INTRODUCTION

Widespread use of alcohol among addicts has been reported by many investigators. Jackson and Richman (1) noted that many narcotic addicts would use alcohol when drugs such as heroin, morphine or cocaine were not available. In New York City during the period from 1950 to 1961 (2) one-tenth of the deaths among narcotic addicts were attributed to the combined use of narcotics and alcohol. Baden (3) reported that more than one-fifth of the heroin addicts who died in New York City had evidence of alcohol abuse. Addicts participating in methadone maintenance programs reported concomitant heavy use of alcohol (4,5). Heroin addicts maintained on prolonged methadone treatment and secondarily addicted to alcohol, develop a ten-fold increase in mortality over those who were on methadone maintenance alone (6). Similar increases in lethality were reported in animals treated with both narcotics and alcohol. In mice pretreated with alcohol, morphine administration markedly increased lethality (7). The depressant effects of alcohol are markedly potentiated by morphine (8). Sinclair et al. (9) observed that the selection of alcohol in Sprague-Dawley rats was suppressed after a single injection of morphine (60 mg/kg). In our laboratory, we have used both rats and mice to study the interaction between narcotics and alcohol.

To further clarify opiate-ethanol interactions, and in particular, to test whether previous exposure to either opiate agonists or antagonist (acute, chronic and withdrawal following Chronic administration) affect alcohol selection, the following experiments were performed. Preliminary results of these studies have been reported elsewhere (10,11).

MATERIALS AND METHODS

ANIMALS

The following strains of rats and mice were used: (a) adult male Sprague-Dawley rats weighing 180-200 g; (b) adult male Long-Evans Hooded rats weighing 180-220 g; (c) adult male C57BL/J6 mice weighing 20-25 g; and (d) neonatal Sprague-Dawley rats of both sexes.

DRUGS AND CHEMICALS

The drugs and chemicals used were: morphine sulfate (Merck Chemical Co.), methadone...
hydrochloride and 1-a-acetylmethadol (LAAM) (Eli Lilly Co.), levorphanol hydrochloride and dextrorphan hydrochloride (Hoffman La Roche Co.), naloxone hydrochloride and naltrexone hydrochloride (Endo Laboratories).

**PROCEDURES**

**Effects of Narcotics and Narcotic Antagonists on Volitional Consumption of Ethanol**

Animals were housed individually in standard, stainless steel wire mesh cages in a laboratory with an ambient temperature of 21.0 ± 0.5 degrees C and a four hour light and ten hour dark cycle. After at least four days of acclimatization to these conditions, the animals were used for experimentation. Except when otherwise specified, animals had free access to food (Purina Lab Chow), water and/or ethanol (prepared fresh daily from 95% ethanol in distilled water). All fluids were placed in graduated Richter type drinking tubes (100 ml, in the case of rats, or 25 ml, in the case of mice) fitted on the outside of each cage. Daily consumption of each of these fluids was recorded between 11 a.m. and 2 p.m. The tubes were randomly rotated each day to prevent the development of position habit. In most experiments, food consumption and body weight were also monitored daily. Under a free-choice situation, stable baseline consumption of water, ethanol (5% in rats and 10% in C57BL mice) and morphine solution was established for at least four days (usually the last four of seven days) prior to drug treatment. In some experiments, the preference-aversion cut-off concentration of ethanol was determined in an individual rat. The concentration of ethanol was increased by 1% daily until the animal consumed no measurable amount of ethanol on two consecutive days.

**Acute Experiments**

The effects of a single injection of various narcotics and narcotic antagonists in doses ranging from 2 to 60 mg/kg on subsequent voluntary consumption of ethanol were studied in both rats and mice.

**Chronic Experiments**

Chronic administration of morphine or methadone by injections, or by pellet implantation in the case of morphine, were performed as described previously (11). In one experiment, morphine was dissolved in drinking water at concentrations of 150 mg/l for thirty-one days, followed by 2 g/l for forty days. The differential selection of water, alcohol or morphine solutions was measured at various periods.

Abstinence signs in morphine dependent animals were observed by injecting the narcotic antagonists, naloxone or naltrexone (0.1 or 0.4 mg/kg s.c.), to precipitate narcotic withdrawal symptoms. The abstinence syndrome included body weight loss, spontaneous withdrawal jumping, diarrhea, teeth chattering, ptosis, wet dog shakes, and irritability to handling and touch.

Physical dependence on alcohol was produced by a chronic forced-drinking schedule described previously (11). The abstinence signs due to ethanol withdrawal were observed in rats using a scoring system as follows:
Loss of 20% or more of the control body weight
1
Mild tremor, hyperreflexia, compulsive drinking
2
Continuous tremor, provoked convulsions (by ringing keys around the animals for one minute)
3
Jumping (provoked by ringing of keys), clonic-tonic convulsions (at least two or more/hour)
4
Severe clonic-tonic convulsions (at least four or more/hour)
5
Coma and/or death
6

Rats were chronically exposed to morphine and methadone and after dependence was established by utilization of naloxone to precipitate jumping, the animals were placed on a forced-drinking alcohol regimen. At various times during the forced drinking schedule, these rats were assessed for withdrawal symptomatology according to the scoring system outlined above. In addition, certain rats were removed from the forced drinking paradigm and allowed to select either alcohol or water according to a three-bottle two-choice method (9).

Thus, withdrawal symptomatology and volitional consumption of ethanol was monitored in animals pretreated with morphine and methadone, and others only saline pretreated.

RESULTS

Acute Experiments

Narcotics and Narcotic Antagonists

Results obtained showed that, in both rats and mice, the acute single injection of active opiates significantly suppressed the voluntary consumption of ethanol in a dose-related manner. The duration of the suppression is only transient, however, as complete recovery to the pretreatment level of ethanol consumption is seen by the next day.

Figure 1 shows the results obtained with various doses of morphine in the C57BL/J6 mice. Water consumption in these mice showed large individual variations. Furthermore, there was no significant alteration in food and water intake or in body weight.

Figure 2 shows the results obtained with methadone in C57BL/J6 mice. Significant depression of ethanol intake was observed with a 30 mg/kg s.c. dose of methadone in the mice. In additional experiments, mice treated with LAAM (2 mg/kg and higher) also experienced a reduction in their alcohol intake. There was significant differences between the acute effects of
levorphanol and its inactive stereoisomer, dextrorphan. Significant reduction in ethanol and food intake were observed with 30 mg/kg and 60 mg/kg doses of levorphanol, whereas treatment with dextrorphan at similar doses failed to cause significant change in ethanol, food or water intake, or in body weight (Figure 3).

In Long-Evans rats, similar results were obtained illustrating that alcohol consumption was significantly reduced by subcutaneous doses of 10 mg/kg and 30 mg/kg morphine (p < 0.01). With methadone (30 mg/kg s.c.) and LAAM (2 mg/kg and higher) significant depression of ethanol intake was observed. However, rats treated with either naloxone or naltrexone showed a small but not significant increase in ethanol intake (Figure 4). No reduction in food and water intake was demonstrated. To test the selectivity of ethanol, the rats were treated with morphine at various doses and allowed to select between water and sucrose (3%). No significant reduction in the intake of sucrose solution was observed.

Neonatal rats were administered 5 mg/kg morphine starting at day three post-partum and increased by 2 mg/kg/day until a dose of 40 mg/kg/day was attained. This plateau was
maintained until morphine administration was terminated on day forty-seven post-partum. After morphine was terminated the rats showed a higher ethanol intake compared to saline controls. Food and water intake were both suppressed in the treated group (Figure 5).

Figure 6 illustrates the effects of morphine withdrawal on the selection of ethanol at different concentrations (5 - 21%). Our results indicate that morphine pretreated animals, as a group, showed a significantly higher preference-aversion cut-off concentration compared to non-treated controls. Between 5 and 7% of morphine pretreated rats selected ethanol compared to less than 75% of the non-treated controls. When the concentration of ethanol was increased to 13% approximately 25% of the morphine pretreated animals continued to select ethanol whereas none of the animals in the control group selected ethanol. Thus, the maximum preference-aversion cutoff for the morphine pretreated group was at 21% compared to 13% for the non-treated controls.

As illustrated in Figure 7, after fifty-one days, both the morphine pretreated and non-treated rats (as previously described in figure 6) were then exposed to ethanol on a forced-drinking schedule for thirty-two days with a graded increment in ethanol concentrations from 10 to 40%. In comparison with the controls, the morphine pretreated rats consumed significantly less ethanol during this period, however, both groups of rats showed similar food intake. Although we have no explanation for these findings, nevertheless, upon withdrawal from ethanol, the morphine pretreated rats showed a greater withdrawal severity, assessed by provoked withdrawal jumping, hyperreflexia, tremor and convulsions, compared to controls. In this regard, after the thirty-two day forced drinking of alcohol, over 50% of the morphine pretreated rats showed jumping, whereas none of the controls showed provoked jumping.
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Figure 8 illustrates that after two months of forced ethanol consumption morphine pre-treatment still exacerbated withdrawal scores. Note, however, that some 10% of the control rats showed provoked jumping, a withdrawal symptom that was not evident in the one-month control animals. In another experiment, two groups of adult rats were similarly treated with morphine (as previously described for the neonatal rats) for six weeks. One group of the animals were allowed free choice between water and quinine solution (4 x 10^{-4} \text{%}) and the other group was given free choice between sucrose (3\%) and a mixture of sucrose (3\%) and ethanol. No significant difference was observed between the morphine treated and the controls in the selection between water and quinine solution. Both groups of rats showed preference for water. On the other hand, the morphine treated rats showed a marked preference for alcohol-sucrose mixture whereas the controls showed a marked preference for sucrose solution (Figure 9).

Chronic Experiments

Effect of Morphine Withdrawal on Ethanol Selection

In this study, adult Sprague-Dawley rats were made dependent by chronic injection of morphine according to the schedule outlined previously for the neonatal rats. To test dependence, some of the rats were injected with naloxone to precipitate characteristic withdrawal symptoms. Rats treated in the above manner were shown to be dependent by day fourteen as evidenced by naloxone-induced jumping, weight loss, diarrhea and wet dog shakes. Our results indicate that there was a decrease in the daily intake of food and water during withdrawal. Ethanol consumption was not depressed, but showed an increase during the four days after morphine withdrawal.

Effect of Ethanol Forced Drinking on Ethanol and Morphine Selection

Rats were exposed to a forced-ethanol drinking paradigm over an eighty-six day period. During this exposure, at certain specified intervals, the animals were removed from the forced drinking condition and allowed to select either water, morphine and ethanol freely. Our results demonstrate that the animals showed a significant increase in the selection of both morphine solution and alcohol (5\%) at the 65th and 82nd days.

DISCUSSION

The results presented in this study tend to confirm our earlier reports that narcotics interact with ethanol after the acute and chronic administration and during the withdrawal state. The fact that narcotics suppressed the voluntary consumption of ethanol after the acute administration and not after chronic treatment, suggests the development of tolerance in the
rats to this effect. Thus, a single injection of morphine, methadone, LAAM, or levorphanol, but not dextrorphan or naloxone, significantly suppressed the voluntary consumption of ethanol in rats, mice or hamsters (9-11). There appears to be some specificity in the opiate-treated animals for ethanol. Dextrorphan has no effect in the volitional consumption of ethanol or blood ethanol level.

It is of interest to note, that rats were able to discriminate ethanol from ethanol-sucrose mixture, and a quinine or sucrose solution from water. Animals furthermore were given free access to food, water and ethanol and there was no schedule-induced influence on the daily consumption of ethanol. The interactions seen after chronic opiates and chronic ethanol are rather striking. The rats treated chronically, as neonates, with morphine or methadone, followed by forced drinking of ethanol, showed lower ethanol intake than untreated controls. These same animals, however, showed more severe and more rapidly developing withdrawal signs after ethanol withdrawal. This may be due to an additive effect on dependence liability, in that the rats previously treated with opiates subsequently require less ethanol to reach a state of ethanol dependence. There appeared in the literature several studies in support of opiate-ethanol interaction(s). Blum et al. (12) reported that naloxone significantly inhibited ethanol induced dependence in mice. This same group in another study (13) also found that morphine administration, significantly suppressed ethanol induced withdrawal symptoms in mice utilizing the Goldstein-Pal (14) inhalation technique. Unlike morphine, dextrorphan was not active in ameliorating these withdrawal reactions in mice (15). Ross et al. (16) showed that in hamsters, both morphine and levorphanol, but not dextrorphan, suppressed alcohol consumption, and this is in agreement with our findings in mice and rats. Along similar lines Blum and co-workers have further advanced the possibility of a common mechanism between ethanol and opiate dependence (17) and suggested that the common link between these two addictive substances may be the dopamine-derived isoquinoline, salsolinol. Furthermore, the thesis proposed by Cohen and Collins (18) concerning the possible involvement of salsolinol, in both ethanol effects and post-ethanol intoxication states has received support from other investigators (19,20).

The lack of tolerance to morphine in mice and humans previously made dependent on ethanol, as evidenced by the increase in lethality under the influence of both opiates and ethanol (3,7) may be partly explained by neurochemical alterations induced by these two commonly abused psychoactive chemicals. Our findings concerning the enhanced severity of withdrawal in animals after prolonged exposure to both morphine and ethanol may be either due to common neurochemical and behavioral mechanisms as suggested in the review by Blum et al. in this volume or to effects on metabolism. With regard to the latter, we are currently looking at penetration of the opiates into the CNS. The fact that, in the rats treated with higher doses of morphine or methadone, the retention of alcohol in the blood is markedly prolonged (11), may be related to an overall increase in the depression of both the circulatory and respiratory systems. There appears to be some circumstantial evidence, from autopsy findings, indicating the presence of high levels of either alcohol or the opiates, or both, in drug related deaths (3). This warrants a more systematic investigation in animals in order to gain further insight into the potential hazards of interactions between opiates and alcohol.

REFERENCES