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Genetic Analysis of Hypothermia Induced by Morphine in Two Strains of Inbred Mice

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MURAKI, T. AND R. KATO. *Genetic analysis of hypothermia induced by morphine in two strains of inbred mice.* PHARMACOL BIOCHEM BEHAV 27(1) 87–91, 1987.—The degree of hypothermia elicited by morphine was greater in DBA/2 than C57BL/6 strain mice of both sexes. Hypothermia elicited by morphine was antagonized by naloxone in both strains of mice, suggesting the involvement of opioid receptors. To examine the role of genetic factors in the strain difference of morphine-induced hypothermia, the effect of morphine on changes in the rectal temperature was studied in the 6 generations of male mice, including the 2 inbred strains, P_1 (DBA/2) and P_2 (C57BL/6), their F_1 and F_2 hybrids, and 2 backcrosses, B_1 ($F_1 \times P_1$) and B_2 ($F_1 \times P_2$). The order of mean temperature decrease determined 40 min after 20 mg/kg morphine injection was $P_1 > B_1 > F_1 = F_2 > B_2 > P_2$. There was no maternal effect on the morphine responses of the F_1 generation. Biometrical analysis revealed that DBA/2 (P_1) is partially dominant over C57BL/6 (P_2) and contribution of polygenes was suggested.

Morphine Hypothermia Mouse Strain difference Genetic analysis

IT has been shown that morphine induces hypothermia in mice depending on the dose of morphine and the ambient temperature [4,14]. Many pharmacological effects of morphine differ among mouse strains, suggesting the contribution of genetic factors in the strain difference of morphine action [8,12]. In previous work, we showed that the hypothermic effect differs among 6 strains of male mice: DBA/2 was the most sensitive, whereas the rectal temperature of C57BL/6 strain decreased rather slightly by the administration of morphine [11]. The difference in the degree of morphine-induced hypothermia among the inbred strains suggests that the morphine responses are genetically determined. In the present study, we examined the effect of morphine on the rectal temperature in the parental strains of DBA/2 and C57BL/6 and their various crosses, and tried to find out which of the 2 inbred strains is dominant in terms of the hypothermic response to morphine and how many pairs of allelic segregating units differentiated between the 2 strains. A part of this study appeared in a preliminary form previously [9].

METHOD

Adult male mice of 8–10 weeks old were usually used. In one experiment (Fig. 1), adult female mice were also examined. The 2 inbred strains, DBA/2N (DBA or P_1), C57BL/6N (C57BL or P_2), and their F_1 generation were purchased from Charles River Japan, Atsugi. F_1 generation was produced by mating female C57BL mice with male DBA, unless otherwise stated. The female F_1 generation was crossed to each parental male strain, P_1 or P_2, to produce backcross generations, B_1 or B_2, and was crossed with male F_1 generation to establish the F_2 generation. Mice were housed in the animal room, which was maintained at 21–23°C and a 12-hour light-dark cycle (lights on at 6:00 a.m.).

Morphine HCl was purchased from Sankyo Co., Tokyo, naloxone HCl was a generous gift of Endo Labs, Inc., Garden City, NY. Drugs were dissolved in 0.9% saline and were administered in a volume of 10 ml/kg body weight subcutaneously. The dose of drugs was expressed as their salt.

All measurements of body temperature was done at 21–23°C between 10:00 a.m. and 4:00 p.m. Usually 6 animals were grouped in a cage, unrestrained except for temperature measurements, when they were held gently by the tail and a thermistor probe (KN-90, Natsume Seisakusho, Tokyo) was inserted 2 cm rectally. Each mouse was used only once. Body temperature was recorded at 10 min and immediately before injection and then at 10, 20, 30, 40, 50, 60, 75, 90, 105 and 120 min after the administration of drugs. The body temperature immediately before injection (at time 0) was taken as the basal and the change from the basal was used as the measure of drug effect on the body temperature.

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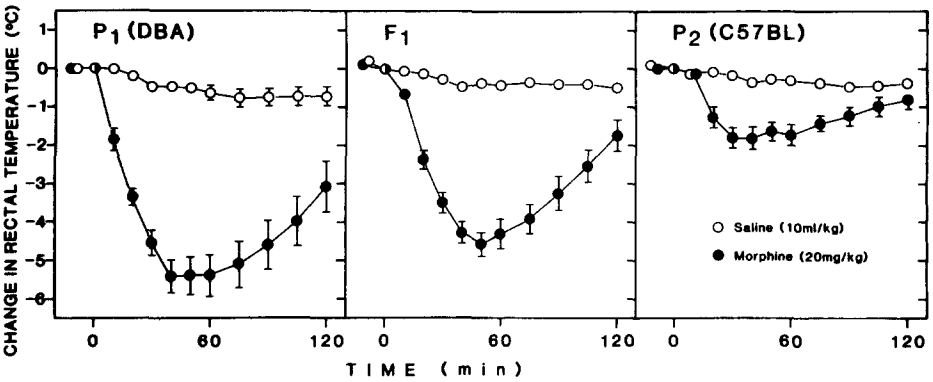

FIG. 1. Hypothermic effect of morphine in female mice. Female mice of DBA (P_1), C57BL (P_2) strains and their cross (F_1) were given saline (10 ml/kg) (open circles) or morphine (20 mg/kg) (filled circles) at time 0. Each point and vertical bar represent mean±SE of 8 mice. Body weight and basal rectal temperature of the pooled mice receiving saline and morphine (mean±SE) are: 23.0±0.4 g, 38.8±0.1°C for P_1; 22.9±0.4 g, 38.5±0.1°C for P_2; 32.4±1.0 g, 38.5±0.1°C for F_1.

TABLE 1

EFFECT OF MORPHINE AND NALOXONE ON THE RECTAL TEMPERATURE OF DBA AND C57BL STRAIN MALE MICE

	Strains	
	DBA	C57BL
Body weight (g)	21.3 ± 0.5 (18)	21.8 ± 0.3 (18)
Rectal temperature (°C) (basal)	37.1 ± 0.2 (18)	37.0 ± 0.2 (18)
Temperature changes (°C) induced by		
Saline (10 ml/kg)	1.40 ± 0.45 (6)	1.25 ± 0.39 (6)
Morphine (20 mg/kg)	−3.65 ± 0.48 (6)*	−0.95 ± 0.36 (6)*‡
Morphine (20 mg/kg) + naloxone (1 mg/kg)	1.50 ± 0.22 (6)†	0.83 ± 0.24 (6)†

Data are expressed as mean ± SE (number of mice). Body weights and the rectal temperature (basal) are the mean of the pooled mice that received saline, morphine, and morphine + naloxone. The basal rectal temperature was determined immediately prior to the subcutaneous administration of drugs. Temperature changes are those during the 30 min after the subcutaneous administration of drugs.

*$p<0.01$ vs. saline; †$p<0.01$ vs. morphine; ‡$p<0.01$ vs. DBA given morphine (Scheffé's test).

The results were evaluated by two-way ANOVA followed by Scheffé's test or by Student's t-test [3].

The biometrical examination of the morphine-induced hypothermia in the 6 populations of male mice, P_1, B_1, F_1, F_2, B_2 and P_2, was accomplished following the method of Bruell [1]. Briefly, an adequate scale was chosen which fulfills Mather's first and second criteria [6]. A measuring scale in which the following relationships hold ($A = 2B_1 - P_1 - F_1 = 0$; $B = 2B_2 - P_2 - F_1 = 0$; $C = 4F_2 - 2F_1 - P_1 - P_2 = 0$; P_1, P_2, F_1, F_2, B_1 and B_2 denote means of respective generations) was considered to fulfil Mather's criteria of adequate scale. We calculate A, B, C and their respective standard errors (SE). The relationships, A, B and C, are not considered to deviate from zero when ratios of A/SE_A, B/SE_B and C/SE_C are not significant. An adequate scale must satisfy Mather's second criteria that variances of P_1, F_1 and P_2 do not differ significantly among each other. Then the dominance was inferred from the relative positions of nonsegregating populations (P_1, P_2, F_1) on a measuring scale, and a measure of dominance ($D = d/a$) was calculated, where a is the distance between P_1 or P_2 and midparent (M) which is the position midway between the 2 parents, and d is the distance between F_1 and M. $D=0$ indicates that dominance is absent; $D=1$ or -1 indicates the complete dominance; $0<|D|<1$ indicates partial dominance. In order to calculate the number of segregating units or allelic gene pairs, k, the presence of a genetic component in the segregating populations (F_2, B_1, B_2) was confirmed by demonstrating that the variances of segregating populations ($s^2_{F_2}$, $s^2_{B_1}$, $s^2_{B_2}$) were significantly larger than environmental variance (s^2_e), which is the weighted

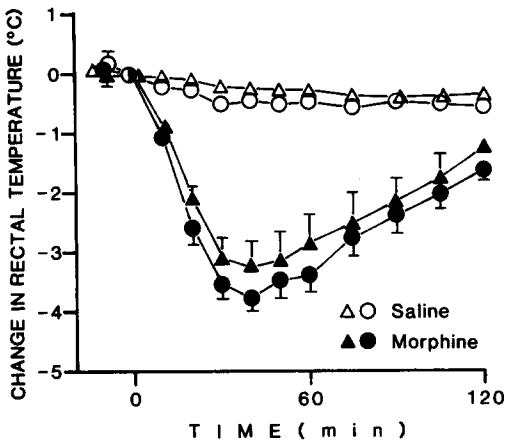

FIG. 2. Effect of morphine on the temperature changes in the reciprocal crosses (F_1) between DBA and C57BL strains. F_1 (D) (filled and open triangles) was obtained from the mating of C57BL females with DBA males, and F_1 (C) (filled and open circles) was from the mating of DBA females with C57BL males. Only the male filial mice were used and were given saline (10 ml/kg) (open symbols) or morphine (20 mg/kg) (filled symbols) subcutaneously at time 0. Each point and vertical bar represent mean±SE of 7–8 mice. Body weight and basal rectal temperature of the pooled mice receiving saline or morphine (mean±SE) are: 30.1±0.7 g, 39.0±0.1°C for F_1 (D); 34.2±0.2 g, 39.2±0.1°C for F_1 (C).

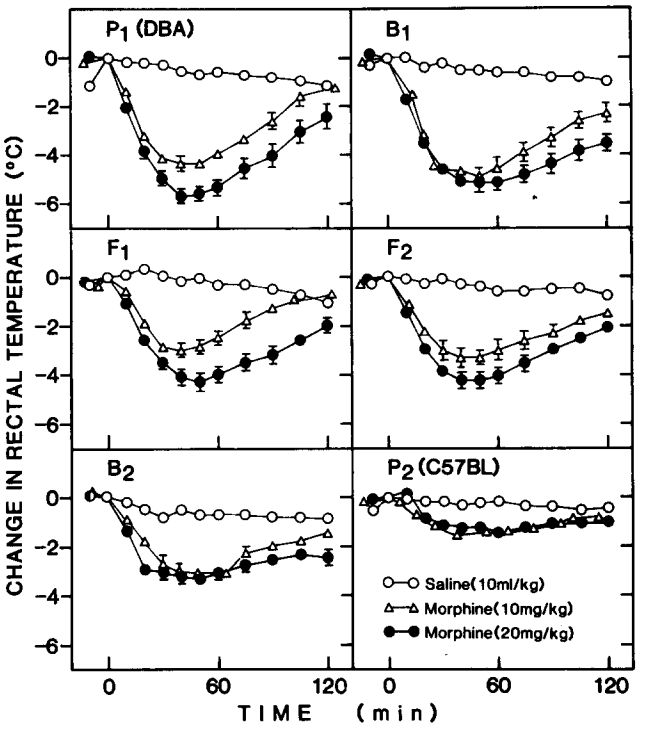

FIG. 3. Difference in the morphine-induced hypothermia in 6 populations of male mice. Male mice were given saline (10 ml/kg) (open circles) or morphine (10 mg/kg) (open triangles) or morphine (20 mg/kg) (filled circles) subcutaneously at time 0. Each point and vertical bar represent mean temperature change from basal±SE of 6–18 mice. Body weight and basal temperature (at time 0) of each population (pooled mice received 3 different treatments) (mean±SE) are: 23.5±0.6 g, 38.9±0.2°C for P_1 (n=25); 26.0±1.0 g, 39.2±0.1°C for B_1 (n=23); 26.5±0.2 g, 38.6±0.1°C for F_1 (n=25); 26.8±0.9 g, 39.1±0.1°C for F_2 (n=30); 24.0±0.4 g, 38.8±0.1°C for B_2 (n=27); 25.7±0.5 g, 38.2±0.1°C for P_2 (n=26).

mean of the variances of nonsegregating populations (P_1, P_2, F_1). The number of segregating units or allelic gene pairs, k, was calculated by the equation: $k_{F_2} = (2a^2 + d^2)/4 \times (s^2_{F_2} - s^2_e)$, $k_{B_1} = (a + d)^2/4 \times (s^2_{B_1} - s^2_e)$, or $k_{B_2} = (a - d)^2/4 \times (s^2_{B_2} - s^2_e)$.

RESULTS

Injection of 20 mg/kg morphine induced hypothermia in both DBA and C57BL strains. However, the effect of morphine was much greater in DBA strain than in C57BL mice, whereas the mean rectal temperature at the time of drug injection and the temperature changes 30 min after the saline injection were not different (Table 1). In both strains of mice, the hypothermia elicited by the subcutaneous administration of morphine (20 mg/kg) was antagonized by the simultaneous administration of naloxone (1 mg/kg), indicating the involvement of the opioid receptor. Two-way ANOVA of the temperature changes determined 30 min after the administration of drugs revealed a significant strain difference, $F(1,30)=4.32$, $p<0.05$, a significant drug action, $F(2,30)=61.42$, $p<0.01$, and a significant strain × drug interaction, $F(2,30)=12.02$, $p<0.01$. The latter result indicates that the 2 strains respond to the drugs differentially.

Injection of 20 mg/kg morphine induced hypothermia in the female mice of both strains and their F_1 hybrids (Fig. 1). In DBA strain, hypothermia reached a maximum of 4.28±0.30°C (mean±SE) 40 min after morphine injection and the body temperature did not return to the saline control levels until 2 hours thereafter. In C57BL mice, the same dose of morphine produced a decrease by 1.81±0.28°C at 40 min and the body temperature returned to the saline control levels in 2 hours. Consistent with the results with male mice (Fig. 3), a greater morphine-induced maximum hypothermia was seen in female DBA than female C57BL strain and the response of female F_1 hybrids resulting from the cross of C57BL females with DBA males was intermediate between the parents.

To examine the maternal effects on the morphine responses on the F_1 hybrids, we examined the effect of 20 mg/kg of morphine in the reciprocal F_1 hybrids (Fig. 2). The 2 reciprocal hybrids did not differ in the basal rectal temperature nor in changes after administration of saline or morphine. This indicates no maternal effect on the morphine response in F_1 generation.

These observations on the difference in the hypothermia elicited by morphine between the 2 inbred strains of mice of both sexes suggest the possible contribution of genetic factors to the strain difference. To investigate the role of genetic factors, we determined the effect of morphine on the rectal temperature of the 6 populations of male mice: parental strains, DBA (P_1) and C57BL (P_2), their F_1 and F_2 hybrids and backcrosses to P_1 and P_2 (B_1 and B_2) (Fig. 3). The rectal temperature of saline control mice of each population showed a slight tendency to decline in the course of investigation. The administration of morphine decreased the rectal temperature in the 6 populations of male mice and the nadir was found at around 40–60 min after morphine injection. The administration of 20 mg/kg morphine produced a greater hypothermia than 10 mg/kg in P_1, F_1 and F_2 generations,

TABLE 2

EFFECT OF MORPHINE ON THE CHANGES OF RECTAL TEMPERATURE IN 6 POPULATIONS OF MALE MICE

Populations	Parents (male × female)	Treatments: Saline (10 ml/kg)	Morphine (10 mg/kg)	Morphine (20 mg/kg)	s^{2*}
		Mean ± SE (n) (°C)			
P_1 (DBA)	$P_1 \times P_1$	−0.57±0.14 (7)	−4.39±0.33 (7)	−5.70±0.30 (11)	1.0145
B_1	$P_1 \times F_1$	−0.53±0.15 (6)	−4.73±0.22 (6)	−5.12±0.29 (11)	0.8342
F_1	$P_1 \times P_2$	−0.15±0.18 (6)	−3.02±0.29 (9)	−4.26±0.21 (10)	0.4064
F_2	$F_1 \times F_1$	−0.28±0.08 (6)	−3.33±0.42 (6)	−4.25±0.33 (18)	1.8291
B_2	$P_2 \times F_1$	−0.47±0.13 (6)	−2.95±0.30 (6)	−3.24±0.30 (15)	1.2410
P_2 (C57BL)	$P_2 \times P_2$	−0.40±0.12 (7)	−1.58±0.17 (8)	−1.06±0.28 (11)	0.6641

Temperature changes 40 min after the administration of saline or morphine were reproduced from Fig. 3.
*Variance of temperature changes elicited by 20 mg/kg morphine.

TABLE 3

MATHER'S SCALING TESTS AND SUMMARY OF DATA NEEDED FOR GENETIC ANALYSIS OF HYPOTHERMIA DETERMINED 40 MIN AFTER ADMINISTRATION OF 20 mg/kg MORPHINE

Mather's Scaling Test 1

$A \pm SE_A$ $(A/SE_A) = -0.28 \pm 0.661$ (0.423)*
$B \pm SE_B$ $(B/SE_B) = -1.16 \pm 0.657$ (1.765)*
$C \pm SE_C$ $(C/SE_C) = -1.72 \pm 1.393$ (1.234)*
*$p>0.05$ (t-test)

Mather's Scaling Test 2

$s^2_{B_1}/s^2_{F_1}$ $F(10,9)=2.496$ ($p>0.05$)
$s^2_{B_1}/s^2_{F_2}$ $F(10,10)=1.527$ ($p>0.05$)

Data for Genetic Analysis

$a=2.32$ $d=-0.88$ $D\,(=d/a)=-0.38$ $s^2_e=0.7040$

$s^2_{B_1}/s^2_e$ $F(10,30)=1.18$ ($p<0.05$);
$s^2_{B_2}/s^2_e$ $F(14,30)=1.72$ ($p>0.05$);
$s^2_{F_2}/s^2_e$ $F(17,30)=2.59$ ($p<0.05$).

although the dose difference was not clear in B_1, B_2 and P_2 generations. The maximum decrease in the rectal temperature elicited by 20 mg/kg dose of morphine was again larger in DBA (P_1) strain than C57BL (P_2) and, as in the case of female mice, the hypothermia found in the F_1 generation was intermediate. The degree of morphine-induced hypothermia of B_1 was less than P_1, and that of F_2 was almost the same as F_1. The hypothermic response to morphine in B_2 population was intermediate between F_1 and P_2. A similar relationship of the intensity of hypothermia among the generations was also found in the mice treated with 10 mg/kg morphine. Distribution pattern of the temperature changes 40 min after the administration of 20 mg/kg of morphine in the individual mouse of segregating populations (B_1, B_2 and F_2) did not show a clear segregation, excluding the possible contribution of a single codominantly expressed genetic factor (data not shown). We, therefore, tried to analyse the mean temperature changes of the 6 populations biometrically (Table 2).

For the biometrical analysis we have chosen the temperature changes determined 40 min after drug administration, because the maximum decrease was frequently observed around that time. For the temperature changes of 6 populations of mice treated with 20 mg/kg morphine, both criteria of Mather for an adequate scale [6] were met by the raw scale (Table 3), therefore, the raw scale (without transformation of data) was used for further analysis of the result. Two-way ANOVA revealed a significant difference by drugs, $F(2,138)=213.9$, $p<0.01$, a significant difference by populations, $F(5,138)=37.5$, $p<0.01$, and a significant drug × population interaction, $F(10,138)=7.75$, $p<0.01$. These results suggest that the genotype as well as the dose of morphine determines the intensity of hypothermia and that the generations of mice differ in the sensitivity to morphine. Although the temperature changes did not differ among the populations after the administration of saline, $F(5,32)=1.36$, $p>0.05$, there were significant strain differences among populations after the injection of both 10 and 20 mg/kg of morphine, $F(5,36)=15.21$, $p<0.01$, for 10 mg/kg; $F(5,70)=25.30$, $p<0.01$, for 20 mg/kg. Since the effect of 20 mg/kg morphine was more marked than that of 10 mg/kg, we examined the temperature changes elicited by 20 mg/kg morphine biometrically as shown in Table 3. The distance (a) between P_1 or P_2 and midparent (M), which is the position midway between the 2 parents, was 2.32, and the distance (d) between F_1 and M, was −0.88. The measure of dominance (D = d/a) was −0.38, suggesting that DBA (P_1) shows a partial dominance over C57BL (P_2).

Among the variances of segregating populations, the variance of F_2 ($s^2_{F_x}$) was significantly larger than the environmental variance (s^2_e) suggesting that $s^2_{F_2}$ contains a genetic component. The number of allelic segregating units estimated from variance of F_2 (k_{F_2}) was 2.56, suggesting that morphine-induced hypothermia is a character controlled by not less than 3 segregating units.

DISCUSSION

We confirmed our previous finding that the decrease in the rectal temperature elicited by morphine in DBA strain

male mice was larger than that found in C57BL strain [11]. We found that there was no sex diffference in the strain difference in morphine-induced decrease in body temperature. The hypothermia elicited by morphine was antagonized by naloxone in these strains of mice in agreement with the previous report with ICR strain [15]. The DBA strain was more sensitive than C57BL to the hypothermic effect of FK33-824, a synthetic opioid peptide, however no strain difference was observed in the degree of hypothermia elicited by oxotremorine (manuscript in preparation). These results suggest that the strain difference in the drug-induced hypothermia may be specific to opioids probably due to the difference in the opioid receptors between the 2 strains. Previously, we found much more naloxone binding in the striatum of DBA strain than that of C57BL strain [11], although the implication of this result in the strain difference in morphine-induced hypothermia is not clear.

We showed that the intensity of the morphine-induced hypothermia of the F_1 hybrids was intermediate between the 2 parental strains in the experiments with either sex and that the maternal effects did not change the hypothermic response of the F_1 hybrids. Biometrical analysis of the morphine responses in the nonsegregating populations revealed that the DBA (P_1) strain mouse is partially dominant over C57BL (P_2) strain. Although we did not demonstrate genetic variation in the B_1 and B_2 populations, the k value obtained from F_2 generation showed the contribution of not less than 3 allelic segregating units, suggesting the role of polygenes in the inheritance of morphine-induced hypothermia.

The results on the morphine-induced hypothermia in DBA and C57BL strains were similar to the genetics of the morphine-induced increase in plasma cyclic nucleotides in that DBA strain is partially dominant over C57BL strain mice [10]. However, this is in contrast to the genetics of the motor effect of morphine, where C57BL strain was partialy dominant over DBA, although the contribution of the multiple allelic pairs of genes was similarly inferred [2,10]. Thus, our study revealed the difference between DBA and C57BL strains in sensitivity to the hypothermic and locomotor effects of morphine as well as the dominance.

Recently, the concept of multiple opiate receptors has been established [5]. It is suggested that a specific effect of opioids may be mediated by different opioid receptors at different central nervous system sites [13]. It is considered that the hypothermic effect of morphine is mediated by its interactions with mu_1 sites [13], whereas mu_1 or mu_2 sites or both may be involved in the locomotor excitatory response to morphine [7]. Therefore, the strain difference in morphine responses between DBA and C57BL may be related to the difference in the amounts of opioid receptor subtypes in the specific brain areas which mediate these morphine effects. Further work is needed to determine the subtypes of opioid receptors and the sites of action of opioids which mediate the hypothermic and locomotor effects of opioids, and also, to demonstrate the differential distribution of opioid receptor subtype bindings in the central nervous system of DBA and C57BL strains.

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