. Siggins, A. R., T. Berger, E. D. French, T. Shier and F. E. Bloom. Ethanol, salsolinol and tetrahydropapaveroline alter the discharge of neurons in several brain regions: comparison to opioid effects. *Proc Clin Biol Res* **90:** 275–287, 1982.
79. Sjoquist, B., S. Borg and H. Kvande. Catecholamine derived compounds in urine and cerebrospinal fluid from healthy volunteers. *Subst Alcohol Actions Misuse* **2:** 63–72, 1981.
80. Summers, M. C. Structural and biological studies of the acetaldehyde adducts of enkephalins and related peptides: a short review. In: *Aldehyde Adducts in Alcoholism*, edited by M. C. Collins. New York: Alan R. Liss, Inc., 1979, pp. 39–49.
81. Tabakoff, B. and P. L. Hoffman. Alcohol interactions with brain opiate receptors. *Life Sci* **32:** 197–204, 1983.
82. Wise, R. A. and M. A. Bozarth. Action of abused drugs on reward systems in the brain. In: *Neurotoxicology*, edited by K. Blum and L. Manzo. New York: Marcel Dekker, 1985, pp. 111–133.