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Tolerance to Ethanol Analgesia Is Not Accompanied by Cross-tolerance to Morphine Analgesia in Rats

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BELL, R. L., R. D. OLSON AND A. L. VACCARINO. *Tolerance to ethanol analgesia is not accompanied by cross-tolerance to morphine analgesia in rats.* PHARMACOL BIOCHEM BEHAV **59**(1) 123–127, 1998.—In the present study, we examined the development of environment-independent and environment-dependent tolerance to ethanol-induced analgesia (EIA) and cross-tolerance with morphine-induced analgesia (MIA). To examine the development of environment-independent tolerance, male Long–Evans rats were given increasing amounts of ethanol (5 days each of 5% (v/v), 10% (v/v), and 15% (v/v)) added to their drinking water over a 15-day period. A control group was given plain tap water to drink. On day 16, all rats were given plain tap water to drink. On day 17, the animals were tested for EIA (2.5 g/kg, IP) or MIA (10 mg/kg, IP) in the hot plate test. To examine the development of environment-dependent tolerance, animals were injected with ethanol (2.5 g/kg, IP) or an equal volume of saline once a day for 2 days. On day 3, the animals received no treatment. On day 4, the animals were tested for either EIA (2.5 g/kg, IP) or MIA (10 mg/kg, IP) in the hot plate test. It was found that rats pretreated with ethanol (both self-administration and IP injections) displayed tolerance to EIA, which was not accompanied by cross-tolerance to MIA.

Ethanol Morphine Pain Analgesia Tolerance Associative Nonassociative Environment dependent Environment independent

ETHANOL-INDUCED analgesia (EIA) has been reported in both humans (4,27,28) and animals (1–3,7–10,16,20,21,29). In an early study, ethanol was found to attenuate naloxone-induced hyperalgesia (1), suggesting a possible interaction between EIA and the endogenous opioid systems. In rodents, there are reports that naloxone either attenuated EIA (2,20,21), or did not attenuate EIA (9). In addition, EIA was related to brain opiate receptor levels in inbred strains of mice (29), and in rats bred for their ethanol sensitivities, it was found that MIA was directly related to ethanol sensitivity (18).

Ethanol–opiate interactions have also been addressed by examining cross-tolerance between EIA and MIA. Using forced gastric administration of ethanol, Fidecka et al. (5) found that tolerance to EIA was accompanied by cross-tolerance to MIA in mice but not in rats. Jorgensen et al. (8) and Jorgensen and Hole (10) found that neither tolerance to EIA nor cross-tolerance to MIA developed in rats injected with 2.5 g/kg ethanol for 8 days. However, tolerance to EIA accompanied by cross-tolerance to MIA was found if the animals were exposed to the analgesic test (i.e., tail flick) during tolerance induction. These results suggest that practice during ethanol administration is necessary for the development of cross-tolerance between EIA and MIA (8). It should also be noted, however, that Jorgensen et al. (8) injected rats in the home cages during tolerance induction and then tested for analgesia in a separate environment. Studies have shown that tolerance to ethanol's effects can be reduced or eliminated if animals are tested in an environment that is different from that which was used to induce tolerance (10,13,24). Therefore, the failure to show tolerance in the absence of testing by Jorgensen et al. (8) may be related to the differences in environmental cues during tolerance induction and subsequent testing.

The development of tolerance to ethanol's effects has been shown to involve both associative and nonassociative mechanisms (14,25). "Associative" or "environment-dependent" tolerance can be shown by administering ethanol by the same route and environment used to test tolerance (25). Such environment-dependent tolerance is suggested to occur because of the presence of environmental cues (i.e., handling, injection procedure, room), which signal the subsequent administration

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of the drug. These cues are believed to induce a compensatory response to the drug, thus reducing its effectiveness (22,23). "Environment-independent" or "nonassociative" tolerance can be shown under conditions in which ethanol is administered by vapor inhalation or voluntary liquid consumption and the animals are then tested for tolerance by IP injections. Because the cues present during tolerance induction and testing are different, and no association is made between environmental cues and the drug effects during tolerance induction, such tolerance is believed to occur independent of learning (25).

In the present study, we examined the development of both environment-independent and environment-dependent tolerance to EIA and cross-tolerance to MIA. To examine environment-independent tolerance to EIA, we added ethanol to the rats' drinking water, and then tested tolerance and cross-tolerance using IP injections of ethanol and morphine. This paradigm should minimize the role of environmental cues in the development of tolerance. Environment-dependent tolerance was assessed by injecting rats with ethanol via the same route (IP) and environment used to test tolerance.

METHOD

Subjects

Male Long–Evans hooded rats (Harlan Laboratories, IN) weighing 300–400 g served as subjects. The rats were housed individually and maintained on a 12 L:12 D cycle (light onset at 0700 h). Rats were given free access to food and water, except as described below.

Environment-Independent Tolerance

Rats were assigned to one of two treatment groups. To examine the development of environment-independent tolerance, one group of rats drank an ethanol solution (tap water mixed with 95% ethanol) for 15 consecutive days. The ethanol solution was given in increasing concentrations: 5 days of 5% (v/v), 5 days of 10% (v/v), and 5 days of 15% (v/v). A control group drank ordinary tap water for 15 days. All tolerance induction procedures were carried out in their home cages, in the vivarium.

On day 16, all animals were given ordinary tap water to drink. At the beginning of day 16, four of the animals were sacrificed by decapitation, and trunk blood was collected for a blood alcohol analysis. The analysis revealed no traces of alcohol.

On day 17, the animals were taken from the vivarium to the test room and injected IP with either alcohol (2.5 g/kg) or morphine (10 mg/kg, Mallinckrodt, USA). Ethanol (95%) was mixed with isotonic saline for a concentration of 30% (v/v) and administered in a volume of 0.83 ml/100 g of body weight. Morphine sulfate was dissolved in isotonic saline and delivered in a volume of 0.1 ml/100 g of body weight. Analgesia was assessed using the hot-plate test. The latency to a hind-paw lick after placement on a hot-plate maintained at 52°C was measured before (baseline) and 30 min after ethanol or morphine injections. A cutoff period of 60 s was imposed to prevent tissue damage.

Environment-Dependent Tolerance

Rats were assigned to one of two treatment groups. To examine the development of environment-dependent tolerance, one group of rats was injected with ethanol (2.5 g/kg, IP) for 2 consecutive days. A control group of rats was injected with an equal volume of saline. All injections were carried out in their home cages in the analgesia testing area. The animals received no treatment on day 3. On day 4, rats were tested for EIA and MIA as described above.

Statistical Analysis

Data were submitted to a 2 × 2 mixed ANOVA (the within-subject factor was baseline latency vs. postdrug latency; the between-subject factor was ethanol pretreatment vs. control, $\alpha = 0.05$ for all tests). Data were analyzed using SPSS version 6.0 for Windows.

RESULTS

Environment-Independent Tolerance

Ethanol-induced analgesia. ANOVA revealed a significant test period × pretreatment interaction, $F(1, 26) = 15.28$, $p < 0.001$, with an effect size of partial $\epsilon^2 = 0.370$ and a power of 0.964. Analysis of simple main effects of baseline latency vs. postdrug latency revealed that ethanol produced significant analgesia in both groups [water pretreatment: baseline vs. postdrug, $F(1, 12) = 5837.13$, $p < 0.001$, ethanol pretreatment: baseline vs. postdrug, $F(1, 14) = 10.08$, $p < 0.01$, Fig. 1]. Examination of posttest scores, however, revealed that the analgesia was significantly less in animals pretreated with ethanol [postdrug latency: ethanol pretreatment vs. water pretreatment, $F(1, 26) = 12.92$, $p < 0.01$, Fig. 1], indicating that the rats developed tolerance to EIA. Differences were also found in body weight for animals pretreated with ethanol vs. water, $F(1, 26) = 13.62$, $p < 0.01$. However, it is unlikely that these differences affected pain responses, as no significant differences were found in baseline pain scores.

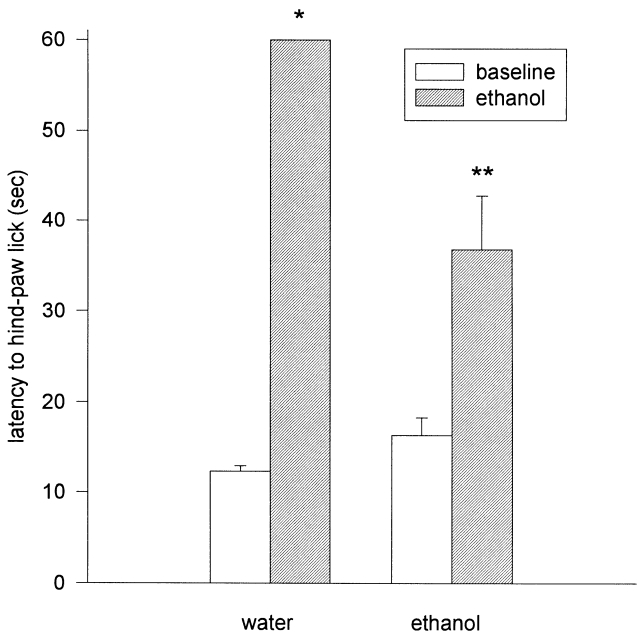

FIG. 1. Analgesic effect of ethanol (2.5 mg/kg, IP) in the hot-plate test after 15 days of drinking an ethanol solution ($n = 15$) or water ($n = 13$). Data are expressed as the mean latency to hind-paw lick (s) (±SEM) in the hot-plate test before (baseline) and after ethanol. *Indicates significant difference from baseline latency. ** Indicates significant difference from baseline latency and between postethanol latencies. See text for significance levels.

Morphine-induced analgesia. ANOVA revealed a significant main effect for baseline vs. postdrug latency, $F(1, 27) = 51.04$, $p < 0.001$, with an effect size of partial $\epsilon^2 = 0.654$ and a power of 0.999, but no significant interaction, indicating that morphine produced significant analgesia in both groups, and that tolerance to EIA was not accompanied by cross-tolerance to MIA (Fig. 2). No significant differences were found in body weight or baseline pain responses for animals pretreated with ethanol vs. water.

Environment-Dependent Tolerance

Ethanol-induced analgesia. ANOVA revealed a significant test period × pretreatment interaction, $F(1, 23) = 6.57$, $p < 0.02$, with an effect size of partial $\epsilon^2 = 0.222$ and a power of 0.687. Analysis of simple main effects of baseline latency vs. postdrug latency revealed that ethanol produced significant analgesia in both groups [saline pretreatment: baseline vs. postdrug, $F(1, 12) = 60.69$, $p < 0.001$, ethanol pretreatment: baseline vs. postdrug, $F(1, 11) = 8.97$, $p < 0.05$, Fig. 3]. Examination of postdrug scores revealed that the analgesia was significantly less in animals pretreated with ethanol, $F(1, 23) = 7.17$, $p < 0.05$, Fig. 3, indicating that the rats developed tolerance to EIA. No significant differences were found in body weight or baseline pain responses for animals pretreated with ethanol or saline.

Morphine-induced analgesia. ANOVA revealed a significant test period × pretreatment interaction, $F(1, 26) = 4.29$, $p < 0.05$, with an effect size of partial $\epsilon^2 = 0.141$ and a power of 0.512. Analysis of simple main effects for baseline latency vs. postdrug latency revealed that morphine produced significant analgesia in ethanol pretreated animals, $F(1, 14) = 12.23$, $p < 0.005$, but not in saline pretreated animals (see Fig. 4). The differences between postdrug latencies approached significance ($p = 0.065$). No significant differences were found in body weight or baseline pain responses for animals pretreated with ethanol or saline.

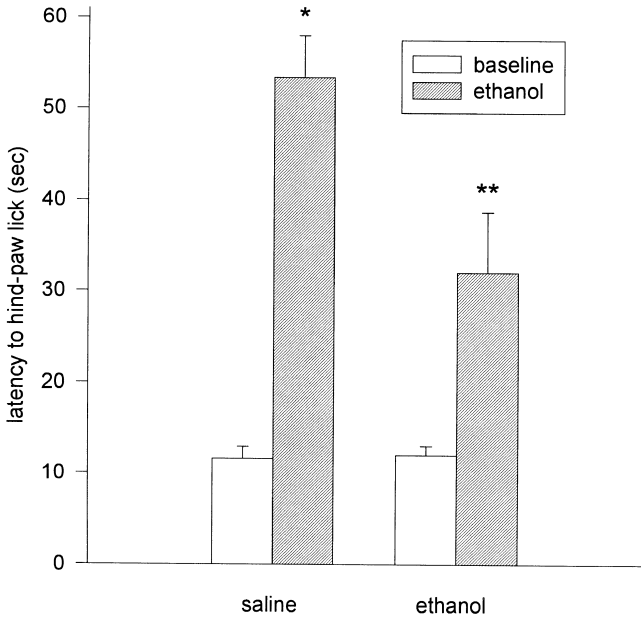

FIG. 3. Analgesic effect of ethanol (2.5 mg/kg, IP) in the hot-plate test after two daily injections of ethanol ($n = 12$) or saline ($n = 13$). Data are expressed as the mean latency to hind-paw lick (s) (± SEM) in the hot-plate test before (baseline) and after ethanol. **Indicates significant difference from baseline latency. *Indicates significant difference from baseline latency and between postethanol latencies. See text for significance levels.

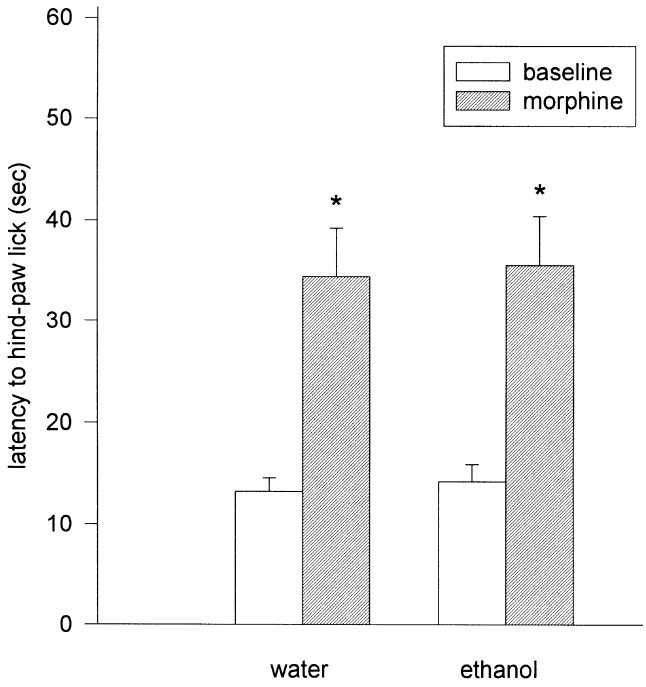

FIG. 2. Analgesic effect of morphine (10 mg/kg, IP) in the hot-plate test after fifteen days of drinking an ethanol solution ($n = 14$) or water ($n = 15$). Data are expressed as the mean latency to hind-paw lick (s) (±SEM) in the hot-plate test before (baseline) and after morphine. *Indicates significant difference from baseline latency. See text for significance levels.

DISCUSSION

The results of the present study show that tolerance to ethanol's analgesic effects can be induced in rats using both nonassociative and associative paradigms. Furthermore, the results of this study do not support an ethanol–opiate interaction for EIA (2,20,21), as neither form of ethanol tolerance was accompanied by cross-tolerance to MIA, and thus was nonopioid in nature. These findings are consistent with the findings of Fidecka et al. (5) that tolerance to EIA following intragastric administration of 40% ethanol in rats was not accompanied by cross-tolerance to MIA. Interestingly, unlike rats, chronically alcoholized mice are cross-tolerant to MIA (5), and naloxone has been shown to antagonize EIA in mice (2), but not in rats (9). Furthermore, studies have shown that rats pretreated with morphine are cross-tolerant to the subsequent analgesic effects of ethanol (16), suggesting that the existence of an ethanol–opiate interaction for EIA may also depend on the direction of treatment [that is, whether animals are pretreated with morphine and then tested for EIA, or whether animals are pretreated with ethanol and then tested for MIA; see (12)]. Asymmetrical or one-way cross-tolerance has also been reported to occur between ethanol and pentobarbital (12). However, such asymmetrical cross-tolerance between ethanol and opiates appears to be specific for their analgesic effects, as symmetrical cross-tolerance occurs for their hypothermic

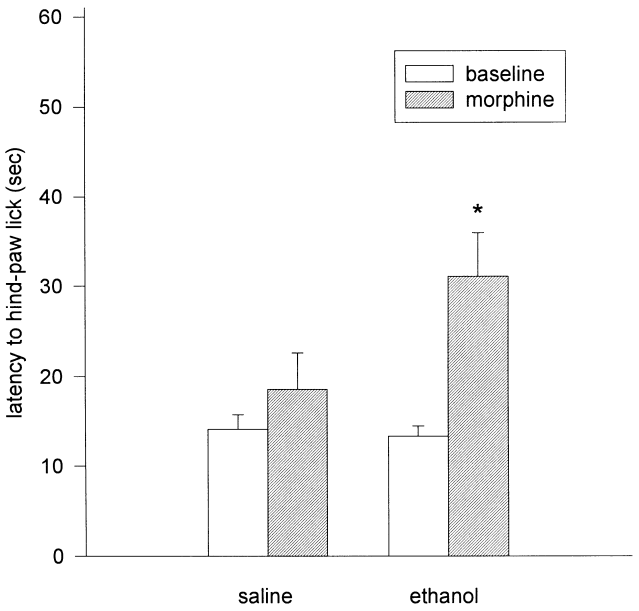

FIG. 4. Analgesic effect of morphine (10 mg/kg, IP) in the hot-plate test after two daily injections of ethanol (n = 15) or saline (n = 13). Data are expressed as the mean latency to hind-paw lick (s) (± SEM) in the hot-plate test before (baseline) and after morphine. *Indicates significant difference from baseline latency. See text for significance levels.

effects (11) and in their ability to inhibit guinea pig ileum muscle contractions (17). Therefore, the existence of an ethanol–opiate interaction may depend on a number of factors including species-related differences (2,5,9), the occurrence of asymmetrical crosstolerance, and the test used to measure opiate–ethanol interactions (12).

Jorgensen et al. (8) found that tolerance to EIA did not develop in rats injected IP with 2.5 g/kg ethanol for 8 days to a test dose of 2.5 g/kg IP. To produce tolerance to 2.5 g/kg daily injections of ethanol, they found that exposing the animals to the analgesic test (tail flick) was necessary during tolerance induction. This was taken to suggest that practice during tolerance induction was necessary for tolerance to EIA to develop (8). In the present study, however, we found that tolerance to EIA to a test-dose of 2.5 g/kg ethanol developed following only 2 days of 2.5 g/kg of ethanol administered IP in the absence of practice with the analgesic test. The reasons for these discrepancies may be related to various methodological differences between our studies, including the strain used (Sprague–Dawley vs. Long–Evans hooded) and/or the analgesic test employed to measure tolerance. In addition, our rats were injected with ethanol in the testing area during tolerance induction, whereas Jorgensen et al. (8) and Jorgensen and Hole (10) injected their animals in their home cages. Therefore, the differences may be related to the additional environmental cues provided to our rats during analgesic testing. Indeed, studies have shown that conditioned tolerance to ethanol's effects can be reduced or eliminated if animals are tested in an environment that is different from that which was used to induced tolerance (10,13,24).

The findings that animals injected with ethanol for 2 days were not cross-tolerant with MIA suggests that the analgesia produced by this volume of solution and concentration (30%) of ethanol is nonopioid in nature. It is known that environmental stressors can produce potent analgesia, which is classified as either "opioid" or "nonopioid," as defined by naloxone-reversibility and cross-tolerance with morphine (15,26). The differential activation of opioid and nonopioid forms of stress-induced analgesia (SIA) has been shown to depend upon the parameters of the stressor employed (15,26). Therefore, as with SIA, the involvements of opioid or nonopioid mechanisms in EIA are also likely to be influenced by the ethanol concentration and/or volume of solution. This raises the possibility that previous studies that reported EIA following ethanol may in fact have been measuring a type of SIA. Indeed, similar to stress, ethanol is known to produce gastric ulcers (19) and activate the hypothalamic–pituitary–adrenal axis (6). Consistent with this hypothesis, we have recently found that ethanol-induced analgesia produced by IP injections is accompanied by cross-tolerance with nonopioid forms of SIA, but not with opioid forms of SIA (unpublished observations). This conclusion is further supported by the finding of Mogil et al. (20) that mice bred for high SIA displayed higher EIA compared with mice bred for low SIA, suggesting that EIA (produced by IP injections) may be related to SIA. It is interesting that in the present study pretreatment with IP saline alone for 2 days resulted in a lack of MIA on day 4 (see Fig. 4). It is possible that IP injections of saline at the relatively high volume used (around 3 ml) were sufficiently stressful to produce SIA, which was accompanied by cross-tolerance with MIA and thus opioid in nature. In fact, Jorgensen et al. (8) found that their saline-injected controls (injected with a volume of 21 ml/kg, IP) showed significantly lower tail-flick latencies following 8 days of injections, compared with day 1, suggesting that tolerance may have developed to the analgesia produced by these injections. The above results suggest, therefore, that the IP injections of saline or ethanol may differentially activate opioid or nonopioid systems.

In conclusion, the present study shows that tolerance to EIA can be produced using both environment-independent and environment-dependent paradigms. Furthermore, neither form of tolerance to EIA was accompanied by cross-tolerance to MIA, suggesting that EIA is mediated by nonopiate mechanisms.

REFERENCES

1. Bass, M. B.; Friedman, H. J.; Lester, D.: Antagonism of naloxone hyperalgesia by ethanol. Life Sci. 22:1939–1946; 1978.
2. Boada, J.; Feria, M.; Sanz, E.: Inhibitory effect of naloxone on the ethanol-induced antinociception in mice. Pharmacol. Res. Commun. 13:673–678; 1981.
3. Brick, J.; Sun, J. Y.; Davis, L.; Pohorecky, L. A.: Ethanol and the response to electric shock in rats. Life Sci. 18:1293–1298; 1976.
4. Cutter, H. S. G.; O'Farrell, T. J.: Experience with alcohol and the endogenous opioid system in ethanol analgesia. Addict. Behav. 12:331–343; 1987.
5. Fidecka, S. E.; Tamborska, E.; Malec, D.; Langwinski, R.: The development of cross-tolerance between ethanol and morphine. Pol. J. Pharmacol. Pharm. 38:277–284; 1986.
6. Gianoulakis, C.: The effect of ethanol on the biosynthesis and regulation of opioid peptides. Experientia 45:428–435; 1989.
7. Jorgensen, H. A.; Berge, O.-G.; Hole, K.: Learned tolerance to

ethanol in a spinal reflex separated from supraspinal control. Pharmacol. Biochem. Behav. 22:293–295; 1985.
8. Jorgensen, H. A.; Fasmer, O. B.; Hole, K.: Learned and pharmacologically induced tolerance to ethanol and cross-tolerance to morphine and clonidine. Pharmacol. Biochem. Behav. 24:1083–1088; 1986.
9. Jorgensen, H. A.; Hole, K.: Does ethanol stimulate brain opiate receptors? Studies on receptor binding and naloxone inhibition of ethanol-induced effects. Eur. J. Pharmacol. 75:223–229; 1981.
10. Jorgensen, H. A.; Hole, K.: Learned tolerance to ethanol in the spinal cord. Pharmacol. Biochem. Behav. 20:789–792; 1984.
11. Khanna, J. M.; Lê, A. D.; LeBlanc, A. E.: Cross-tolerance between ethanol and morphine with respect to their hypothermic effects. Eur. J. Pharmacol. 59:145–149; 1979.
12. Khanna, J. M.; Lê, A. D.: Cross-tolerance: Theoretical and experimental issues. In: Begleiter, H.; Kissin, B., eds. The pharmacology of alcohol and alcohol dependence. New York: Oxford University Press; 1996:110–142.
13. Lê, A. D.; Poulos, C. X.; Cappell, H.: Conditioned tolerance to the hypothermic effect of ethyl alcohol. Science 206:1109–1110; 1979.
14. Lê, A. D.; Mihic, S. J.; Wu, P. H.: Alcohol tolerance: Methodological and experimental issues. In: Boulton, A.; Baker, G.; Wu, P. H., eds. Neuromethods, vol. 24, Animal models of drug addiction. Totowa, NJ: Humana Press; 1992:95–124.
15. Lewis, J. W.; Cannon, J. T.; Liebeskind, J. C.: Opioid and nonopioid mechanisms of stress analgesia. Science 208:623–625; 1980.
16. Malec, D.; Kotlinska, J.; Langwinski, R.: Cross-tolerance between morphine and ethanol and their nociceptive effects. J. Stud. Alcohol. 48:507–510; 1987.
17. Mayer, J. M.; Khanna, J. M.; Kalant, H; Spero, L.: Cross-tolerance between ethanol and morphine in the isolated guinea-pig ileum myenteric plexus preparation. Eur. J. Pharmacol. 63:223–227; 1980.
18. Mayer, J. M.; Khanna, J. M.; Kim, C.; Kalant, H.: Differential pharmacological response to ethanol, pentobarbital and morphine in rats bred for ethanol sensitivity. Psychopharmacology (Berlin) 81:6–9; 1983.
19. Mezey, E.: Effects of alcohol on the gastrointestinal tract. In: Pattison, E. M.; Kaufman, E., eds. Encyclopedic handbook of alcoholism. New York: Gardner; 1982:245–254.
20. Mogil, J. S.; Marek, P.; Yirmiya, R.; Balian, H.; Sadowski, B.; Taylor, A. N.; Liebeskind, J. C.: Antagonism of the nonopioid component of ethanol-induced analgesia by the NMDA receptor antagonist MK-801. Brain Res. 602:126–130; 1993.
21. Pohorecky, L. A.; Shah, P.: Ethanol-induced analgesia. Life Sci. 41:1289–1295; 1987.
22. Poulos, C. X.; Hunt, T.; Cappell, H.: Tolerance to morphine analgesia is reduced by the novel addition or omission of an alcohol cue. Psychopharmacology (Berlin) 94:412–416; 1988.
23. Siegel, S.: Classical conditioning, drug tolerance, and drug dependence. In: Israel, Y.; Glaser, F. B.; Kalant, H.; Popham, R. E.; Schmidt, W.; Smart, R. G., eds. Research advances in alcohol and drug problems, vol. 7. New York: Plenum; 1983:207–246.
24. Siegel, S.; Sdao-Jarvie, K.: Attenuation of ethanol tolerance by a novel stimulus. Psychopharmacology (Berlin) 88:258–261; 1986.
25. Tabakoff, B.; Cornell, N.; Hoffman, P. L.: Alcohol tolerance. Ann. Emerg. Med. 15:1005–1012; 1986.
26. Terman, G. W.; Yehuda, S.; Lewis, J. W.; Cannon, J. T.; Liebeskind, J. C.: Intrinsic mechanisms of pain inhibition: Activation by stress. Science 226:1270–1277; 1984.
27. Wolff, H. G.; Hardy, J. D.; Goodell, H.: Studies on pain: Measurement of the effect of ethyl alcohol on the pain threshold and on the "alarm" reaction. J. Pharmacol. Exp. Ther. 75:38–49; 1942.
28. Woodrow, K. M.; Eltherington, L. G.: Feeling no pain: Alcohol as an analgesic. Pain 32:159–163; 1988.
29. Yirmiya, R.; Taylor, A. N.: Genetic differences in opiate receptor concentration and sensitivity to ethanol's effects. Pharmacol. Biochem. Behav. 33:793–796; 1989.