Family History of Alcoholism and Hypothalamic Opioidergic Activity

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Background: This study was designed to assess whether nonalcoholic offspring from families with a high density of alcohol-dependent individuals have altered endogenous central nervous system opioid activity. Naloxone hydrochloride stimulates plasma cortisol by blocking opioidergic input on the corticotropin-releasing factor neuron, thereby providing a noninvasive method for measuring hypothalamic opioid tone.

Methods: Forty-eight nonalcoholic subjects aged 18 to 25 years were enrolled in a protocol to measure endogenous opioid activity by inducing opioid receptor blockade with the receptor antagonist, naloxone. Twenty-six subjects were offspring from families with a high density of alcohol dependence and were designated as family history-positive subjects. Twenty-two subjects were biological offspring of nonalcohol-dependent parents and designated as family history-negative subjects. Subjects received naloxone hydrochloride (0, 125, and 375 µg/kg) in double-blind, randomized order. Serum cortisol levels were monitored.

Results: Family history–negative subjects had a graded cortisol response to each dose of naloxone. In contrast, family history–positive subjects achieved a maximal cortisol response to the 125-µg/kg dose of naloxone hydrochloride with no further increase in cortisol levels observed following the 375-µg/kg dose. Family history–negative subjects had a diminished cortisol response to the 125-µg/kg dose compared with the family history–positive subjects. Plasma naloxone concentrations did not differ between family history groups.

Conclusions: Individuals from families with a high density of alcohol dependence are more sensitive to naloxone compared with offspring of nonalcohol-dependent parents. This implies that individuals with a family history of alcohol dependence have diminished endogenous hypothalamic opioid activity.

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Alcohol dependence is characterized by loss of control over alcohol drinking, tolerance, and physical dependence. Twin, adoption, and cross-fostering studies and pedigree analyses indicate that there are inherited as well as environmental determinants for the pathogenesis of this disorder. Important biological and psychological differences have been found as a function of a family history of alcoholism. Evidence suggests that the brain opioid system is part of a neurocircuitry involved in heavy alcohol drinking. Initially, pharmacological studies indicated that opioid receptor antagonists, such as naloxone hydrochloride and naltrexone, decreased alcohol self-administration in animal models. Subsequent clinical trials demonstrated that the opioid antagonist naltrexone reduced alcohol drinking, alcohol craving, and relapse rates in alcoholic subjects. In fact, naltrexone was approved by the Food and Drug Administration as a pharmacotherapeutic agent for the treatment of alcohol dependence.

Current information linking the opioid system to the actions of alcohol has generated models speculating that alcoholic subjects and their offspring have defective brain opioid activity that results in abnormal reinforcement following ingestion of alcohol. To determine the validity of an opioid model for alcoholism, it would be necessary to generate measurements of endogenous opioid activity within the central nervous system. One technique that can measure opioid tone is the induction of receptor blockade with an opioid receptor antagonist. To understand how opioid receptor antagonists can measure opioid activity, it is necessary to understand how modulation of cortisol is opioid dependent.

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SUBJECTS AND METHODS

SUBJECTS

Subjects were recruited from the Baltimore, Md, area. After obtaining informed consent, 250 subjects between ages 18 and 25 years underwent a comprehensive screening interview to determine final eligibility. Information regarding respondents' alcohol and substance use and psychiatric status was obtained using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) I/II. All diagnoses were formulated on the basis of DSM-IV criteria using assessment information obtained from the SSAGA I/II interview. Subjects who were assessed to be free of a diagnosis of alcohol and substance abuse or dependence and free from selected psychiatric diagnosis as assessed by the SSAGA were administered the Family History Assessment Module (FHAM) to determine the number of first-, second-, and third-degree paternal and maternal relatives reported to demonstrate symptoms indicative of alcohol and substance abuse or dependence diagnoses as well as selected psychiatric diagnoses. The FHAM interview with each subject was followed up by telephone interviews conducted with father, mother, and 1 other first-, second-, or third-degree relative identified for the purpose of validating FHAM reports. The father and mother were administered the SSAGA to validate diagnostic status of proband and diagnostic status of other relatives. The SSAGA interviews were completed with 90% of fathers and 96% of mothers. However, if the father or mother could not be interviewed, their diagnostic status was validated by the FHAM interview with 3 first- or second-degree relatives. Following this procedure, 48 of the original 250 respondents were enrolled. A subject designated as family history negative had no history of alcohol or dependence in any first- or second-degree relative. A family history-positive subject was one whose biological father and at least 1 additional relative met diagnostic criteria for alcohol dependence. Subjects with mothers meeting criteria for alcohol dependence or abuse were excluded from this study to avoid potential complications of fetal alcohol exposure on central nervous system function. The following additional assessments were administered: (1) 90-Day Time Line Follow Back to provide supplemental confirmation of recent alcohol and other drug use patterns; (2) Fagerstrom test to provide supplemental confirmation of nicotine dependence; and (3) medical history, physical examination, and laboratory tests (complete blood cell counts, electrolyte tests, liver and renal function tests, and glucose determination) to assess health status and to exclude subjects who were pregnant, had a serious medical condition, had abnormal liver functions, had central nervous system or endocrine disorders; and had received treatment within the past 10 years with antidepressants, neuroleptics, mood stabilizers, sedative hypnotic medications, isoniazid, glucocorticoids, and appetite suppressants.

NEUROENDOCRINE PROTOCOL

Subjects reported for sessions at 12:30 PM after fasting since 9 AM. At each session, an intravenous catheter was inserted into a forearm vein at 1 PM. One hour following intravenous line placement, naloxone hydrochloride dissolved in 0.9% isotonic sodium chloride solution, cosyntropin, or placebo (0.9% isotonic sodium chloride solution) was administered for 1 minute as a bolus dose. Baseline blood samples were obtained 15 minutes before and immediately before drug administration. After drug or placebo administration, blood samples were drawn at 15, 30, 45, 60, 90, and 120 minutes. Naloxone hydrochloride (0, 125, or 375 µg/kg) and cosyntropin (0.25 µg) was selected to include a dose previously shown to be submaximal based on ability to stimulate cortisol secretion in healthy subjects. Plasma concentrations of cortisol were measured by radioimmunoassay (Diagnostic Products Corporation Inc, Los Angeles, Calif). Intra-assay and interassay coefficients of variation were 5.2% and 8.0%, respectively. Plasma naloxone concentrations were determined by high-performance liquid chromatography with dual-electrode electrochemical detection (National Medical Services, Willow Grove, Pa). Intra-assay and interassay coefficients of variation were 3% and 6%, respectively.

STATISTICAL ANALYSES

Several statistical procedures were used to evaluate the hypothesis that family history–positive subjects are more sensitive to naloxone relative to family history–negative subjects. First, a dose × family history × time analysis of variance (ANOVA) (with time as the repeated measure) was performed to test for the main effects of family history, dose, and session time, and for the interaction of the 3 effects on the cortisol time curve. Wilks Λ, resulting in an exact F statistic, was used to determine significance levels. Significant multivariate effects were analyzed by univariate tests. Significant effects from univariate analyses were probed with multiple comparison tests using the Bonferroni method to control for the type I error rate. Two summary indicators of cortisol response—peak and delta—were calculated on the basis of the cortisol time curve. The peak response was defined as the highest cortisol value reached after naloxone administration; delta was defined as the difference between peak response and the zero time cortisol value (pre-naloxone administration). A dose × family history repeated-measures ANOVA (with dose as the repeated measure) was performed to test for the main effects of family history, dose, and interaction effects on peak and delta responses. Descriptive statistics (mean, median, skewness, and kurtosis) indicated that raw data tended to be nonnormal for most of the cortisol measures. The square root transformation achieved normality for all study variables; thus, for all the study variables statistical tests were performed on square root transformed data. Significance was evaluated at P ≤ .05.

releasing factor (CRF) neurons of the hypothalamus. This input induces the glucocorticoid component of the stress response. The CRF neurons receive this stress signal through several major neurotransmitter systems: stimulatory input from serotonergic and noradrenergic inner-
Naloxone, a nonselective opioid receptor antagonist, induces a rise in corticotropin and cortisol levels by blocking the opioid component of the inhibitory activity directed at the CRF-producing neurons (Figure 1). As a result of opioidergic modulation of CRF neurons, this test can identify alterations in endogenous opioid activity. For example, persons with less opioid activity (less inhibitory tone directed at the CRF neuron) would be maximally blocked (plateau in cortisol levels) by a lower dose of naloxone compared with individuals with greater opioid activity (more inhibitory tone) who require higher doses of naloxone to induce blockade. Thus, opioid receptor blockade by naloxone provides a functional assessment of opioid activity.

We used opioid receptor blockade with naloxone to explore the hypothesis that offspring from families with a high density of alcoholism (family history positive) have altered endogenous opioid activity compared with offspring from families with no history of alcoholism (family history negative).

RESULTS

Subjects averaged 21 years of age, completed at least 1 year of college, and were predominantly white. Typically, subjects drank 1 day per week, averaging 3 drinks per episode. Groups did not differ in age, sex, racial representation, body mass index, educational status, and alcohol drinking histories (Table). All family history–positive subjects had fathers meeting diagnostic criteria for alcohol dependence; 21 family history–positive subjects (81%) had second-degree paternal relatives meeting diagnostic criteria for alcohol dependence; 21 family history–negative subjects had 23 second-degree maternal relatives meeting diagnostic criteria for alcohol dependence (Table).

Figure 2 presents the cortisol time curves generated within each family history group in response to varying doses of naloxone. Multivariate results yielded a significant main effect for time (F7,114 = 25.26, P < .001), time × dose (F14,228 = 9.83, P < .001), and time × family history (F7,114 = 2.55, P < .05). Tests of between-subject effects yielded significant main effects for dose (F7 = 14.79, P < .001) and family history (F1 = 2.63, P < .05). Family history–negative subjects displayed a dose-dependent cortisol response to each dose of naloxone (Figure 2, left). Results of multiple comparison tests revealed significant differences in cortisol response between placebo and low-dose naloxone at 30 (P < .01), 45 (P < .001), 60 (P < .001), and 90 (P < .01) minutes. At these time points, cortisol levels were higher following administration of low-dose naloxone relative to placebo. Moreover, there were significant differences in cortisol responses between low- and high-dose naloxone at 15 (P < .04), 30 (P < .001), 45 (P < .003), 60 (P < .001), 90 (P < .001), and 120 (P < .002) minutes. At these time points, cortisol levels were higher after administration of high-dose naloxone compared with low-dose naloxone.

In contrast, while family history–positive subjects displayed a dose-dependent cortisol response to low-dose naloxone compared with placebo, no cortisol response differences were observed between those receiving low- and high-dose naloxone (Figure 2, right). Results of multiple comparison tests revealed significant differences in cortisol response between placebo and low-dose naloxone at 30 (P < .01), 45 (P < .001), 60 (P < .002),
and 90 (P<.01) minutes. At these time points, cortisol levels were higher following administration of low-dose naloxone relative to placebo. However, there were no significant differences in cortisol responses between low- and high-dose naloxone at any time points. To assure that adjustment for type I error by the multiple comparisons procedure did not mask small but significant cortisol time point differences between low- and high-dose naloxone in family history–positive subjects, t tests were also performed on the cortisol–time point data set. The results of this additional analysis once again showed no cortisol–time point differences between low- and high-dose sessions for the family history–positive group.

Figure 2 presents differences in cortisol responses between family history groups for each dose of naloxone. Significant differences between family history groups in cortisol responses to placebo were identified at 60 minutes (F = 4.15, P < .05) and 90 minutes (F = 7.02, P < .01). At these time points, family history–positive subjects had increased cortisol levels relative to family history–negative subjects.

Significant differences between family history groups in cortisol responses to low-dose naloxone were identified at all time points following naloxone administration: 15 (F = 9.51, P < .004), 30 (F = 7.70, P < .008), 45 (F = 6.19, P < .02), 60 (F = 7.10, P < .01), 90 (F = 11.01, P < .002), and 120 (F = 7.50, P < .009) minutes. At all time points, family history–positive subjects had increased cortisol levels relative to family history–negative subjects (Figure 2).

In contrast to cortisol responses following administration of placebo and low-dose naloxone, there were no significant differences in cortisol responses between family history groups to high-dose naloxone at any time point (Figure 2). To assure that the multiple comparisons procedure did not mask small but significant family history differences, t tests were performed on the data set. The results of this more liberal analysis showed no differences between family history groups during the high-dose session.

Determination of peak and delta cortisol responses following each dose of naloxone provides supplemental support for these findings. A repeated-measures ANOVA examining the effects of family history and dose on peak and delta responses (with dose as the repeated measure) yielded a significant main effect for dose (peak: F1,38 = 8.33, P < .01; delta: F1,38 = 15.36, P < .001) and a significant interaction effect for dose × family history (peak: F1,38 = 4.34, P < .05; delta: F1,38 = 4.67, P < .04). Tests of within-subjects effects yielded a significant main effect for dose (peak: F1 = 8.33, P < .01; delta: F1 = 15.36, P < .001) and dose × family history (peak: F1 = 4.34, P < .05; delta: F1 = 4.67, P < .04). In family history–negative subjects, mean ± SEM peak cortisol responses were significantly greater following the high dose of naloxone (966 ± 110 nmol/L) compared with the low dose (690 ± 55 nmol/L). Similarly, delta cortisol responses were significantly greater following the high dose of naloxone (579 ± 55 nmol/L) compared with the low dose (276 ± 27 nmol/L). In contrast, family history–positive subjects had similar peak (low dose, 883 ± 83 nmol/L; high dose, 883 ± 83 nmol/L) and delta cortisol (low dose, 441 ± 55 nmol/L; high dose, 497 ± 55 nmol/L) responses to both low- and high-dose naloxone.

In total, these analyses indicate that family history–negative subjects had a statistically unique cortisol response to each dose of naloxone. Cortisol responses to high-dose naloxone were greater than cortisol responses to low-dose naloxone, which in turn were greater than cortisol responses to placebo. However, cortisol responses of family history–positive subjects were maximally stimulated following low-dose naloxone administration, with no further increase in cortisol following high dose.

Age, race, body mass index, educational status, and alcohol drinking histories were not associated with cortisol responses to naloxone, and sex did not qualify the effect. Furthermore, these findings cannot be explained by group differences in naloxone metabolism since plasma naloxone concentrations did not differ between family history groups during the entire 120 minutes (data not shown). Finally, these findings cannot be explained by group differences in adrenal responsiveness to endogenous corticotropin generated by naloxone administration.
tion since cortisol responses to the corticotropin analog, cosynotropin did not differ between family history groups (peak: family history positive, 690 ± 66 nmol/L vs family history negative, 635 ± 77 nmol/L; delta: family history positive, 243 ± 27 nmol/L vs family history negative, 254 ± 33 nmol/L).

COMMENT

This study extends findings from our previous work demonstrating significant differences in hypothalamus-pituitary-adrenal (HPA) axis dynamics as a function of family history of alcoholism and the results are in line with those of other studies demonstrating family history differences in corticotropin, β-endorphin, and cortisol levels following low-dose ethanol ingestion. The HPA axis may serve as a marker to distinguish individuals at increased risk for alcoholism. This is not surprising when one considers that paraventricular CRF neurons, which are responsible for initiating the HPA axis stress response, are also modulated by the “candidate” neurotransmitter systems implicated in genetic vulnerability for alcoholism: opioidergic, GABAergic, serotoninergic, and catecholaminergic systems. These 4 neurotransmitter systems not only alter hypothalamic CRF neurons but also modulate mesolimbic dopamine generation and reward (Figure 1).

These findings lend further support to the contention that alcohol-dependent individuals and their offspring have a defect in brain opioid activity. In this regard, several opioid hypotheses have been proposed to explain the biological vulnerability to alcohol dependence. Although several neurotransmitters influence alcohol reward, dopamine has been proposed as the key neurotransmitter interacting with reward centers of the brain to induce the reinforcing effects of many drugs of abuse, including alcohol. It has been proposed that opioid peptides and dopamine act sequentially in this reward pathway. Ethanol administration induces opioid release, which in turn triggers the release of dopamine at the level of the nucleus accumbens. This release is blocked by the opioid antagonist naltrexone, implicating the endogenous opioid system in this reward process.

There are at least 4 interpretations for our findings. First, the observations may imply that family history–positive subjects have less opioid activity compared with family history–negative subjects (opioid deficiency hypothesis). In this model, subjects at high risk for excessive alcohol consumption have inherited or acquired a deficiency in activity of the endogenous opioid system, ie, less synaptic opioid content and/or reduced opioid receptor density. If this were true, then subjects with less opioid activity and, therefore, less inhibitory tone on CRF would be maximally blocked by a lower dose of naloxone, resulting in a plateau in serum cortisol levels compared with individuals having greater opioid activity. Since alcohol enhances opioidergic activity, the high-risk subject consumes greater quantities of alcohol to compensate for this basal deficiency. More specifically, reduced opioid tone may result in diminished accumulation of dopamine within the nucleus accumbens at baseline or following low-dose ethanol ingestion. Thus, family history–positive individuals would require higher blood ethanol levels to stimulate the opioid-mesolimbic dopamine cascade compared with family history–negative individuals. Although we speculate that this is the meaning of our findings, it is acknowledged that other interpretations are also possible.

A second explanation is that family history–positive subjects have opioid receptors with greater binding affinity for naloxone compared with control subjects (opioid sensitivity hypothesis). It is plausible that family history–positive subjects have an opioid receptor mutation resulting in more avid binding of ligand to receptor. In this scenario, the higher binding affinity allowed our lower naloxone dose (125 µg/kg) to fully block opioid receptors, thereby stimulating maximal cortisol release. In this model, increased receptor affinity results in enhanced sensitivity to ethanol-induced opioid secretion, effecting the magnitude of mesolimbic dopamine release. This opioid abnormality would result in family history–positive individuals generating more mesolimbic dopamine per drinking episode (eg, more reward) compared with family history–negative individuals. However, the observation that placebo cortisol levels are higher rather than lower in family history–positive subjects relative to family history–negative subjects, argues against this model. One would assume that greater receptor affinity (eg, more inhibitory tone on CRF) would result in lower placebo cortisol levels.

A third explanation for the finding is that high- and low-risk offspring may differ in the ratio of µ and δ opioid receptors regulating CRF inhibitory input. Last, it is also possible there are no differences in endogenous opioid activity as a function of family history of alcoholism. Rather, the induction of opioid blockade, resulting in the disinhibition of CRF neurons, merely unmasked family history differences in serotonin, GABA, and/or noradrenergic tone on the CRF neuron. Regardless of cause, we speculate that family history difference in neurotransmitter input on CRF neurons is part of a neurochemical milieu generating altered reinforcement from alcohol. While our findings may reflect genetic determinants for endogenous opioid tone, recent findings have shown that chronic stress can also alter opioid activity and this perhaps could cluster in dysfunctional families.

In summary, nonalcoholic offspring from families with a high density of alcohol-dependent individuals have altered neurotransmitter activity directed at hypothalamic CRF neurons, possibly representing group differences in opioid activity. We speculate that a subset of family history–positive individuals acquire a higher rate of alcohol-seeking behavior in adulthood to compensate for this abnormality.

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