Antagonism of the non-opioid component of ethanol-induced analgesia by the NMDA receptor antagonist MK-801

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Recent evidence from our laboratory suggests that the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (dizocilpine) selectively antagonizes non-opioid (i.e. naloxone-insensitive) mechanisms of stress-induced analgesia in mice. For example, we have recently demonstrated that a low dose of MK-801 (0.075 mg/kg, i.p.) antagonizes the non-opioid component of a mixed opioid/non-opioid swim stress-induced analgesia (SSIA) resulting from forced swimming for 3 min in 20°C water. Since ethanol-induced analgesia (EIA) has been found to be only partially attenuated by naloxone, we hypothesized that MK-801 would similarly block the non-opioid component of EIA. The effects of MK-801 and of the opioid receptor antagonist naloxone (10 mg/kg, i.p.) on analgesia produced by ethanol (2.5 g/kg in 20% vol/vol, i.p.) were studied in control mice and in mice selectively bred for high (HA) or low (LA) SSIA. HA mice showed significantly more, and LA mice significantly less, EIA than controls. Naloxone and MK-801 significantly attenuated EIA in control and HA mice, and in these lines the combined administration of both antagonists blocked EIA completely. In LA mice, which displayed very little EIA, naloxone but not MK-801 reversed EIA completely. These findings provide additional evidence for the role of the NMDA receptor in non-opioid mechanisms of analgesia. The finding that mice selectively bred for high and low SSIA also display high and low EIA suggests common mediation of the effects of stress and ethanol on antinociceptive processes.

In order to investigate the hypothesis that humans consume ethanol for its anxiety-reducing properties, much attention has been paid to the study of stress/ethanol interactions at both the biochemical and the behavioral level. This interaction, however, has proven to be complex. Ethanol affects a number of physiological variables (e.g. corticosterone, ACTH, catecholamines, β-endorphin) in ways similar to stress itself6,50,51. However, ethanol has also been shown to antagonize stress-induced changes in the levels of these same substances6,14,15,28,39,42,50. Similarly, behavioral research into the interaction of stressors with ethanol has been contradictory, featuring reports of both synergism and antagonism40.

Another commonality between stress and ethanol is that they both have antinociceptive properties. The phenomenon of stress-induced analgesia (SIA) has received a great deal of attention over the last two decades, and many environmental stressors have been found that induce SIA49,52. Research into the neurochemical mechanisms of SIA has revealed that two major forms of SIA exist, referred to as ‘opioid’ and ‘non-opioid’ based upon their naloxone-reversibility, susceptibility to tolerance development, and cross-tolerance with opiates such as morphine. Ethanol induces analgesia in rodents2,5,7,25,30,41,56 and humans9,11,12,24,53,54 that has been reported to be at least partially opioid mediated5,12,30,41 or non-opioid2,25. It is possible, therefore, that the neurochemical mediation of EIA, by analogy to SIA, crucially depends on the ethanol dose and/or the specifics of the testing situation. That is, in different experimental paradigms, EIA might be opioid, non-opioid, or mixed opioid/non-opioid in character.
Fig. 1. Effects of naloxone (NAL), MK-801 (MK), and a combination of these drugs (NAL+MK) on analgesia in the hot-plate test produced by injection of 2.5 g/kg ethanol in mice selectively bred for low (LA) and high (HA) swim stress-induced analgesia, and in unselected controls (C). Bars represent mean percent maximum possible effect ± S.E.M. *P < 0.05 compared to saline. #P < 0.05 compared to all other groups.

We have recently demonstrated the ability of a low dose of the specific, non-competitive, N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 ( dizocilpine; 0.075 mg/kg, i.p.) to attenuate the non-opioid component of a mixed opioid/non-opioid SIA resulting from 3 min forced swimming in 20°C water. The major aim of the present study was, therefore, to examine the effect of MK-801 on the non-opioid component of EIA.

Recently it has been demonstrated that the analgesic, as well as the hypothermic, effects of ethanol are correlated with genetic differences in opiate receptor concentration. In that study, the recombinantly inbred CXBH strain, which has been found to display high brain opiate receptor density, manifested more EIA than the opiate-receptor deficient CXBK strain. The parental mouse strains used to develop the CXB series, C57BL/6 and BALB/c, displayed intermediate EIA. In fact, these strains display dramatic differences in the magnitude of many forms of opioid-mediated analgesia (for reviews, see). A similar divergence in analgesic magnitudes following swim stress, morphine, and brain stimulation-produced analgesia is observed in mice that have been bred specifically for high (HA) and low (LA) swim SIA (SSIA). Thus, another aim of this study was to compare EIA in HA versus LA mice.

Subjects were naive 8-10 week old HA, C (unselected controls) and LA mice of both sexes bred using two-way artificial selection for 21 generations in the Institute of Genetics and Animal Breeding, Polish Academy of Sciences (Jastrzebiec, Poland). The breeding strategy has been described previously. At 6 weeks of age, animals were flown to Los Angeles, where all subsequent testing occurred. Animals were housed 5 to a cage, and fed and watered ad libitum. The housing colony was maintained on a 12:12 h light/dark cycle, with lights on from 08.00 to 20.00 h. Testing began at 12.00 h and was completed by 15.30 h. Pain sensitivity was assessed using the hot-plate test. Animals were placed on a 54°C ± 0.2°C metal surface, and the latency was recorded of one of the following behavioral end-points, vigorous hindpaw shake or lick, whichever occurred first. A cut-off of 60 s was imposed; animals not responding within this time period were removed from the hot plate in order to avoid the possibility of tissue damage.

Animals were injected with either naloxone (10 mg/kg; Research Biochemicals Inc., Natick, MA) or isotonic saline. Twenty min later, baseline hot-plate latency was measured as described above. Following baseline assessment, all animals were injected intraperitoneally with ethanol (2.5 g/kg in 20% vol/vol). Immediately following ethanol administration, animals were injected with either MK-801 (0.075 mg/kg; Research Biochemicals Inc., Natick, MA) or isotonic saline. Thus, the 4 drug conditions in the experiment were: saline + ethanol + saline (SAL), naloxone + ethanol + saline (NAL), saline + ethanol + MK-801 (MK), and naloxone + ethanol + MK-801 (NAL + MK). Naloxone and MK-801 were delivered intraperitoneally in a volume of 10 ml/kg. Twenty min later, hot-plate latency was measured again.

For the purposes of data analysis, elevations of post-ethanol hot-plate latencies over baseline levels were expressed as percentages of the maximum possible effect (%MPEs), as follows:

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%\text{MPE} = \frac{(\text{post-ethanol latency} - \text{baseline latency})}{(\text{cut-off latency} - \text{baseline latency})} \times 100
\]
In order to assess the effect of ethanol on pain sensitivity, a repeated measures ANOVA was performed using raw latency data of the SAL animals, with genetic line (HA, C, LA) treated as a between-subject comparison and baseline latency versus post-ethanol latency as the repeated measure. In further statistical analyses, only %MPE values were considered. Data were subjected to a square-root transformation in order to achieve equality of variance between groups. A two-way ANOVA was performed on the transformed %MPE data, with both genetic line and drug condition treated as between-subject comparisons. Post-hoc analyses were performed where appropriate using the Student-Newman-Keuls procedure and a significance level of \( P < 0.05 \) in all cases.

Ethanol produced significant analgesia in saline-treated animals of all lines, and the magnitude of EIA differed significantly among the lines in the rank-order: LA < C < HA. The two-way ANOVA revealed significant main effects of genetic line \( (F_{2,99} = 33.49) \) and drug \( (F_{3,99} = 36.44) \). The line \( \times \) drug interaction was not significant \( (F_{6,99} = 1.20, P = 0.32) \). Significant simple main effects of drug condition were revealed by one-way ANOVAs for each genetic line (LA: \( F_{3,99} = 33.49 \)) and drug \( (F_{3,99} = 36.44) \). Post-hoc analyses revealed that MK and NAL produced significant antagonism of EIA in both C and HA mice. NAL + MK produced significantly more antagonism of EIA than either drug alone in these two groups. NAL and NAL + MK, but not MK alone, significantly attenuated EIA in LA mice. Significant differences in baseline nociception among genetic lines \( (F_{1,105} = 30.67) \) were observed, as have previously been noted\(^{31,32}\). By converting raw hot-plate latencies to %MPEs, how-ever, it was possible to assess analgesic magnitudes unaffected by these baseline differences. No obvious sedation or ataxia was observed in any of the subjects.

There is a great deal of evidence that ethanol exerts a number of its myriad behavioral and physiological effects via brain opioid mechanisms\(^4\). In addition to the partial or complete attenuation of a wide variety of ethanol effects by naloxone, cross-tolerance between ethanol and morphine has been demonstrated with respect to their effects on nociception\(^{20}\), body temperature\(^{27}\), brain calcium concentration\(^{43}\), and guinea pig ileum/rat vas deferens contractility\(^{35,36}\). The acute administration of ethanol has been found to change levels of endogenous opioid peptides, including \( \beta \)-endorphin\(^{20,39,42,44}\) and Met-enkephalin\(^44,45\), and to alter opiate receptor binding\(^{22,48}\). A direct mechanism for the interaction between ethanol and opiates has also been proposed. Tetrahydroisoquinolines (TIQs), alkaloid condensation products of the primary metabolite of ethanol, are in fact synthesis intermediates in the formation of morphine, and have been demonstrated to have naloxone-reversible effects similar to both ethanol and the opiates, including analgesia\(^{17,34}\).

The fact that in the present study as well as in others\(^5,41\) naloxone only exhibited a partial antagonism of EIA suggests a need to look elsewhere for a complete understanding of its mechanisms. Serotonergic, but not adrenergic, systems have been implicated in the mediation of non-opioid EIA\(^7\). A literature is developing that documents the existence of ethanol/glutamate interactions. Ethanol inhibition of NMDA ion currents in brain slice preparations has been reported\(^{1,8,19,23,29}\), and these findings have been replicated in vivo\(^47\). This type of interaction is unlikely to explain the present results, however, since ethanol must activate, not inhibit, NMDA receptors in order to produce EIA that can be antagonized by MK-801. There has been at least one report that micromolar concentrations of ethanol increase the affinity of \( [^{3}H]MK-801 \) binding in rat brain, although much higher concentrations (200 mM) decrease binding affinity\(^13\).

When selective breeding for one trait produces line differences in another trait, these are known as correlated responses\(^{10,16}\). In the present study it was demonstrated that HA mice, bred for high SSIA, display high EIA as well; and LA mice, bred for low SSIA, display low EIA. Such correlations can occur by chance (i.e. genetic drift), and the lack of replicate HA/C/LA lines could pose interpretive problems; however, Henderson\(^{21}\) argues that correlated responses are in fact trustworthy when the correlated mean difference exceeds 25% of the equivalent difference on the original selected trait. This is clearly the case with respect to EIA; HA mice have %MPE scores that are 300% higher than those of LA mice, which is almost as large a difference as that routinely observed between these lines with respect to 20°C SSIA\(^31\). Such findings strongly support the contention that SIA and EIA share genetic determinants. Similarity in genetic determination, in turn, implies that the traits have common physiological mediation.

Although many attempts have been made in recent decades to demonstrate directly that organisms voluntarily consume ethanol to attenuate stress, there also exists physiological evidence suggesting that, at least in a rodent naïve to the drug, ethanol might itself be a stressor (see ref. 40). Ethanol-induced stress may be due to intoxication per se and/or peritoneal discomfort caused by the i.p. injection\(^19\). Ethanol, like stressors, can produce gastric lesions in mice\(^26\). Thus, it may be that the opioid and NMDA-mediated analgesic systems identified for SIA\(^{31,32}\) are the same as those...
activated by ethanol. This hypothesis is supported by the present observation that mice selectively bred for a mixed opioid/non-opioid SIA, 20°C swim, show striking similarities in both their quantitative and qualitative responses to SIA and EIA, magnitude of analgesia and neurochemical mediation, respectively.

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