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Morphine Withdrawal in Mice Selectively Bred for Differential Sensitivity to Ethanol

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HOROWITZ, G. P. AND A. M. ALLAN. *Morphine withdrawal in mice selectively bred for differential sensitivity to ethanol.* PHARMAC. BIOCHEM. BEHAV. 16(1) 35–39, 1982.—Mice which have been selectively bred for differences in sensitivity to acute doses of alcohol have also been shown to differ in severity of seizures upon withdrawal from chronic alcohol administration. We investigated the responsiveness of these mice to withdrawal from chronic morphine treatment. Mice were made dependent on morphine via pellet implantation, and withdrawal was precipitated with naloxone challenge. Mice which are less sensitive to the hypnotic effects of ethanol (short sleep: SS) displayed more jumping and wet dog shakes during withdrawal than did the more sensitive long sleep (LS) mice. In addition, the amount of jumping was dependent on the dose of naloxone in both lines. Differences between lines in naloxone precipitated withdrawal may reflect differences in alterations in extrapyramidal dopaminergic activity, but other substrates for the observed differences cannot be discounted. Finally, the observed difference between SS and LS mice in severity of morphine withdrawal parallels the previously reported difference between these lines in seizure severity during withdrawal from alcohol.

Opiate dependence Opiate withdrawal Alcohol-opiate commonalities Pharmacogenetics Selective breeding

THE tools of genetic breeding and analysis can be fruitfully applied to elucidate mechanisms underlying individual differences in behavioral and pharmacological responses to psychoactive agents [4]. For example, genetic factors have been shown to be an important source of individual differences in responses to both alcohol and opiates (see [7] and [14] for respective reviews). To a lesser extent, these genetic techniques have been used to investigate possible commonalities between these two classes of drugs. Some of these studies using various types of genetically defined rats and mice have shown a positive relationship between alcohol and morphine related phenotypes (e.g., [8, 18, 21]), while other studies have demonstrated the independence of certain measures of alcohol and morphine responsiveness (e.g., [11,26]). These disparate findings are probably to be expected, given the diversity of subject populations and the difficulty in translating conceptual similarities into objectively measurable phenotypes. Nonetheless, the demonstration of concomitant responses to alcohol and opiates in genetically defined subjects could prove to be an important initial step in elucidating possible genetic substrates for particular responses common to these drugs of abuse. In the present study, we report that mice which have been selectively bred for differences in response to acute administration of ethanol and which have been previously shown to differ in severity of alcohol withdrawal [10], also differ in a predictable direction in severity of opiate withdrawal.

Several nongenetic studies have demonstrated alcohol-opiate commonalities in both mice and rats. For example, acute morphine treatment has been shown to decrease voluntary alcohol ingestion in both species [12,22] and to decrease the severity of withdrawal from chronic alcohol administration in mice [1]. In addition, opiate antagonists such as naloxone have been reported to block or attenuate a number of acute responses to ethanol (e.g., [13, 17, 20]. A general review of alcohol-opiate interactions has been presented elsewhere [2].

If genetic factors influence responses to alcohol and to opiates, and if there are commonalities between these two classes of drugs, then it might be predicted that genetically defined mice that differ in some response to alcohol might also differ in an analogous response to opiates. McClearn and Kakihana [18] have successfully bred mice for differential effects of alcohol. Specifically, these two lines of mice, designated long sleep (LS) and short sleep (SS), were bred to differ in the duration of the loss of righting reflex following a hypnotic dose of ethanol. Subsequent research has demonstrated that LS and SS mice also differ on a number of other alcohol related phenotypes (see [5]).

With respect to the present hypothesis, it is of interest that SS and LS mice have been shown to differ in the severity of seizures induced by abrupt withdrawal from chronic ethanol exposure [10]. Specifically, SS mice displayed more severe seizures than did LS mice when withdrawn from three days of continuous exposure to ethanol vapor. As noted by Goldstein [9] the observed difference may be spe-

cific to this particular measure rather than representing a general difference in the degree of dependence on ethanol. Furthermore, the differential sensitivity to seizures induced by alcohol may not be genetically related to the selection criterion of alcohol induced "sleep time," since the two traits assorted independently in mice of heterogeneous genetic stock [10]. However, given the observed phenotypic difference between lines in the degree of seizure severity during withdrawal from chronic alcohol exposure, the present study was conducted to see if LS and SS mice also differed in behavioral sequelae of withdrawal from chronic morphine administration.

In mice, the behavioral responses exhibited during withdrawal from alcohol may be quite different from those observed during morphine withdrawal. As noted above, alcohol withdrawal is, in part, characterized by seizures. In mice dependent on morphine, jumping is a characteristic response to withdrawal. Jumping can be precipitated in dependent mice by either naloxone challenge or by abrupt termination of morphine administration, is easily quantifiable and has been shown to be correlated with duration of prior morphine treatment [24]. Furthermore, genetic differences among inbred strains of mice in the amount of jumping following morphine withdrawal have previously been reported [3]. Therefore, naloxone precipitated jumping was selected as one primary index of withdrawal severity in the present study. In addition, wet dog shakes, a behavior associated with opiate withdrawal in mice and rats (e.g., [25]) was also measured.

METHOD

Subjects

The subjects were descendents of the mice selectively bred by McClearn and Kakihana [18] for differential sensitivity to hypnotic doses of ethanol. Mating pairs were obtained from the Institute for Behavioral Genetics at generation 18 and have been maintained at the present laboratory with relaxed selection within each line. It should be noted that the colony of LS and SS mice at the University of Colorado were also maintained under relaxed selection within lines between generations 19 and 24 [17]. A minimum of 15 mating pairs have been maintained for each line, with the restriction that, when possible, the progeny of each pair did not share common grandparents.

In the present study, 32 SS and 32 LS male mice, naive to any previous drug exposure, served as subjects. The mice were representatives of a total of 23 different litters. Subjects were housed as like sexed littermates from weaning at postnatal day 21 until their first use in the experiment. Throughout the experiment, subjects were maintained in a temperature controlled (21±1°C) vivarium on a 12:12 hr light dark cycle (light onset at 0800 hr), and were given continuous access to Purina mouse chow and tap water. At the time of their initial use in the experiment, all mice were 90–120 days of age.

Drugs and Apparatus

Morphine pellets for chronic treatment were modified from the preparation outlined by Way *et al.* [24]. A previous study [3] has reported mortality ranging from 23% to 84% following morphine pellet implantation in inbred mouse strains represented in the initial founding population of the LS and SS mice. Therefore, the amount of morphine in pellets used in the present experiment was 37.5 mg, compared to 75 mg in the pellets used in previous studies (e.g., [3,24]). The amounts of other ingredients in our pellets were similarly reduced to half those employed by Way *et al.* [24]. Placebo pellets were prepared in a similar fashion, except that morphine was replaced by lactose (37.5 mg). Naloxone hydrochloride was dissolved in 0.9% (w/v) saline and administered in a volume of 0.01 cc/g body weight. The doses of naloxone, expressed as the salt, were 0 (saline vehicle), 3 or 10 mg/kg. Naloxone hydrochloride was generous gift from Endo Laboratories (Garden City, NY).

The home/holding cage was made of clear plastic and measured approximately 16×30×13 cm deep. The floor of the cage was covered with a shallow layer of pine chips as bedding. During the observation period, the cage was covered by a stainless steel grated top.

The testing apparatus was a cylinder constructed of clear acrylic plastic 30.2 cm in height and 10 cm in diameter fitted with a clear acrylic top and bottom. Holes were drilled in the top to allow air flow. The top and bottom were easily removed for cleaning.

Procedure

On the morning of its first use in the experiment, each animal was weighed and individually caged. Approximately 1–2 hrs later, the mice were transported to a separate room for pellet implantation. Each mouse was lightly anesthetized with ether, a morphine (n=24/line) or placebo (n=8/line) pellet was implanted subcutaenously just behind its neck and the incision was closed with wound clips. Following surgery, all subjects were returned to their home cages and transported back to the colony vivarium.

Evaluation of morphine dependence took place 72 hours after pellet implantation. All subjects were again weighed just prior to testing. The morphine implanted animals were randomly assigned to receive either 0, 3, or 10 mg/kg naloxone, while placebo implanted subjects received only the highest dose of naloxone (10 mg/kg). Thus, each treatment group consisted of 8 mice of each selected line.

The subjects were injected IP and placed back in their home/holding cage for 3 min. Immediately following the 3 min waiting period, the subjects were placed in the testing apparatus and observed for a total of 5 min, during which jumping, wet dog shakes and several other behaviors were monitored continuously and recorded by the experimenters. These responses included the number of times the mouse jumped, latency to the first jump, the number of bouts of wet dog shakes and forelimb tremors, the number of rearings (both supported and unsupported rears) and the number of fecal boli. At the end of this 5 min session, each subject was returned to its home cage and the apparatus was rinsed with tap water and dried with a paper towel.

The experiment was conducted in eight successive replications. Thus, each treatment group and line was represented by one animal in each replication. Within line, subjects were randomly assigned to treatment groups, with the restriction that each litter contributed only one mouse to any morphine treatment group.

RESULTS

Naloxone-Precipitated Jumping

SS mice pretreated with morphine showed significantly more jumping than LS mice following challenge with either 3 or 10 mg/kg naloxone, as presented in Fig. 1. No mouse of

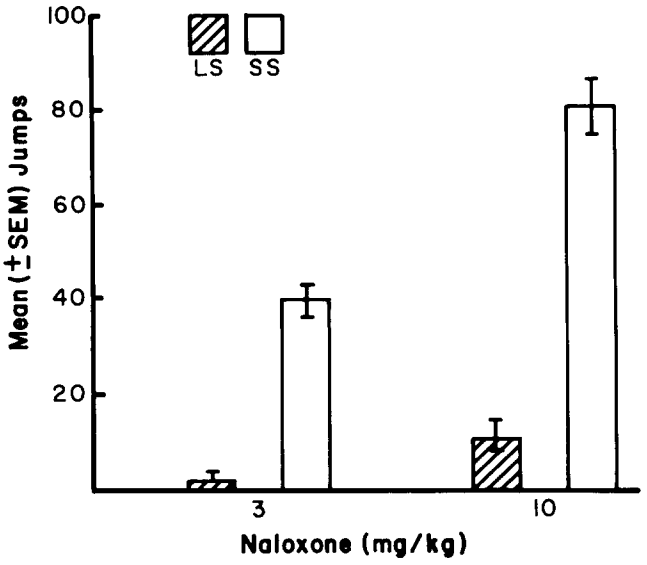

FIG. 1. Withdrawal jumping in LS and SS mice pretreated with morphine pellets and injected with naloxone. Each bar represents the mean and standard error for 8 mice.

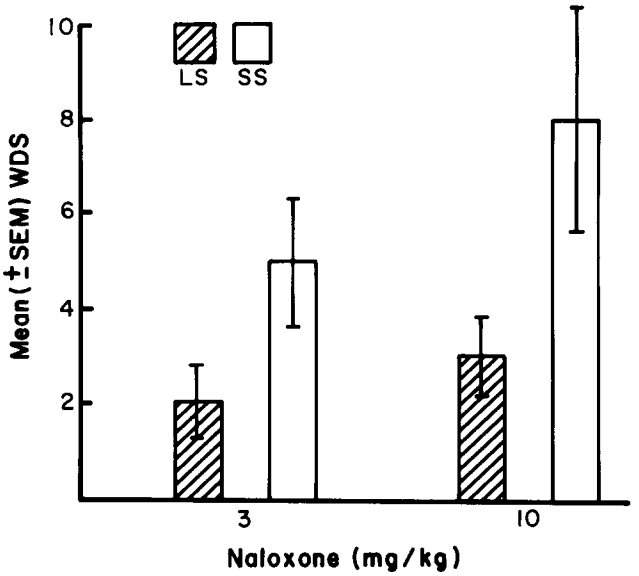

FIG. 2. The number of bouts of wet dog shakes (WDS) during naloxone precipitated withdrawal in morphine dependent LS and SS mice. Each bar represents the mean and standard error for 5 mice.

either line receiving a placebo pellet followed by 10 mg/kg naloxone jumped during the course of the experiment. Similarly, mice receiving morphine pellets followed by 0 mg/kg naloxone did not jump, regardless of genotype. Therefore, animals in these treatment groups are not represented in Fig. 1, and are excluded in subsequent analyses.

For mice receiving morphine pellets followed by naloxone (3 or 10 mg/kg) challenge, jumping scores were subjected to a 2(Line)×2(Dose) analysis of variance (ANOVA). This analysis revealed a significant main effect of both line and dose, as well as a line×dose interaction. As is evident in Fig. 1, SS mice showed more jumping than LS mice, regardless of dose of naloxone, $F(1,28)=229.75$, $p<0.001$. Similarly, the higher dose of naloxone precipitated more jumping than did the lower dose, regardless of genotype, $F(1,28)=49.87$, $p<0.001$. Furthermore, the difference in jumping between lines was greater following 10 mg/kg naloxone than that following 3 mg/kg naloxone, as evidenced by a significant line×dose interaction, $F(1,28)=19.34$, $p<0.001$. However, it should be noted that LS mice jumped 5.5 times more frequently following the higher naloxone dose, relative to the amount of jumping exhibited by this line following 3 mg/kg naloxone. The same comparison with SS mice showed that jumping following 10 mg/kg naloxone was only greater than that following the lower naloxone dose by a factor of two.

Genotypic and dose differences were also apparent in two other measures related to jumping. All SS mice receiving morphine and either 3 or 10 mg/kg naloxone jumped at least once during the test, while 3 out of 8 and 6 of 8 LS morphine dependent mice jumped after receiving 3 and 10 mg/kg naloxone, respectively. Finally, the latency to first jump was shorter for SS than LS mice receiving 3 mg/kg (75±15 sec vs 196±37 sec) or 10 mg/kg (51±14 sec vs 138±50 sec) naloxone.

Wet Dog Shakes (WDS)

During the initial replications of the experiment, WDS were only recorded during the 5 min test in the observation chamber. However, it soon became apparent that the vertical orientation of this apparatus and/or the competing response of jumping might be reducing the overall level of WDS. Therefore, we decided to score this behavior in both the 3 min post injection period in the home cage and in the observation chamber. Thus, WDS scores for both periods are available for only 5 mice of each genotype in each treatment group. As was the case for jumping, no mouse of either genotype in any control group (i.e., placebo-naloxone or morphine-saline) exhibited this response during the course of the experiment and were excluded from subsequent illustration or analysis. The remaining groups are presented in Fig. 2.

WDS scores of the mice for whom both periods were recorded were subjected to 2(Line)×2(Dose) ANOVA. As can be seen in Fig. 2, there was a significant main effect of line, $F(1,16)=5.56$, $p<0.05$, with morphine dependent SS mice displaying more WDS than LS mice following either dose of naloxone. Neither a significant effect of dose, nor a line×dose interaction could be reliably detected, given the relatively small sample size.

Other Responses

The remaining behaviors recorded in the observation chamber provided a discrimination between animals in the control groups vs morphine-naloxone groups rather than between LS and SS mice. In general, control animals displayed more grooming and supported rearing, and less forepaw tremors, than did morphine dependent mice receiving either 3 or 10 mg/kg naloxone. Among morphine-naloxone animals, there were no consistent differences between LS and SS

mice for any of these behaviors. Finally, there was no difference between LS and SS mice in the loss of body weight following morphine pellet implantation, with both lines losing approximately 6% of initial body weight during morphine administration (but prior to withdrawal).

DISCUSSION

SS mice made dependent on morphine by pellet implantation and challenged with naloxone exhibited significantly greater signs of opiate dependence than did LS mice. Significant differences between lines were observed for both jumping and wet dog shakes, but not for other behaviors. The observed differences cannot be attributed to differences in baselines for these measures, since the behaviors were never observed in mice of either genotype receiving placebo pellets followed by the highest dose of naloxone.

The hypothesis that SS mice may be more sensitive to withdrawal from chronic exposure to morphine is consistent with recent findings in our laboratory that these mice are also more sensitive to acute morphine administration, at least as measured by the effect of the drug on body temperature [15]. Although line differences in sensitivity to acute and chronic morphine exposure are opposite in direction to differences observed in response to the hypnotic effects of acute alcohol administration [5,17], the direction of the differences observed in the present study is consistent with the previous report that SS mice showed greater seizure susceptibility during alcohol withdrawal [10]. Interestingly, there was no difference between lines in amount of weight loss during chronic alcohol (10) or morphine administration, with both lines losing about 6% of their original body weight in both studies.

The generality of the results reported herein should be tempered by two cautions. The LS and SS mice in our laboratory have been maintained under relaxed selection for several generations, and may represent a different subpopulation from those maintained at the University of Colorado or other laboratories. Therefore, it would be of interest to test LS and SS mice from Colorado for possible differences in severity of opiate withdrawal. It should be noted, however, that LS and SS mice from our colony continue to differ markedly in ethanol induced sleep time and hypothermia.

A second caution should be made against inferring from these data that morphine dependence in general is greater in SS mice than LS mice. A similar view has been expressed for the observed difference in seizure susceptibility during alcohol withdrawal [9]. That is, the evaluation of degree of dependence may be limited to the particular measure of withdrawal. In the present study, SS mice showed greater responsiveness on two standard measures of opiate dependence in rodents. In addition, the amount of jumping elicited by a given dose of naloxone is related to the duration of prior exposure to morphine (which presumably is related to the degree of dependence) [24]. Nonetheless, the differences observed between LS and SS mice in the present study may reflect differences in sensitivity to naloxone-induced jumping and wet dog shakes, rather than differences in the absolute degree of physical dependence.

It should be further noted that the differences reported herein between LS and SS mice in severity of morphine withdrawal induced jumping and wet dog shakes may not be related to the original differences in response to the selection criterion of duration of loss of righting reflex following hypnotic doses of alcohol. As previously noted [10], alcohol-induced sleep time and severity of seizures following withdrawal from chronic alcohol exposure do not appear to be genetically correlated characters. Thus, the results of the present study do not address the issue of the relationship between the degree of acute tolerance and physical dependence for either alcohol or the opiates.

The neurochemical and neuroanatomical substrates of differences between LS and SS mice in these two measures of opiate dependence remain to be determined. Previous studies have suggested that the extrapyramidal dopamine system may subserve jumping induced in morphine dependent mice by naloxone or abrupt withdrawal. Increases in whole brain [23] and striatal [16] dopamine levels have been found in morphine dependent mice treated with naloxone. The administration of apomorphine, a dopamine agonist, just prior to naloxone challenge, reduced the incidence of jumping in morphine dependent mice. Furthermore, among two different genotypes of mice, differences in withdrawal induced increases in brain dopamine levels correlated positively with the differences in the incidences of jumping [23]. Thus, it is suggested that withdrawal from morphine produces an increase in dopamine levels and a decrease in dopamine utilization, which may reflect a decrease in the release of dopamine synaptically [23]. This suggestion that a decreased release of dopamine is involved in naloxone induced jumping by morphine dependent mice predicts that postsynaptic dopamine receptor agonists should attenuate this response. Apomorphine, in doses that are known to stimulate postsynaptic dopamine receptors, has been shown to antagonize withdrawal induced jumping [23]. Pretreatment with haloperidol blocked the attenuating effect of apomorphine. SS mice have been reported to have greater whole brain dopamine levels than LS mice [6]. Therefore, differences in withdrawal induced jumping observed in the present study may be related to differences in dopamine neurochemistry between these lines.

Other mechanisms might also be postulated which involve opiate receptors, permeability of the blood-brain barrier to morphine and naloxone, and the time course of development or expression of opiate dependence. Differences among inbred strains of mice have previously been reported for brain morphine levels following either acute or chronic morphine administration [3]. However, brain morphine levels at the time of withdrawal did not correlate significantly with the amount of withdrawal induced jumping. Similarly, even when blood alcohol levels were equated in LS and SS mice, seizures induced by alcohol withdrawal were greater for SS mice [10]. Future research should prove useful in elucidating the mechanisms underlying differences in the severity of opiate withdrawal between these lines of mice. Finally, although LS and SS mice appear to differ in severity on measures of both alcohol and opiate withdrawal, these findings do not necessarily imply that alcohol and opiate withdrawal share a common neuroanatomical or genetic substrate. An appropriate test of a common genetic mechanism would be to evaluate whether or not these two traits covary among individual animals of heterogeneous genetic origin.

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