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Genetic Determinants of Morphine Activity and Thermal Responses in 15 Inbred Mouse Strains

J. K. BELKNAP,* J. RIGGAN,* S. CROSS,* E. R. YOUNG,*
E. J. GALLAHER*† AND J. C. CRABBE*†

*Research Service (151W), VA Medical Center, and
†Departments of Behavioral Neuroscience and Department of Physiology and Pharmacology,
Oregon Health Services University, Portland, OR 97201

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BELKNAP, J. K., J. RIGGAN, S. CROSS, E. R. YOUNG, E. J. GALLAHER AND J. C. CRABBE. *Genetic determinants of morphine activity and thermal responses in 15 inbred mouse strains*. PHARMACOL BIOCHEM BEHAV **59**(2) 353–360, 1998.—Mice from 15 standard inbred strains were tested for sensitivity to two effects of acute morphine administration, open-field activity, and body temperature changes, at doses of 0, 4, 8, 16, and 32 mg/kg, IP. Large strain differences were consistently observed, indicating a substantial degree of genetic determination of these traits. For morphine-induced activity, some strains were markedly insensitive to all doses (e.g., C3H/He, CE), while others showed increases and some decreases at the same morphine dose. For thermal responses, one strain was insensitive to all doses employed (C3H/He), while others showed marked hypothermia and some hyperthermia at the same dose. Although strains differed in brain morphine concentrations at time of behavioral testing, pharmacokinetic differences were unrelated to both measures of morphine sensitivity. Correlations among strain means (estimates of genetic correlations) were rather high across doses within each measure, indicating that strain differences to a given effect of morphine were rather stable across doses. This suggests substantial commonality in genetically mediated mechanisms across the dose range used for activity, and also for thermal responses. In contrast, genetic correlations between activity and thermal responses were not significant at any dose, indicating that these two traits are largely genetically independent. © 1998 Elsevier Science Inc.

Morphine Hypothermia Hyperthermia Thermoregulation Inbred mouse Genetics Locomotor activity

IT is well known that sensitivity to several effects of morphine in rodents is determined, at least in part, by genotypic factors [reviews: (5,8,9,14,16,19,24,34)]. Genetic sensitivity to morphine is not, however, a monolithic phenomenon. Some studies report that sensitivity to one effect of morphine is genetically unrelated to sensitivity to other effects (see above reviews); this suggests separate genetic determinants mediated by independent biochemical and neural pathways.

In Swiss–Webster mice, substantial doses of morphine and similar drugs have long been known to produce a marked increase in activity ("running fit") (21), a trait that was shown to be heritable (23). However, over 2 decades ago, it was shown that among inbred strains, this effect is highly strain dependent (10,18,27,28,35). The C57BL/6 strain has been reported to show marked increases in open-field or "toggle-box" activity in response to a wide range of morphine doses, whereas the DBA/2 strain remains at baseline or decreases [reviews: (5,19)]. Essentially the same findings were seen with the ICV route of administration (33), suggesting that morphine, rather than a metabolite, is the principal active agent. The C3H/He and BALB/c strains appear to be "runners," although to a lesser extent than C57BL/6 mice, while the A and AKR strains tend to be "nonrunners" [review: (5)]. It has been hypothesized that morphine-induced behavioral activation is due, at least in part, to increased dopaminergic activity in mesocortical and mesolimbic pathways (38). In support of this, Racagni et al. (32) found that morphine-induced striatal dopamine release, as reflected in 3-methoxytyramine levels, was enhanced in C57BL/6J mice and unchanged in DBA/2J mice.

Morphine has long been known to cause pronounced thermoregulatory effects in rats and mice [reviews (1,11,12)]. In mice, marked inbred strain differences have been reported in

Requests for reprints should be addressed to John K. Belknap, Ph.D., Research Career Scientist, Research Service (151W), VA Medical Center, Portland, OR 97201.

morphine-induced hypothermia assessed by rectal probe. Muraki and Kato (25,26) studied hypothermia induced by 10 or 20 mg/kg of morphine in six strains of mice. DBA/2N animals were most sensitive to this effect, followed in descending order by A/J, BALB/c, ICR, C57BL/6N, and C3H/HeN mice. Belknap et al. (4) reported similar strain rankings for the DBA/2J, C57BL/6J, and C3H/HeJ strains. C57BL/6J mice are more likely to show Straub tail, an index of muscular rigidity, than are DBA/2 or C3H/He mice (4). This may confer some resistance to hypothermia via thermogenesis compared to the other two strains (30).

While the number of opioid studies employing inbred strains is extensive [reviews: (5,9,19,24,34)], the number of strains that has been well characterized has been few. This is largely due to the frequent and often exclusive use of C57BL/6J and DBA/2J mice, presumably because of the large strain differences often reported. The next most commonly studied strains are BALB/cJ and C3H/HeJ, which rank well below the first two strains in frequency of use. Also well studied is the CXBK recombinant inbred strain, which has been shown to be relatively insensitive to a number of morphine effects and deficient in mu opioid receptors [reviews: (2,5,9,24)]. This concentration on a few strains can be viewed as both a strength and a weakness. One strength is that there is a wealth of data on C57BL/6J and DBA/2J genotypes across many measures and many laboratories, allowing the emergence of reasonably consistent patterns of strain differences. For example, following opiate administration, the C57BL/6 strain, when compared to DBA/2 strain, has distinguished itself as a marked "runner" (hyperactive) in the open field, low on hot plate-assessed analgesia, high incidence of Straub tail, relatively sensitive to naloxone-induced jumping after chronic morphine exposure, and very high on intake of morphine-saccharin solutions in preference to water [reviews: (5,24,34)].

A major weakness of exhaustively studying only a few strains stems from the lack of statistical power to assess the degree to which two measures share common genetic influences, i.e., to detect genetic correlations (13). These estimates are usually based on the correlation among inbred strain means where the degrees of freedom are two less than the number of strains. Thus, for only two strains, no genetic correlation estimates are possible, and for studies with three to six strains, two variables must be extremely highly correlated ($r > 0.81$, $n = 6$) before the result is statistically significant. The present study was undertaken to increase the power for genetic correlation estimates by testing 15 standard inbred strains for morphine sensitivity. In particular, we wished to assess the genetic relationship between morphine-induced activity and thermal responses, two of the more common sensitivity measures in the literature. This relationship has not been directly studied at the genetic level. We also wished to initially characterize 10 of these strains not previously studied for either trait, and to compare them to the better characterized strains.

METHOD

Animals and Husbandry

Male mice ($n = 618$) of 15 inbred strains (average $n = 40$/strain) were purchased from The Jackson Laboratory, Bar Harbor, ME, and shipped to Portland at age 5 weeks, and tested beginning at 7 weeks. A brief history and summary characteristics of these strains is given by Festing (17). Mice of the following strains were tested in two blocks: block 1 = A/HeJ, CBA/J, CE/J, C57L/J, PL/J, SJL/J; and block 2 = AKR/J, BALB,cJ, C3H/HeJ, C57BR/cdJ, DBA/1J, SWR/J. Mice of strains C57BL/6J (C57) and DBA/2J (DBA) were included in both blocks to enable detection of any seasonal or procedural variations associated with shipment block. Total ns were 39–40 mice/strain except C57 (60/strain), DBA (60/strain), and C57L (20/strain). Mice were group housed (four/cage) in clear polypropylene cages (28 X 18 X 13 cm) on corncob bedding (changed twice weekly) in a filtered rack system (Thorens). Water and Purina rodent block food were available ad lib, ambient temperature was maintained at 21 ± 1°C, and fluorescent lighting was on from 0600–1800 h daily. All procedures followed USDA and NIH guidelines for the care and use of laboratory animals.

Experimental Design

The general scheme of testing is described here: specifics follow in later sections. All mice were first tested for open-field activity after IP injection with either saline, 4, 8, 16, or 32 mg/kg morphine sulfate at an ambient temperature of 21 ± 1°C. Each mouse was injected only once with one dose. Body temperature via rectal probe was determined immediately before and after activity testing using brief restraint. Some mice from the higher dose groups were sacrificed immediately after activity and temperature testing for morphine brain and plasma assays.

Activity and Thermal Response Testing

Previously-published procedures were employed for testing (29). Briefly, 1 h prior to testing, all animals were moved into the room where testing would occur. Each animal was restrained briefly in a 50 mm i.d. Plexiglass tube and a lubricated 0.5 mm diameter rectal probe was inserted 2.5 cm. A baseline temperature was recorded 5–10 s later using a rapid response sensor (Sensortek Thermalert Th-8 Digital Thermometer). The animal was injected with morphine sulfate (4, 8, 16, or 32 mg/kg, IP, in 0.9% NaCl) or saline and placed immediately into the center of an illuminated, sound-attenuated Omnitech Activity monitor. Infrared beam interruptions in the horizontal plane were recorded automatically each 5 min for 30 min, yielding a measure of horizontal distance traversed for each animal (ACT). When removed from the activity chamber, a single postinjection temperature was taken 30 min after injection using the same procedure as the preinjection measurement. Only a single postinjection measurement was taken to minimize possible confounding effects of stress associated with multiple rectal temperature measurements (3,15).

Brain and Plasma Morphine Determinations

Because we wanted to test some mice from each strain for other studies, only mice given either 16 or 32 mg/kg were sacrificed by cervical dislocation for brain and plasma assays immediately after completion of the activity/body temperature test. Trunk blood (200 μl) was collected, and brains were rapidly dissected free and frozen in tightly capped vials until assay. Assays were carried out by HPLC using a UV detector (210 nm) and a flow rate of 1.0 ml/min at 1500 psi. The extraction procedure was modified from that of Foltz et al. (20). Blood samples were diluted into 100 mM potassium phosphate buffer (pH 7.0) containing 1 μg of naloxone (internal standard) and extracted into chloroform:heptane:isopropanol (50:33:17). After recovery and drying of the organic phase, the residue was dissolved in 40 μl of mobile phase, washed with heptane, and injected into the HPLC. The brain samples were

homogenized in 100 mM potassium phosphate buffer, pH 7.0, containing 1 μg of naloxone. Under saturating NaCl, the homogenates were extracted into toluene:heptane:isoamyl alcohol (70:20:10). After centrifugation, the organic phase was recovered and extracted into 0.2 N sulfuric acid. The latter was recovered and washed twice with heptane. The aqueous layer was neutralized with 6 N NaOH, and saturating NaCl added in phosphate buffer. This solution was extracted with toluene:heptane:isoamyl solvent. After centrifugation, the organic phase was dried in nitrogen and the sample dissolved in mobile phase (acetonitrile:50 mM phosphate buffer, pH 7.0, 30:70) and injected onto the LC-CN Supelcosil column. Free base morphine concentrations were calculated based on morphine:naloxone ratios of detector response areas reported by integration.

Data Analysis

Data were analyzed by factorial ANOVAs using the factors strain and dose for the 30-min activity and body temperature data corrected for postinjection saline (strain mean) values. An alpha level of 0.05 was adopted for significance testing. Post hoc comparisons were made using simple main effects. The heritability was estimated by r^2, or $SS_{strain}/(SS_{strain} + SS_{within})$ from a one-way ANOVA. This value represents the proportion of the total variation due to strain, and estimates the proportion of the phenotypic (total) variance due to genetic influences.

Because DBA/2 and C57BL/6 mice were tested in both blocks, block effects were tested in a strain × dose × block ANOVA for these two strains.

Genetic Correlational Analyses

To determine the extent of genetic codetermination of the different morphine sensitivity measures studied, the strain means for each dose were correlated with the other doses within each trait (either activity or thermal responses, $df = 13$) and between traits. This procedure provides estimates of genetic correlations (13), which index the degree to which each pair of doses within traits, or the same dose between traits, share common genetic influences. Thus, two traits with identical genetic influences (determination by the same genes) would show a genetic correlation of 1.0, while those with completely independent gene effects would show a correlation of zero. Put another way, a high genetic correlation between activity and hypothermia, for example, would imply that the strains most sensitive to one trait will also tend to be most sensitive on the other.

The goal of the correlational analyses was essentially exploratory. Because we were examining several correlations, some may be significant simply by chance. On the other hand, with 13 *df*, our power to detect real genetic associations was limited (significance levels for $df = 13$ are $r > 0.52$ for $p < 0.05$ and $r > 0.64$ for $p < 0.01$). Our strategy, therefore, was to use an alpha level of 0.05 as an initial index of their potential importance. Finally, all correlations were verified by examination of scatterplots to insure that they were not unduly influenced by a single extreme scoring strain.

RESULTS

Plasma and Brain Morphine Concentrations

Concentrations of morphine in plasma and brain 32 min after injection at each dose are given in Table 1. Strains differed significantly for brain and plasma concentrations ($p < 0.05$). However, because these values did not correlate systematically with any morphine sensitivity measure (see below), they were not used in interpreting the sensitivity data.

Block Effects

When postinjection saline values were subtracted from postinjection morphine values, no significant block effects were seen for either activity or thermal responses ($p > 0.15$).

Locomotor Activity (ACT)

Significant strain differences were seen for the saline groups alone ($r^2 = 0.45$, $p < 0.001$, not shown); therefore, all morphine responses were corrected for the saline strain differences by subtraction of the appropriate strain mean. The

TABLE 1

FREE MORPHINE CONCENTRATIONS (±SEM) 32 MIN POSTINJECTION IN THE INBRED STRAINS EXPRESSED IN μg/g FOR BRAIN AND μg/ml FOR PLASMA

Strain	Brain, 16 mg/kg Dose	Brain, 32 mg/kg Dose	Plasma, 32 mg/kg Dose
A/HeJ	0.61 ± 0.01	1.53 ± 0.07	1.99 ± 0.21
AKR/J	0.59 ± 0.05	1.57 ± 0.11	2.21 ± 0.24
BALB/cJ	0.64 ± 0.04	1.61 ± 0.09	2.29 ± 0.16
CBA/J	0.61 ± 0.02	1.89 ± 0.32	2.66 ± 0.11
CE/J	0.69 ± 0.03	1.74 ± 0.02	2.84 ± 0.07
C3H/HeJ	0.53 ± 0.08	1.55 ± 0.15	2.53 ± 0.23
C57/BL/6J	0.67 ± 0.03	1.68 ± 0.10	2.38 ± 0.11
C57BR/cdJ	0.73 ± 0.07	1.61 ± 0.15	2.33 ± 0.15
C57L/J	0.63 ± 0.05	1.49 ± 0.06	2.68 ± 0.07
DBA/1J	0.77 ± 0.04	1.63 ± 0.24	3.08 ± 0.07
DBA/2J	0.77 ± 0.05	1.56 ± 0.23	2.46 ± 0.32
PL/J	0.77 ± 0.20	1.62 ± 0.08	2.47 ± 0.14
SJL/J	0.58 ± 0.04	1.34 ± 0.07	1.51 ± 0.16
SWR/J	0.51 ± 0.06	1.72 ± 0.07	2.17 ± 0.18
129/J	0.64 ± 0.03	1.57 ± 0.11	1.79 ± 0.35

n = 7–12 per strain except C57L, where $n = 4$.

results pooled across all 15 strains was for morphine to significantly decrease activity at the lowest dose and to increase activity at the two higher doses relative to saline values. The morphine-induced changes from saline activity (means ± SEM) for the 4, 8, 16, and 32 mg/kg doses were −448 ± 201, −186 ± 212, +1501 ± 334, and +3569 ± 401 photocell interruptions, respectively. All are significantly different from zero, the saline response, except the 8 mg/kg dose. These values can be seen as responses of the "average" strain, with an n of 124 mice per dose. The correlation between these saline-corrected activity scores and log dose was 0.95, indicating good linearity of dose response. Twelve of the 15 strains showed rs > 0.90. Dose–effect curves for each strain are shown in Fig. 1a and 1b and time–effect curves are shown in Fig 2a–c. The main effects of strain, $F(14, 543) = 48.9$, dose, $F(4,543) = 60.6$, and their interaction, $F(56,543) = 5.6$, were highly significant ($p < 0.001$). The heritability (r^2) was 0.39 for saline-corrected morphine activity, indicating a substantial degree of genetic control of this trait. Post hoc analyses showed that strains differed significantly at all doses ($p < 0.001$). All but two strains showed dose-dependent changes in activity ($p < 0.01$). In contrast, strain CE ($p = 0.40$) and C3H ($p = 0.12$) did not respond significantly to morphine across all doses, or at any one dose, although significance was approached for the highest dose employed ($p \approx 0.10$).

The pattern of morphine responses differed markedly across strains (see Fig. 1). Three strains (DBA/2, DBA/1, A) showed only morphine-induced reductions in ACT compared to saline values. Seven strains (AKR, 129, BALB, CBA, C3H, C57BL/6, C57BR) showed either no change or only stimulation of ACT across doses. Three strains (SJL, PL, C57L) showed significant depression of activity at the lower doses while higher doses enhanced ACT compared to saline values. All strains showing increased ACT displayed the largest increase at 32 mg/kg dose compared to the other doses. However, the dose producing maximal decreases in ACT, when it occurred, varied from strain to strain.

Because strains differed markedly in saline group activity, percent increases and decreases vs. saline were examined for each dose. Strains showing the greatest percent increases in ACT after 32 mg/kg morphine were 129 (205%) and BALB (176%) relative to saline values. The strains maximally sensitive to reductions in ACT were A/He (−78%), DBA/2 (−74%), and DBA/1 (−56%).

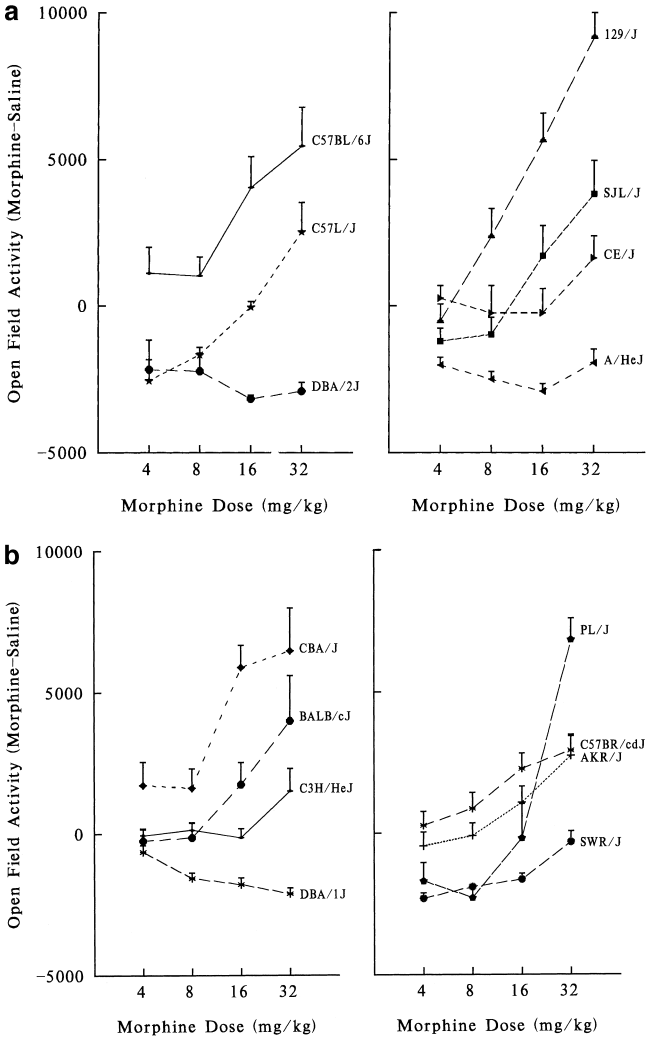

FIG. 1. Dose–response curves for open field activity in 15 inbred mouse strains. Plotted points represent the number of beam interruptions (mean ± SEM) for morphine groups minus corresponding saline values per strain from 0 to 30 min postinjection.

Thermal Responses

Thirteen of 15 strains were hyperthermic 30 min after 4 mg/kg morphine compared to preinjection body temperatures (within-subject comparison). However, when 30 min postinjection body temperatures for the morphine groups were compared to 30 min postinjection values for the saline group for each strain (between group comparison), rather than preinjection values, only two strains (CBA, CD) showed hyperthermia at the 4 mg/kg dose. This discrepancy is largely due to the significant increase in temperature seen in the saline group between pre- and postinjection rectal probe measurements (mean of +1.2°C over all 122 saline-injected mice), indicating that the injection and testing regimen itself caused an increase in temperature in the absence of morphine, and this effect varied greatly across strains ($r^2 = 0.29$, $p < 0.001$). The PL, A, and 129 strains showed ~2°C increases following saline injection, while the AKR, CE, SWR, and BALB/c strains showed less than 0.8°C increases (not shown). Thus, when the morphine-treated groups are examined, the within-subjects comparison confounds the injection and testing regimen (stress?) effects in the absence of drug with the effects of morphine. We, therefore, elected to analyze all of the thermal response data reported below in terms of changes from saline group postinjection values for each strain as baseline to correct for this saline treatment effect, much as was done for the activity data described above. Thus, all analyses for both activity and thermal responses are between-group comparisons throughout using measures taken postinjection. Saline treatment effects have been noted and discussed by Wenger (37).

Significant strain differences in body temperature were seen for the saline groups alone at 30 min postinjection ($r^2 = 0.36$, $p < 0.001$, not shown); therefore, all morphine responses were corrected for the saline strain differences by subtraction of the appropriate strain mean. The results pooled across all 15 strains was for morphine to significantly decrease body temperature (TMP) in a dose-dependent manner relative to saline values. The overall morphine-induced changes from saline TMP (means ± SEM) for the 4, 8, 16, and 32 mg/kg doses

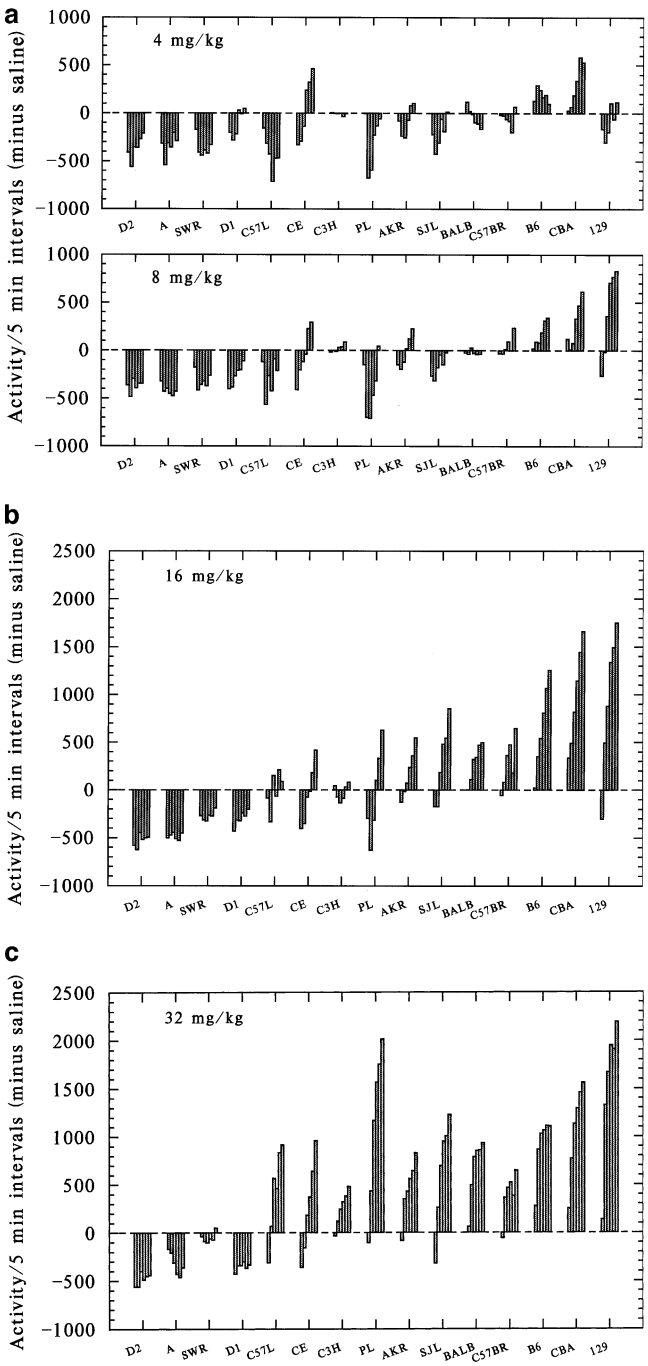

FIG. 2. (a) 4 and 8 mg/kg; (b) 16 mg/kg; (c) 32 mg/kg: depict the mean activity for each strain (morphine minus saline) at 5-min intervals throughout the 30-min testing period. The morphine injection (IP) was given just before the first time interval shown. Activity is given as the number of beam interruptions for each of six 5-min intervals per strain. The strains are plotted in rank order (left to right) from the least to the most sensitive strains to the activating effects of morphine (minus corresponding saline values) summed over all doses and time intervals.

were -0.29 ± 0.09, -1.12 ± 0.13, -2.24 ± 0.18, and -3.03 ± 0.19°C, respectively (all are significantly different from zero, the saline response). These values can be seen as responses of the "average" strain, with an n of 124 mice per dose. The correlation between these saline-corrected body temperatures with log dose was -0.998, indicating excellent linearity of dose response across all strains. Twelve of the 15 strains showed $rs > 0.90$. Strains differed significantly in their acute TMP responses to morphine 30 min postinjection, $F(14, 536) = 31.7$, $p < 0.0001$, as shown in Fig. 3a and 3b. The estimate of the heritability (R^2) was 0.47 for morphine-induced TMP changes. The effect of morphine was dose-dependent $F(4, 536) = 265$, $p < 0.0001$, and differed among strains as a function of dose, $F(46, 536) = 7.1$, $p < 0.0001$. Post hoc analyses of simple main effects showed that strains differed significantly at all doses ($p < 0.0001$). All strains except one (C3H) showed dose-dependent TMP changes (all $F > 6.1$, $p < 0.001$, except strain CE, for which $F = 3.0$, $p < 0.05$). Strain C3H was unique in that it did not respond significantly to the thermal effects of morphine across doses ($p = 0.10$) or at any one dose, a similar lack of response as was seen for activity.

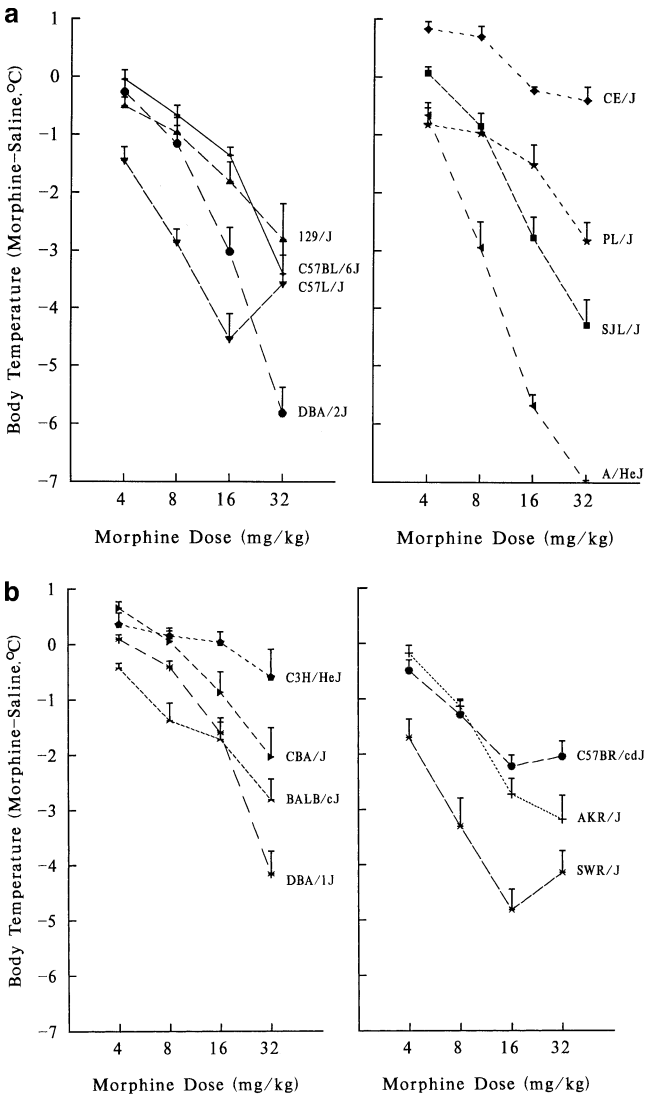

FIG. 3. Dose–response curves for thermal responses (TMP) to morphine in 15 inbred mouse strains. Plotted points represent mean (±SEM) rectal body temperatures (°C) minus corresponding saline values per strain taken 30 min postinjection.

At the 4 mg/kg dose, the CE (+0.83°C) and CBA (+0.65°C) strains showed the most hyperthermia, while the SWR (−1.7°C) and C57L (−1.45°C) showed the most hypothermia relative to saline group values. At the higher doses, hypothermia predominated, as shown in Fig. 3a and 3b. For most strains, the hypothermic response was greatest after 32 mg/kg compared to the other doses. At the 32 mg/kg dose, the most sensitive strains were A/He (−7.1°C) and DBA/2 (−5.8°C). Least sensitive were the CE strain (−0.4°C) and C3H (−0.6°C) strains.

Genetic Correlations Among Responses

For genetic correlation purposes (13), ACT was assessed at 0–30 min and also at 25–30 min postinjection. The latter reflects the 5-min period immediately prior to body temperature determination and brain harvest. The correlations between the two ACT measures ranged from 0.94 to 0.99 over the four doses (pooled $r = 0.96$), so they are nearly interchangeable. For this reason, only ACT for 0–30 min is shown in Table 2. Activity responses to 4, 8, 16, and 32 mg/kg morphine, measured as changes from saline values, were all significantly positively intercorrelated (six correlations) for both ACT time intervals. A pooled genetic correlation can be obtained by making an r to z transformation for each of the six correlations (36): the mean z value was then calculated, and transformed back to r. This pooled r was 0.82, $p < 0.001$, for 0–30 min ACT and 0.83, $p < 0.001$, for 25–30 min ACT, indicating that the strains showing the most activity at any one dose tended to do so for the other doses as well. Basal (saline) activity was not significantly correlated with any morphine-induced activity measure (not shown).

Temperature responses to each of the four doses were also highly intercorrelated. All six genetic correlations among the four doses were statistically significant except one −4 mg/kg with 32 mg/kg ($r = 0.27$). The pooled r among the six correlations was 0.72, $p < 0.005$. This pattern of significant positive intercorrelations indicates that the strains showing the most hypothermia at any one dose tended to do so for the other doses as well. The thermal responses to all four doses of morphine were not significantly correlated with saline body temperatures, although significance was approached for all doses (rs between −0.40 and −0.48, $p = 0.14$ to 0.07 NS).

The effects of morphine on activity were slightly positively related to the thermic responses, but this trend was not significant. Of the 16 possible genetic correlations between ACT and TMP for the four doses shown in Table 2, none was significant at the 0.05 alpha level for either 0–30 min ACT or 25–30 min ACT. The pooled genetic correlation across all 16 correlations between activity and thermal responses was 0.33 for 0–30 min ACT and 0.37 for 25–30 min ACT (ps ≈ 0.2, NS).

DISCUSSION

Studies of morphine thermal responses using the laboratory mouse are fewer compared to the rat, but the available data suggest considerable similarity between these two rodent species. In the rat, hyperthermia has often been reported after low doses of morphine, while hypothermia often results from higher doses at standard laboratory ambient temperatures [review: (1)]. For example, small acute morphine doses cause hyperthermia in CD-1 mice, and larger doses cause hypothermia (31). However, several reports show that when restraint is used during body temperatures measurements, a shift toward hypothermia is seen, which can completely reverse the hyperthermia observed without restraint [review: (1)]. This may explain why only hypothermia was seen in the present study when all 15 strains were pooled (averaged). In addition, we assessed thermal responses only at 30-min postinjection; later time points may have revealed hyperthermia (4). Ambient temperature is also an important determiner of the incidence of hyperthermia (1). For example, C57BL/6 mice showed significant hyperthermia at 25°C in a previous study from our laboratory (4), but only hypothermia or no change was seen at 21°C in the present study.

Linear dose–response curves (rs > 0.90) were seen in 12 of the 15 strains for both activity and thermal responses, indicating that an orderly dose–response relationship exists across the range of doses studied. Moreover, when genetic correlations (13) were determined for either activity or thermal responses across the four doses, high positive correlations were

TABLE 2

GENETIC CORRELATIONS AMONG ACTIVITY (ACT) AND THERMAL (TMP) RESPONSES AT EACH OF FOUR MORPHINE DOSES

	ACT 4	ACT 8	ACT 16	ACT 32	TMP 4	TMP 8	TMP 16	TMP 32
ACT, 8 mg/kg	0.84							
ACT, 16 mg/kg	0.75	0.92						
ACT, 32 mg/kg	0.56	0.78	0.90					
TMP, 4 mg/kg	0.20	0.14	0.12	−0.05				
TMP, 8 mg/kg	0.36	0.30	0.27	0.20	0.87			
TMP, 16 mg/kg	0.35	0.32	0.32	0.30	0.66	0.92		
TMP, 32 mg/kg	0.40	0.45	0.41	0.42	0.27	0.58	0.76	
Brain Mor, 16	0.21	0.07	0.00	0.04	0.11	0.27	0.23	−0.11

All ACT and TMP variables were calculated as differences from saline group scores (between groups) per strain. All correlations were based on 15 strain means and thus have $df = 13$: $r > 0.52$ were significant at the $p < 0.05$ level, and are underlined, while $r > 0.76$ were significant at the $p < 0.001$ level, and are double underlined. Correlations shown are for 0–30 min ACT; values for 25–30 min ACT were closely similar.

seen within each trait, indicating that the strains showing relatively high activity (or body temperatures) relative to saline at one dose tended to do so for the other doses as well. (The sole exception was the nonsignificant *r* between the 4 and 32 mg/kg doses for thermal responses.) The pooled genetic *r*s across doses within each trait were 0.82 for activity and 0.72 for thermal responses, indicating that the strain differences across doses for both activity and thermal responses are reasonably stable. In contrast to these high within-trait correlations, none of the correlations between traits was significant at any dose, indicating that the genetic influences on these two traits are largely independent. This suggests that these two traits have largely different genetic influences mediated by different mechanisms. In their studies in the rat, Dafters and Taggart (15) argue that these two responses are the result of independent underlying mechanisms with different time courses. Our results are fully consistent with their contention.

Following equivalent IP or SC doses of morphine in the 10–20 mg/kg range, C57BL/6J mice have been reported to show 70–80% higher plasma concentrations (22) and 40–70% higher brain concentrations of morphine than did DBA/2J mice (4, 8, 22). Thus, pharmacokinetic differences are quite evident for these two strains, although the mechanism (e.g., differential absorption, hepatic biotransformation) remains unknown. These findings prompted our inclusion of brain and plasma morphine determinations close to the time when the activity and thermal response measures were taken. However, correlations between drug concentrations and the morphine sensitivity measures were not significant across all 15 strains, indicating that pharmacokinetic contributions to the strain differences observed were too small to be detected. Because the range in blood or brain concentrations was less than 30% among the 15 strains, while the morphine sensitivity differences ranged over 600% for both ACT and TMP, we conclude that the strain differences seen were predominantly pharmacodynamic in origin.

In a morphine study using C57BL/6, DBA/2, and C3H mice tested at 15-min intervals for 90 min (4), the time of peak analgesia and hypothermia was at 30 min postinjection, but for activity, the peak morphine effect was at 30–60 min, depending on the strain. In a study similar to this one with 20 BXD RI strains (6), the peak thermal response and analgesic effects were seen at about 30 min, but activity peaked between 30 and 60 min postinjection at the same doses used in the present report. The range of strain differences was quite large in the BXD study, and approached that seen in the present study. One interpretational problem for this study, unlike the earlier studies, stems from the fact that we limited our data collection up to only 30 min postinjection which appears appropriate for the thermal response data, but this cutoff is before the time of peak locomotor activation in most strains, especially at the higher doses. This can be seen in Fig. 2a, b, and 2c. However, in the BXD study involving 20 strains, the correlation between activity at 30 min and activity much closer to the time of peak morphine effects was 0.95 at 8 mg/kg, 0.91 at 16 mg/kg and 0.85 at 32 mg/kg. These findings show that the strain rank orders were closely similar when the 30 min values were compared to values taken much closer to the peak morphine activity effects. Therefore, the strain differences reported here for activity must not be interpreted as the peak effect, although we presume it is highly correlated with the peak effect.

A biphasic response has often been reported as a function of time after a single dose of morphine; hypoactivity soon after injection, followed later by hyperactivity when brain drug levels are higher [e.g., (15)]. The time–effect curves for activity (Fig. 2a–c) do tend to support this pattern for those strains showing hypoactivity initially, in that many of them show hyperactivity, relative to saline values, later in test period. A more general pattern, however, even for strains that show hyperactivity at first, is for a gradual increase in activity to be seen in almost all strains as a function of time over the 30-min test period. In other studies, a biphasic response has also been seen for thermal responses; hyperthermia followed later in time by hypothermia [review: (1)]. However, this could not be assessed in the present study due to our use of only a single postinjection temperature determination to minimize possible confounding effects of stress associated with multiple rectal probes (3, 15). The use of radio telemetry would be ideal for biphasic response studies, because it eliminates the need to handle the animals, but at a practical level, this method is not well suited to testing hundreds of mice in a short space of time. However, we intend to conduct such studies on the more interesting strains identified, such as those showing the most marked biphasic responses in activity (CE, PL, SJL) compared to those that show little or no biphasicity (A, SWR), as shown in Fig. 2.

What are the presumed mechanisms underlying biphasic effects? A widely accepted hypothesis for thermal responses is that hyperthermia is due primarily to μ receptor activation, while hypothermia reflects κ receptor activation [review: (1)]. Because morphine is a largely a μ-preferring ligand, hyperthermic effects predominate at the lower morphine concentrations at the site of action, but is reversed at the higher concentrations when κ-mediated effects become prominent.

Screening relatively large numbers of inbred strains often leads to the identification of genotypes (strains) that possess useful and/or interesting characteristics. For example, the BALB/c, 129, C57BL/6, PL, and CBA strains were markedly activated in the open field by the higher doses of morphine. In contrast, the DBA/1, DBA/2, and A/He strains were consistently depressed by the same doses, and the C3H and CE strains were insensitive in either direction. For thermal responses, the CE and C3H strains were also remarkably resistant; the C3H strain in particular showed no significant morphine effect at any dose compared to saline. [However, the C3H strain appears to be normally sensitive to morphine analgesia, and shows normal whole brain naloxone binding (4).] In contrast, the A/He, C57L, and SWR strains were exquisitely sensitive to the hypothermic effects of even the lowest dose employed (4 mg/kg). These extreme responders immediately suggest several recombinant inbred (RI) sets or F_2 crosses derived from these strains to pursue quantitative genetic and chromosomal mapping studies [e.g., 6, 7], or to assess genetic correlations with neurochemical processes that may be etiologically related.

In summary, this study showed that morphine-induced activity and thermal responses exhibit linear dose–response relationships for most strains, and the strain differences are highly correlated across doses despite the bidirectional responses (some strains above saline values, some below) seen at some doses, indicating substantial genetic commonality throughout the dose range employed. However, it remains to be seen whether lower doses than 4 mg/kg might show a departure from this overall trend in some strains. Moreover, morphine activity and thermal responses are themselves not significantly genetically correlated, suggesting that they are determined by largely different genetically mediated mechanisms.

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