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Genetic Differences in Opiate Receptor Concentration and Sensitivity to Ethanol's Effects

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YIRMIYA, R. AND A. N. TAYLOR. *Genetic differences in opiate receptor concentration and sensitivity to ethanol's effects.* PHARMACOL BIOCHEM BEHAV **33**(4) 793–796, 1989.—The hypothesis that genetic differences in opiate receptor concentration are involved in determining the sensitivity to some of the effects of alcohol was studied by comparing the hypothermic and analgesic effects of ethanol in four strains of mice that can be divided into three groups on the basis of their brain opiate receptor concentration: high (CXBH), low (CXBK) and intermediate (C57BL/6By and BALB/cBy). In the first experiment, animals within each strain were injected with either saline or 1.5 g/kg ethanol and their pain sensitivity was assessed 20 min later by the hot-plate test. The same procedure was repeated 10 days later with a higher dose of ethanol (2.5 g/kg). The lower dose produced analgesia only in CXBH mice, whereas the higher dose produced analgesia in CXBH, C57BL/6By and BALB/cBy mice, but had no effect in CXBK mice. In the second experiment, animals within each strain were injected with either saline or naloxone, followed 20 min later by an injection of 3.5 g/kg ethanol. CXBH mice were significantly more hypothermic and CXBK mice were significantly less hypothermic than all other strains. Naloxone attenuated ethanol's hypothermic effect in all strains except CXBK. These results suggest that the hypothermic and analgesic effects of ethanol are at least partly mediated by opiate receptors and are correlated with genetic differences in opiate receptor concentration.

Ethanol Opiates Opioid receptors Hypothermia Analgesia Naltrexone Genetic differences Mice

SEVERAL lines of evidence indicate the existence of an interaction between opiates and ethanol. Opiate antagonists suppress ethanol consumption and preference, whereas low doses of the opiate agonist morphine increase ethanol intake and preference (38). Ethanol administration alters several endogenous opioid peptide systems, including β-endorphin (11,12) and Met-enkephalin (40), and alters opiate receptor characteristics (16,41). Cross tolerance between opiates and alcohol develops with respect to many of their effects (22, 25, 28). Finally, a neurochemical basis has been proposed for the opiate-ethanol interaction (3,7).

Additional evidence for an interaction between alcohol and opiates was provided by studies demonstrating an attenuation or reversal of ethanol's behavioral and physiological effects by administration of opiate antagonists. In experiments with animals, naloxone attenuates the analgesic effect of ethanol (6,36), attenuates ethanol-induced hypothermia (35), antagonizes ethanol-induced narcosis and lethality (17), blocks the stimulatory and depressant effects of a low and a high dose of ethanol, respectively, on locomotor activity, open-field external ambulations and shuttle-box performance (30,37), and attenuates the activation of adrenocortical function during withdrawal from ethanol (13). In clinical studies, naloxone reverses ethanol-induced coma (20), inhibits the psychomotor effects of mild ethanol intoxication (19), and ameliorates ethanol-induced impairment of performance in a vigilance task involving decision making and motor response (9).

Studies in both humans and animals provide evidence for a genetic contribution to the susceptibility to ethanol's effects. Some of this evidence derives from studies showing differential preference for alcohol and differential responsiveness to the behavioral, physiological and biochemical effects of alcohol in inbred strains of mice (21) or after selective breeding for ethanol preference (8) or responsiveness (5,24). The sources for the genetic variability in vulnerability to ethanol's effects are not clear. One such source may be related to genetic alteration in the brain's opioid system.

The present study was based on the hypothesis that genetic differences in brain opiate receptor concentration are involved in determining the sensitivity to at least some of the effects of ethanol. This hypothesis was tested by comparing the analgesic and hypothermic effects of ethanol in four inbred strains of mice, C57BL/6By (C57), BALB/cBy (BALB), CXBK and CXBH. These strains can be divided into 3 groups on the basis of brain opiate receptor concentration: high (CXBH), low (CXBK) and

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intermediate (C57 and BALB) (1,31). Previous behavioral studies on these strains demonstrated that their opiate receptor concentrations correlate with the analgesic and locomotor (1,33) effects of exogenous opiate administration, and with endogenous opioid-mediated processes, such as the analgesic effects of stress (26), defeat (29), electroacupuncture (34), D-amino acid administration (2), and saccharin preference (42). We now report that the sensitivity to the analgesic and hypothermic effects of ethanol correlates positively with the opiate receptor concentrations found in these four strains.

METHOD

Subjects

Subjects were C57, BALB, CXBK and CXBH male mice (Jackson Laboratories, Bar Harbor, ME), weighing 27–36 g. Mice were housed in individual cages at 22 ± 1°C and had free access to food (Wayne Lab Blox) and water. Testing was conducted in the first half of the light portion of a 12-hr light/dark cycle.

Procedure

Assessing the analgesic effect of ethanol. Animals within each strain were divided into two groups (n = 8–11), matched for mean body weight. Mice of the first group were injected with ethanol (1.5 g/kg; IP, diluted in saline to an injection volume of 10 ml/kg); the others with an equal volume of saline. A similar dose of ethanol was previously found to produce naloxone-attenuated analgesia (36). Pain sensitivity was assessed 20 min after ethanol/saline injection by the hot-plate method. Each animal was placed on a 56°C metal surface and the latency to display a characteristic hind-paw flick was measured. To avoid tissue damage, animals that did not display hind-paw flick were removed from the plate after 60 sec. The same procedure was repeated 10 days later with a higher dose of ethanol. The results were analyzed by a 3-way analysis of variance with the strain and drug as between subjects factors and the ethanol dose as repeated measures, followed by post hoc tests with the Newman-Keuls procedure ($p<0.05$).

Assessing the hypothermic effects of ethanol. The hypothermic effects of ethanol were assesed 10 days after the last analgesia experiment. Animals within each strain were divided into two groups matched for mean body weight and counterbalanced in terms of their exposure to ethanol/saline in the analgesia experiment. Six to eight mice of each strain were injected with naloxone (10 mg/kg; IP); the others with saline. Twenty min later, all animals were injected with ethanol (3.5 g/kg; IP, diluted in saline to an injection volume of 10 ml/kg). Rectal temperature was monitored by a telethermometer and insertion of a lubricated probe 2 cm into the rectum 30, 45 and 90 min after the ethanol injection. Baseline core temperature was measured in a separate group of mice (14–21 of each strain). Ambient room temperature was 22 ± 1°C. The results were analyzed by a 3-way analysis of variance with the strain and drug (naloxone vs. saline) as between-subjects factors and the time after the injection as repeated measures. Differences between individual strains and between saline- and naloxone-injected animals with each strain were analyzed by planned comparisons ($p<0.05$).

RESULTS

Analgesic Effects of Ethanol in the Four Strains

Mean paw-flick latencies on a hot plate 20 min after administration of either saline or ethanol to CXBK, BALB, C57 and CXBH mice are presented in Fig. 1. The ANOVA revealed overall significant effects of the strain, $F(3,67)=36.66$, $p<0.001$, ethanol treatment, $F(1,67)=41.01$, $p<0.001$, and a significant strain by drug interaction, $F(3,67)=7.78$, $p<0.001$. Additionally, a significant drug by trials interaction was shown, $F(1,67)=7.62$, $p<0.01$, indicating that the higher ethanol dose produced significantly greater analgesia than the lower dose. Post hoc tests indicated that the lower dose of ethanol produced significant analgesia (compared to saline-injected animals) only in CXBH mice. The higher dose of ethanol produced significant analgesia in CXBH, C57 and BALB mice. The analgesic effect of this dose was significantly larger for CXBH mice than for C57 and BALB mice. Even at this higher dose, ethanol had no analgesic effect in CXBK mice.

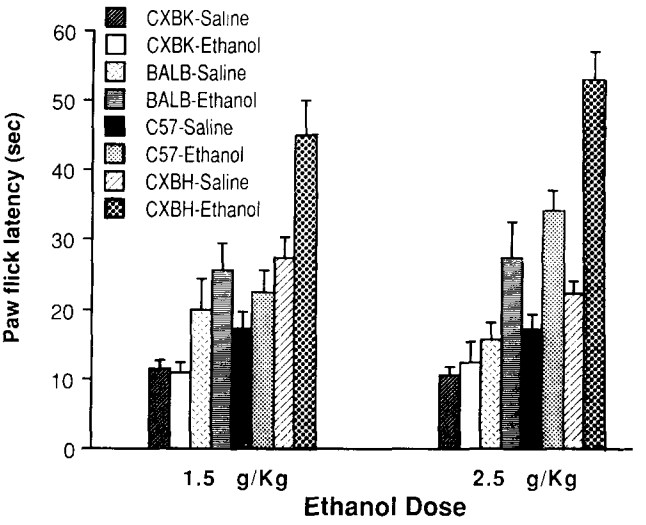

FIG. 1. Mean (±S.E.M.) paw-flick latencies on a hot plate 20 min after administration of either saline or ethanol to CXBK, BALB, C57 and CXBH mice. Ethanol-injected mice received 1.5 g/kg ethanol during the first session and 2.5 g/kg ethanol during the second session, conducted 10 days later.

Hypothermic Effect of Ethanol in the Four Strains

Mean core temperature of CXBK, BALB, C57 and CXBH mice 30, 45 and 90 min after administration of 3.5 g/kg ethanol is presented in Fig. 2. The ANOVA revealed an overall significant difference among strains, $F(3,66)=21.80$, $p<0.001$, and a significant attenuation of hypothermia by naloxone pretreatment, $F(1,66)=16.73$, $p<0.001$. Further planned comparisons showed that CXBH mice were more hypothermic, whereas CXBK mice were less hypothermic than all other strains. Naloxone attenuated the hypothermic effect of ethanol in all strains except CXBK. Mean baseline temperature in the four strains ranged between 37.2–37.5 and did not differ among the strains, $F(3,69)=2.05$, $p>0.1$. For all strains, baseline temperature was significantly higher than the temperatures measured 30, 45 and 90 min after ethanol injection ($p<0.05$).

DISCUSSION

The results indicate that both the analgesic and hypothermic effects of ethanol are correlated with genetic differences in opiate receptor concentration. These results suggest that both effects are at least partly mediated by brain opiate receptors and that genetic differences in opiate receptor concentration account for some of the variability in the sensitivity to ethanol-induced analgesia and hypothermia. These conclusions are in accord with studies dem-

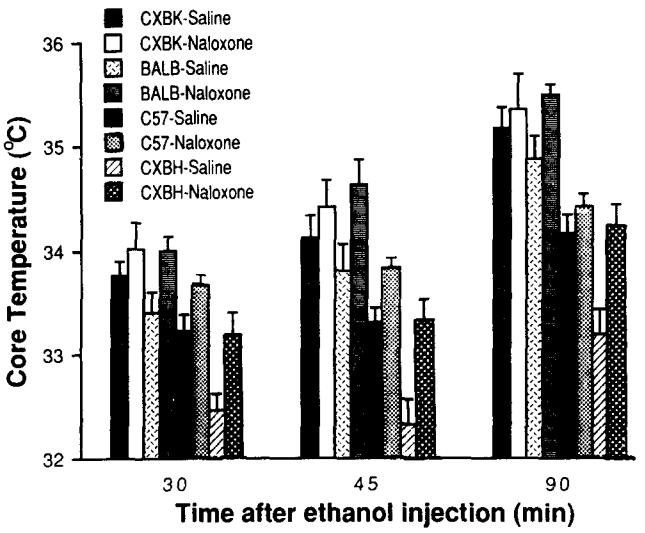

FIG. 2. Mean (±S.E.M.) core temperature of CXBK, BALB, C57 and CXBH mice 30, 45 and 90 min after administration of 3.5 g/kg ethanol. Mice within each strain were preinjected with either naloxone or saline 20 min before ethanol administration.

onstrating attenuation of ethanol-induced analgesia (6,36) and hypothermia (35) by naloxone, and development of cross tolerance between morphine and ethanol in terms of their analgesic (25) and hypothermic (22) effects. Additionally, the correlation between opiate receptor concentration and ethanol's effects found in the present study is identical to the correlation between opiate receptor concentration in these mice strains and exogenous opiate administration (1,33) or endogenous opioid-mediated manipulations (26, 29, 34, 42). Altogether, these results support the notion that ethanol exerts at least some of its behavioral and physiological effects by interacting with the brain opioid system, and thus the opioid system, in general, and genetic differences in opiate receptor concentration, in particular, may be implicated in the etiology of alcoholism and alcohol's effects.

A neurochemical basis for the interaction between ethanol and opiates was proposed by pointing out that tetrahydroisoquinolines (TIQs), alkaloid condensation products formed as a consequence of alcohol metabolism, are requisite intermediates in the synthesis of morphine in the poppy plant, and may contribute to the acute and chronic effects of alcohol intoxication (3,7). This hypothesis is supported by evidence indicating that TIQs are formed in the brains of animals exposed to alcohol (14) and in human alcoholics (4), that TIQs have both ethanol- and opiate-like effects (10, 27, 39), and that intracerebroventricular (ICV) infusion of TIQs dramatically increases ethanol intake and preference (32). Of particular relevance to the present study is the demonstration that TIQs produce a naloxone-reversible analgesia in both rats and mice after either systemic or ICV administration (10,27). These findings, together with the results of the present study, suggest that the sensitivity to the effects of ethanol are at least partly determined by the number of receptors that interact with TIQs, which may be formed as a result of ethanol administration.

Acute administration of ethanol has been found to stimulate the release of β-endorphin into the blood (11,12), to either decrease (11) or not change (40) the levels of β-endorphin in the pituitary and to either decrease or not change the level of β-endorphin in the hypothalamus (12). Thus, it could be postulated that ethanol induces its analgesic and hypothermic effects indirectly, by changing β-endorphin levels and that the differences among the strains derive from differential activation of β-endorphinergic systems. This possibility is unlikely, however, since C57 and BALB strains, which have different baseline levels of β-endorphin and differ in their hypothalamic β-endorphin response to acute ethanol administration (12), did not manifest differential responsiveness to ethanol in the present study.

Previous studies demonstrated that ethanol alters opiate receptor characteristics when administered either in vitro (16,41) or in vivo (18). The reduction of μ receptor number (23) and affinity (18) observed with chronic ethanol administration may be specifically relevant to the results of the present study, as the concentration of these receptors is genetically decreased in CXBK mice (31). Thus, it is possible that tolerance to at least some of the effects of ethanol (e.g., reduced hypothermic and analgesic responses) results from a reduction in μ receptor affinity or number, which creates a 'CXBK-like' animal.

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