Pharmacology Biochemistry & Behavior, Vol 16, pp 473–479, 1982 Printed in the U S A

Alcohol and Morphine Induced Hypothermia in Mice Selected for Sensitivity to Ethanol[1]

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Received 8 July 1981

BRICK, J AND G P HOROWITZ *Alcohol and morphine induced hypothermia in mice selected for sensitivity to ethanol* PHARMAC BIOCHEM BEHAV 16(3) 473–479, 1982 —We have used changes in body temperature as an index of responsiveness to alcohol and morphine in mice selectively bred for differential sensitivity to ethanol In agreement with other laboratories, we found that mice which show longer duration of loss of righting reflex following hypnotic doses of ethanol (long sleep, LS) also showed greater loss in body temperature following subhypnotic doses of ethanol than did the less sensitive short sleep (SS) mice This effect was dose dependent in both lines In contrast, SS mice were more sensitive than LS mice to the hypothermic effects of morphine, although the difference was only evident 30 min after morphine administration Naloxone attenuated morphine induced hypothermia in mice of both genotypes, but attenuated alcohol induced hypothermia only in SS mice Thus, SS mice may be more sensitive to an opiate agonist and an antagonist, at least as indexed by changes in body temperature, and may prove to be a useful population for evaluating both alcohol-opiate interactions and genetic differences in opiate responsiveness

Alcohol-opiate interactions Pharmacogenetics Selective breeding Morphine-induced hypothermia Alcohol-induced hypothermia

CONSIDERABLE data now suggest that individual differences in behavioral and biochemical responses to opiates and to alcohol, as well as to many other drugs, are mediated in part by genetic differences among subject populations Recent reviews of the role of genetic factors influencing responses to psychoactive agents in general [7], and to opiates [22] and alcohol [15] in particular, have been compiled Briefly, genetic factors have been shown to contribute to differences among mice to a number of diverse responses to alcohol, including (but not limited to) preference for alcohol in a choice situation (e g , [31,41]), acceptance of alcohol using a forced ingestion regimen [28], the metabolism of ethanol (e g , [44]), and neural sensitivity to ethanol (e g , [13]) Similarly, genetic factors have been shown to contribute to differences among mice or rats to both acute and chronic administration of opiates, including preference for morphine adulterated drinking solutions (e g , [24]), acceptance of morphine solutions using a forced ingestion regimen [33], opiate induced locomotor hyperactivity and analgesia (e g , [9,25]) neurochemical responses to opiates (e g , [39] and the development of physical or behavioral dependence [6,22])

To a lesser extent, basic research into the etiology of opiate and alcohol dependence has yielded results that suggest commonalities between the effects of, and responses to, these drugs of abuse In part, these commonalities are evidenced in three independent lines of research the biochemistry of alcohol metabolism and opiate synthesis (e g , [14,36]), behavioral studies in which both classes of drugs are given concomitantly or sequentially (e g , [4,19, 20, 45]), and studies investigating the effects of opiate antagonists on acute or chronic responses to ethanol (e g , [21, 34, 42])

If individual differences in responses to opiates and to alcohol are contributed to by genetic differences in subject populations, and if opiate and alcohol share certain behavioral and biochemical characteristics, then opiate-alcohol commonalities might also be influenced by genetic factors If such commonalities between the effects of opiates and alcohol are, in part, genetically mediated, then responses to these drugs might be expected to covary among genetically diverse subjects

To date, most of the evidence for a genetic substrate for opiate-ethanol commonalities must be derived from individual studies of the genetic influence on responses to either class of drug These studies have employed both inbred and selectively bred mice and rats Thus, relationships can be suggested for preference for ethanol [31,41] and morphine

[1]Supported in part by UAC award 0219-03-240-78 from the Research Foundation of the State of New York to GPH
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adulterated solutions [22,24], readdiction susceptibility to alcohol and to morphine [16,37], and ethanol preference and central levels of endogenous opiates [3]

Another population of animals which are of potential value for investigations of alcohol-opiate commonalities are the Colorado "sleep lines" These two lines of mice have been selectively bred for differential sensitivity to ethanol, as measured by the duration of the loss of the righting reflex following hypnotic doses of ethanol After 18 generations of selection for short (SS) or long (LS) sleep times, there was virtually no overlap in ethanol induced narcosis between the two lines (see [31] for a more complete description of the history of these mice)

SS and LS mice have been shown to differ on a number of alcohol related phenotypes (see [12] for a review), including alcohol induced hypothermia [35] Morphine has also been shown to induce hypothermia in mice, although the effect is influenced by a number of factors [11] Thus, changes in body temperature induced by alcohol and by morphine could prove to be a useful index of genetic aspects of commonalities or interactions between these agents The purpose of the present experiments was to investigate the effect of alcohol and of morphine on body temperature in mice of the LS and SS lines The first experiment was conducted to establish dose-response relationships for both drugs in each line The second experiment was designed to explore the effect of naloxone, an opiate antagonist, on both morphine and alcohol induced hypothermia in these mice

GENERAL METHODS

Subjects

The subjects were descendents of the mice selectively bred by McClearn and Kakihana [29] 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 A minimum of 15 mating pairs have been maintained for each line, with the restriction that the progeny of each pair did not share common grandparents

In the present studies, equal numbers of adult male and female mice of the LS and SS lines served as subjects All mice were 60–100 days of age when tested Subjects were housed as littermates (no more than 6 per cage) segregated as to sex and kept in a clear plastic cage from weaning at postnatal day 21 throughout the experiment In an effort to reduce possible litter effects, no more than 2 subjects per litter were included in any one treatment group Subjects were maintained in a temperature controlled environment (21°±1°C) on a 12 12 hour light-dark cycle (lights on 0700 hrs) Purina lab chow and tap water were available ad lib throughout the experiment

Drugs and Apparatus

Morphine sulfate doses are expressed as the salt concentrations The drug was prepared in normal saline from a concentrated stock solution which was made fresh each week Morphine was stored in the dark to retard oxidation All injections were at a volume of 0 01 cc/g body weight Morphine was supplied by Merck (Rahway, NJ)

Ethyl alcohol was a 20% (w/v) concentration made from 95% ethanol diluted in saline for doses of 2 0 g/kg or more For doses below 2 0 g/kg the drug was prepared as a 10% solution so that the injection volumes were 0 01 cc/g body weight Injection solutions were made fresh every week

Naloxone hydrochloride was dissolved in saline and administered in a volume of 0 01 cc/g body weight A naloxone dose of 10 mg/kg, expressed as the salt, was used when indicated Naloxone hydrochloride was a generous gift from Endo Laboratories (Garden City, NY)

Body temperature was measured using a telethermometer (Yellow Springs Instruments #43TA) and a distal small animal rectal probe (YSI #402) The probe was lubricated lightly with mineral oil prior to each temperature determination

Procedures

All injections were given intraperitoneally (IP) For body temperature determination, all animals were gently restrained by lifting the tail The lubricated probe was inserted 2 cm into the rectum and the temperature was recorded after 30 sec

Due to the relatively large number of mice tested in both experiments, each experiment was conducted over several successive days Within practical limits, treatment groups were represented on each day of each experiment

EXPERIMENT 1

Both morphine and ethanol produce changes in body temperature in many species The direction, as well as the magnitude, of this change depends on a number of interactive factors, including ambient temperature of the testing environment, dose of the drug and time since injection (see [11]) With respect to LS and SS mice, several studies (e g , [1,35]) have demonstrated that LS mice are more hypothermic than SS following doses of ethanol of 2 0 g/kg or greater when body temperature is measured at least 30 min post-injection In fact, LS mice do not recover baseline temperatures for up to 5 hr following 2 0 g/kg ethanol

In the first experiment, the effects of varying doses of ethanol and morphine on body temperature were examined in LS and SS mice The ethanol dose-response curve was evaluated for two reasons First, it extends the work of other laboratories to additional doses of ethanol In addition, a replication of results from other laboratories would support the hypothesis that possible differences in response to morphine are not due to random genetic factors associated with maintaining a separate colony of LS and SS mice in our laboratory

METHOD

A total of 180 mice of both genotypes and sexes, naive to any previous drug treatment, served as subjects For both the alcohol and morphine treatment groups, 5 mice/sex/genotype/dose were tested, with mice being randomly assigned to treatment groups except as noted in the general methods

For each drug, the treatment groups were defined by the dose of the drug the animal received Each mouse in the ethanol groups was injected with either 0, 1 0, 1 5, 2 0 or 2 5 g/kg ethanol, while mice in the morphine groups received either 0, 5, 10 or 20 mg/kg morphine sulfate

Each subject was weighed and injected with the appropriate drug and dose, then returned to its home cage Each mouse was removed from the cage for determination of body

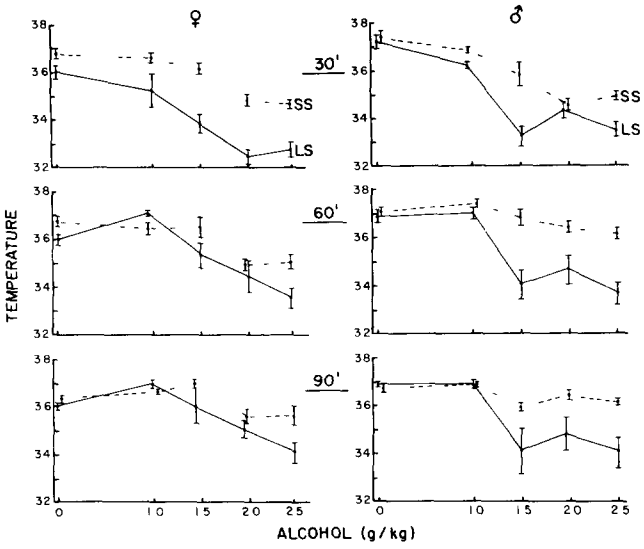

FIG 1 Mean (±SEM) body temperature (°C) of female and male LS and SS mice 30, 60 and 90 minutes after administration of alcohol

temperature at 30, 60 and 90 min after the administration of the drug

Body temperature at each post-injection time point served as the dependent variable These scores were analyzed separately for alcohol and morphine treatment groups by subjecting them to a 2(Line) × 2(Sex) × 5(Doses of alcohol) or 4(Doses of morphine) × 3(Post-injection times) repeated measures analysis of variance Newman-Keuls post-hoc analyses were applied when indicated to specify further the results obtained

RESULTS

Response to Ethanol

The dose response curve to ethanol is presented in Fig 1 for each line, sex and post-injection time Analysis of the variance in body temperature revealed significant main effects of line, sex, dose of ethanol and time since injection In general, LS mice were more hypothermic than SS mice, $F(1,93)=72\ 68$, $p<0\ 001$ Although female mice displayed significantly lower body temperature than did male mice, $F(1,93)=5\ 82$, $p\leq 0\ 05$ a large part of this difference may be due to differences in the baseline (0 dose ethanol) condition The degree of hypothermia increased with increasing doses of ethanol, $F(4,93)=48\ 53$, $p\leq 0\ 01$, while the effect waned from 30 to 90 min after drug administration, $F(2,186)=46\ 34$, $p\leq 0\ 001$ Interpretation of these main effects, however, should be tempered by evaluation of a number of significant interactions

There was a significant line × dose interaction, $F(4,93)=8\ 56$, $p\leq 0\ 001$ Averaged over the three post-injection measurements, the greatest difference between lines occurred following 1 5 g/kg ethanol ($p\leq 0\ 01$), but the most prominent decrease in body temperature in both lines was evident following the highest dose of ethanol

A significant post-injection time × dose interaction, $F(8,186)=6\ 54$, $p\leq 0\ 001$, indicated maximum hypothermia was found 30 min post-injection with more rapid return towards baseline with decreasing doses (see Fig 1) Subjects receiving 1 0 g/kg returned to baseline within 60 min of injection whereas higher doses had a longer lasting hypothermic effect

A significant sex × dose interaction was also found, $F(4,93)=3\ 58$, $p\leq 0\ 001$ Females had lower baseline (0 dose) levels than males and greater hypothermia than males to the 2 0 g/kg dose, although males were more hypothermic to the 1 5 g/kg dose ($p\leq 0\ 01$) Some of these effects may, however, be due to differences in baseline

There was a significant post-injection time × line × sex interaction, $F(2,186)=11\ 32$, $p\leq 0\ 001$ From Fig 1, it can be seen that female LS mice were more hypothermic than female SS mice at 30 min ($p\leq 0\ 01$) Male LS mice were more hypothermic than male SS mice at 60 and 90 min post-injection ($p\leq 0\ 05$) LS females showed a significant return towards baseline by the 90 min post-injection period compared to other subjects ($p\leq 0\ 05$)

Response to Morphine

Analysis of variance in body temperature following morphine administration revealed significant main effects of dose and post-injection time In general, increasing doses of morphine produced greater decreases in body temperature, $F(3,70)=75\ 02$, $p\leq 0\ 001$ The main effect of post-injection time, $F(2,140)=7\ 58$, $p\leq 0\ 001$, cannot be attributed in simple fashion to a waning of the effect across time, since post-injection time interacted significantly with two other factors The morphine dose-response curve is presented in Fig 2 Since analyses revealed no significant main or interactive effect of sex, values are pooled across this variable

Analysis of variance indicated a significant time × line interaction, $F(2,140)=11\ 65$, $p\leq 0\ 001$ This effect was due primarily to the SS subjects displaying greater sensitivity than LS mice to the hypothermic effects of morphine at 30 min post-injection In contrast to alcohol induced hypothermia, the SS mice were more hypothermic than LS mice to 5, 10 and 20 mg/kg doses of morphine ($p\leq 0\ 05$) It can be seen from Fig 2 that this difference was seen only at the 30 min post-injection period The absence of any apparent line difference at 60 min is due to both an increase of body temperature in SS mice as well as a decrease in LS mice, relative to their respective temperatures 30 min post injection

Analysis of variance also revealed a significant post-injection period × dose interaction, $F(6,140)=6\ 56$, $p\leq 0\ 0001$ Increasing doses of morphine produced an increasing degree of hypothermia Body temperature at the 90 min time point, compared to the 30 min period, shows a slight decrease in body temperature with saline, but an increase or return towards baseline with the 20 mg/kg dose The decrease in body temperature evident in SS mice 90 min after saline administration may be a spurious effect of sampling error, since only a slight decrease was seen in the SS mice in the analogous group in the alcohol dose response curve (see Fig 1)

DISCUSSION

In the present study, LS mice were more sensitive than SS mice to the hypothermic effects of ethanol when the dose was 1 5 g/kg or greater These findings are in general agreement with previous research from other laboratories (e g , [1,35]), suggesting that relaxed selection in our colony has

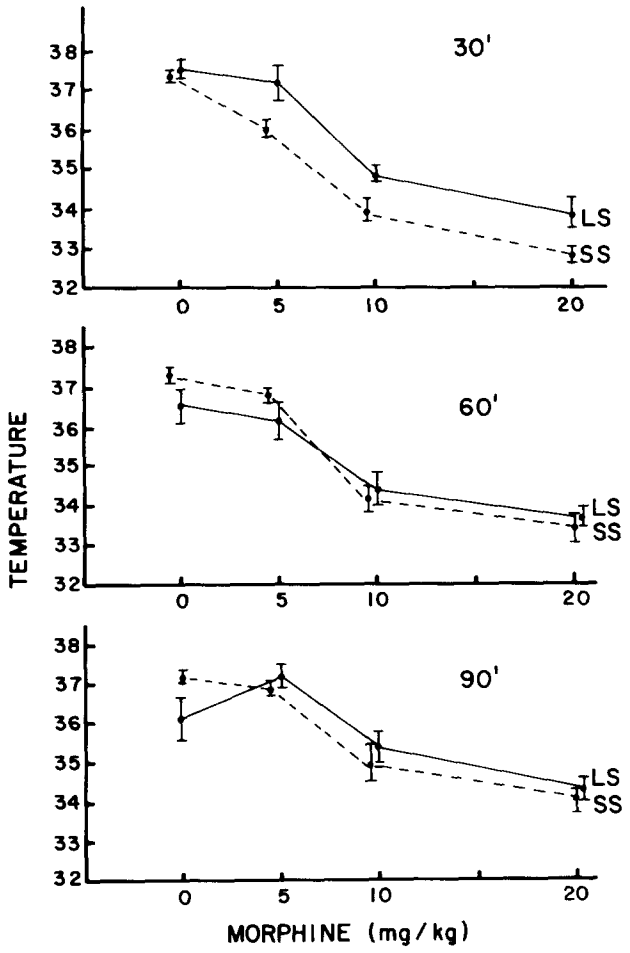

FIG 2 Mean (±SEM) body temperature (°C) of LS and SS mice 30, 60 and 90 minutes after administration of morphine Each data point represents 10 subjects

not obscured previously reported differences between these lines in the effects of alcohol on body temperature However, hyperthemic responses to 1 0 g/kg ethanol reported previously [35] were not evidenced in the present study The reason for our failure to replicate one point on the dose-response curve is not readily apparent Random genetic drift or founder effects are possible sources of variability It should be noted, however, that past [10] and present research employing subjects from our colony of LS and SS mice has demonstrated consistent differences in the appropriate direction in the selection criterion (i e , ethanol induced narcosis)

Morphine-induced hypothermia was also different between the two lines However, the differences in sensitivity to morphine were reversed relative to alcohol That is, SS mice displayed a significantly greater sensitivity to morphine than did LS mice, although the difference was only observed 30 min after morphine administration

It is unclear whether or not this difference in morphine sensitivity is related to the selection criterion of ethanol induced narcosis The reversal in sensitivity might suggest that the two phenotypes are not related, which would indicate the absence of opiate-ethanol commonalities with respect to the hypothermia produced by these agents in mice However, cross tolerance to alcohol and morphine induced hypothermia following chronic drug administration has been demonstrated in rats by Khanna *et al* [26], and in SS (but not LS) mice in our laboratory [23] Thus, the possibility of commonalities between alcohol and opiates with respect to changes in body temperatures cannot be discounted In the following experiment, this possiblity was further examined in the context of acute responses to alcohol and morphine using a pharmacological intervention technique

EXPERIMENT 2

One of the most useful tools for demonstrating that physiological or behavioral responses are, in part, mediated by opiate systems is the use of relatively specific opiate antagonists Although it is no longer safe to say that such antagonists are totally devoid of any behavioral activity in the absence of either exogenous or endogenous opioids, the attenuation by an opiate antagonist of responsiveness to an agent which is not itself an opioid is a necessary, but not sufficient, demonstration of an interaction between that agent and an opioid system [43] It is possible that any such interaction may be directly at the level of the opiate receptors, but given the complex connectively of the nervous system, this does not have to be the case

With respect to possible alcohol-opiate interactions, naloxone (a relatively pure antagonist) has been shown to block or attenuate a number of responses to ethanol in both mice and rats, including naloxone inhibition of ethanol dependence [5], attenuation of alcohol narcosis [21] and alcohol enhanced self-stimulation [27], and the blockade of brain calcium depletion produced by ethanol and salsolinol [42] The growing body of literature demonstrating the possibility of ethanol-opiate interactions adds support to the hypothesis that the effects of naloxone on alcohol responsivity is mediated either directly or indirectly via opioid neuromodulator systems [43] The purpose of the following study was to evaluate the effects of naloxone on both morphine and alcohol induced hypothermia, and to correlate the effects of naloxone on the differences between LS and SS mice to both agents which was observed in the first experiment

METHODS

A total of 60 adult mice of both sexes and lines served as subjects Subjects were weighed and randomly assigned to one of six conditions which were formed by the factorial arrangement of three initial drug treatment groups (saline, 2 g/kg ethanol or 10 mg/kg morphine) and two post-drug treatment groups (saline or 10 mg/kg naloxone) Thus, 5 mice of each sex and genotype were tested in each of the six groups The doses of morphine and ethanol were selected as intermediate doses from the curves presented in Experiment 1 The dose of naloxone selected may be considered moderately high relative to that needed to attenuate an opiate effect, but is one commonly used to alcohol-opiate interaction studies (e g , [21])

Subjects were injected with saline, ethanol or morphine at 5 min intervals Thirty minutes after the initial injection, a second injection of either saline or naloxone was administered Rectal body temperature was recorded 5 min after the second injection as described in the general methods

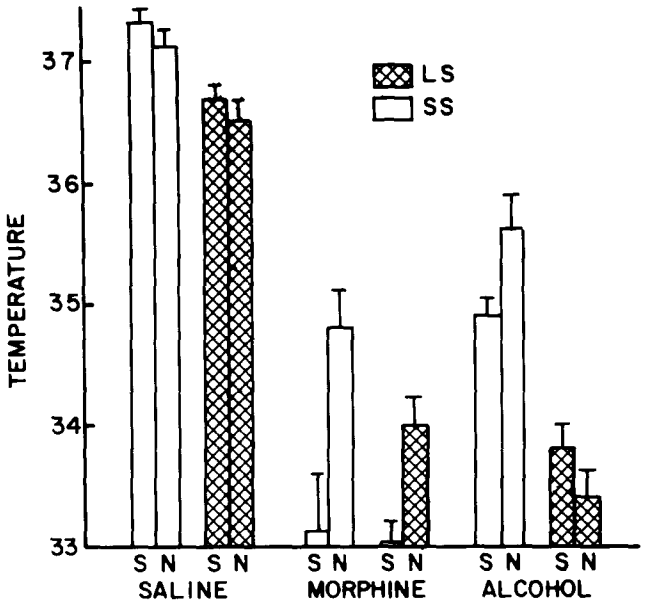

FIG 3 Mean (±SEM) body temperature (°C) of LS and SS mice injected with saline, morphine (10 mg/kg) or alcohol (2 g/kg), followed by either saline or naloxone (10 mg/kg) Each mean represents 10 subjects

Body temperature scores were subjected to a 2(Line) × 2(Sex) × 3(Initial drug treatment) × 2(Post-drug treatment) analysis of variance Newman-Keuls tests or *t*-tests were used for specific comparisons of interest

RESULTS AND DISCUSSION

Analysis of the variance in body temperature did not reveal any significant main or interactive effect of sex Therefore, the results summarized in Fig 3 are pooled across this variable There were significant main effects line, $F(1,96)=31\ 81$, $p\leq 0\ 001$, initial drug treatment, $F(2,96)=161\ 19$, $p\leq 0\ 001$, and post drug treatment, $F(1,96)=10\ 05$, $p\leq 0\ 005$ As is evident from Fig 3, these latter two effects are contributed to significantly by the inclusion of the saline groups in both initial and post-drug treatment groups

It should be noted that there was no significant difference between LS and SS mice in morphine induced hypothermia when the morphine injection was followed by a second injection of saline Thus, the results of the first experiment (i e , greater sensitivity to morphine induced hypothermia in SS mice) were not replicated under the present conditions It is possible that the original effect was reversed by the saline injection just prior to testing A somewhat similar effect of saline injection has been reported in mice by Riffee *et al* [40] Furthermore, we have replicated the results of the first experiment in other experiments in our laboratory (see General Discussion)

The line × initial drug interaction was significant, $F(2,96)=5\ 72$, $p\leq 0\ 005$ This effect was due largely to the greater hypothermia produced by ethanol in LS mice ($p\leq 0\ 01$) In addition, there was a significant initial drug × post-drug interaction, $F(2,96)=9\ 06$, $p\leq 0\ 001$ This effect was due in large part to the fact that, while naloxone had no significant effect on mice first receiving saline, it significantly attenuated morphine induced hypothermia in mice of both lines

The line × post-drug interaction approached, but did not reach, statistical significance, $F(1,96)=3\ 79$, $p=0\ 054$ However, given a main effect of line and an initial drug × post-drug interaction, it is of interest to compare LS and SS mice in response to naloxone challenge following either morphine or alcohol As indicated, naloxone significantly attenuated morphine induced hypothermic in both lines However, of greater interest is the finding that naloxone significantly attenuated alcohol induced hypothermia in SS mice, $t(18)=2\ 08$, $p\leq 0\ 05$, but not in LS mice, $t(18)=-1\ 24$, $p>0\ 05$

The fact that SS, but not LS, mice exhibited an alcohol-opiate interaction in the form of a reduction by naloxone of the decrease in body temperature produced by prior ethanol treatment is compatible with the suggestion from Experiment 1 that SS mice were more responsive to opiates, at least when the index of responsivity is changes in body temperature Since SS mice appear to be more sensitive to both an opiate agonist (morphine) and an antagonist (naloxone), it is tempting to speculate about possible differences between these lines in some property of opiate receptors Other studies using different genetically defined subjects have demonstrated genetic substrates for differences in the density of opiate receptors in mice [2] However, in the absence of any characterization of opiate receptors in LS and SS mice, the differences in physiological responses to morphine observed in the present studies are not necessarily directly related to opiate receptors *per se* Other explanations are equally as plausible, some of which are reviewed in the general discussion

GENERAL DISCUSSION

Previous research from different laboratories has demonstrated that mice originally bred for differential sensitivity to hypnotic doses of ethanol also differ in a number of other alcohol related phenotypes [12], including alcohol induced changes in body temperature [1,35] The present studies have, in general, replicated this latter finding and have extended it to additional doses In addition, the results reported herein have suggested that SS mice are more sensitive that LS mice in their responsiveness to morphine and to ethanol-naloxone interactions

Although the differential response to both an opiate agonist and an antagonist might intimate differences between these lines in either density or affinity of opiate receptors, such possible differences have not been examined In addition, other possibilities might also be suggested For example, the systemic administration of morphine employed in these studies does not preclude the possiblity of differences in peripheral effects of the drug However, previous studies using rats have shown that a quaternary derivative of morphine, which is unable to cross the blood-brain barrier, did not produce changes in core temperature following systemic administration, although significant hypothermia resulted from intracerebral administration of this agent [18]

Differences in the permeability across the blood-brain barrier cannot be dismissed as a possible source of the reported differences between LS and SS mice in repsonse to morphine or naloxone Possible differences in the access of morphine to the central nervous system could be reflected in

both the time course and the absolute amount of the drug reaching the brain The difference between lines is evident at 30, but not 60, min after morphine administration (see Fig 2) The lack of differences between lines at later time points appears to be due to both a decrease in SS and an increase in LS mice in responsiveness, relative to the effect at 30 min post-injection However, subsequent research in our laboratory [23] using a within-subject difference score (pre-injection minus post injection body temperature) has indicated a slightly different pattern of recovery Morphine (10 mg/kg) produced a 3°C decrease in body temperature in SS mice 30 min after injection, while at 60 min post-injection, there was a 2°C decrease, relative to their pre-injection baseline temperature On the other hand, the body temperature of LS mice was lowered by 2°C at both 30 and 60 min post-injection trials Furthermore, previous studies have demonstrated that brain morphine levels do not correlate significantly with other acute responses to morphine, such as analgesia and locomotor activities [6] Nonetheless, in the absence of the determination of morphine levels in the brain at various time points after administration, absorption and permeability factors cannot be discounted

Finally, the finding that naloxone attenuated alcohol-induced hypothermia in SS, but not LS mice, may suggest opiate receptor differences betwee these lines However, opiate receptors are known to occur on the synaptic terminals of neurons forming a number of different neurotransmitter systems Therefore, differential responsiveness to morphine may reflect differences in the neurotransmitter systems modulated by these opiate receptors, rather than differences in the opiate receptors *per se* For example, hypothermia induced by both ethanol [38] and morphine [8] appear to involve alterations in the functional activity of serotonergic neurons, although the specific mechanisms might be quite different To our knowledge, no systematic investigation of possible differences between LS and SS in serotonergic neurotransmitter systems has yet been conducted However, since it has been previously suggested that endogenous tryptophols, their aldehydes, or both, may play an important role in the loss of righting reflex induced by ethanol [17], it is tempting to speculate that differences in serotonergic systems may play a role in the differences reported in the present experiments In addition, both ethanol and morphine cause depletion of brain Ca++ levels [42], an ion which is important in synaptic transmission (see [32]) Thus, the differences between LS and SS mice in responsiveness to morphine may be mediated by underlying differences in one or more neurotransmitter systems

The LS and SS lines have been selectively bred for one phenotype, that of duration of loss of righting reflex following hypnotic doses of ethanol Since they are theoretically randomly bred for all other phenotypes, any significant differences observed in other phenotypes may be genetically related to the selection criterion However, fortuitous associations of genes are also possible Although the differences in morphine responsiveness suggested from the present study may be a genetically (albeit inversely) correlated response to selection, future research is needed to evaluate whether morphine induced hypothermia and alcohol induced narcosis covary or assort independently in mice of heterogeneous genetic origin

Finally, it should be further acknowledged that the LS and SS mice maintained in our laboratory represent a different genetic population from those presently reared at the University of Colorado, due to relaxed selection and the relatively small number of mating pairs from which they are derived Nonetheless, LS and SS mice from our colony show consistent differences in responses to alcohol and to opiates It is hoped that they will be useful tools in elucidating genetic and neurochemical factors that influence responsiveness to these agents

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