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Inheritance of Amphetamine-Induced Thermoregulatory Responses in Inbred Mice

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SEALE, T. W., J. M. CARNEY, P. JOHNSON AND O. M. RENNERT. *Inheritance of amphetamine-induced thermoregulatory responses in inbred mice.* PHARMACOL BIOCHEM BEHAV **23**(3) 373–377, 1985.—Two inbred strains of mice, DBA/2 and C57BL/6, differ in their responses to d-amphetamine-induced alteration of core temperature. At low doses of amphetamine (e.g., 2 mg/kg IP), both strains become markedly hypothermic within 10–20 minutes. High doses (e.g., 20 mg/kg IP) induce significant hyperthermia (+1.8°C) in DBA/2 mice but have only a slight hyperthermic effect (+0.2–0.3°C) effect on C57BL/6 mice. The phenotype of the F_1 hybrid strain derived by crossing C57BL/6 by DBA/2 is indistinguishable from its C57BL/6 parent at a dose of 20 mg/kg IP, i.e., reduced responsiveness to amphetamine-induced hyperthermia is dominant. Analysis of the thermoregulatory responses of recombinant inbred derivatives (lines BXD-9, 11, 15, 19, 20, 21, 23, 27, 28, 30) suggest that the relative responses to amphetamine-induced hyperthermia is inherited in a simple Mendelian fashion. These results differ from other pairs of inbred mouse strains which have been compared. These findings identify yet another neuropharmacological difference between mouse strains C57BL/6 and DBA/2 and are reviewed in terms of neuroregulatory mechanisms effecting thermoregulation.

Amphetamine Thermoregulation Hyperthermia Catecholamines Dopamine Pharmacogenetics Inbred mice Recombinant inbred mice

AMPHETAMINE exerts its behavioral effects, in part, through an increased release of catcholamines from neuronal storage vesicles [2,13]. Dopamine rather than norepinephrine is associated with the stereotyped behavior and locomotor activity stimulation induced centrally by this drug [6,11]. In rodents, low doses of d-amphetamine produce hypothermia but higher doses produce hyperthermia [8,15]. Hypothermia induced by IP or ICV d-amphetamine has been attributed to a central mechanism [8, 12, 19] which appears to be mediated by released dopamine [12,19]. In contrast, the hyperthermia induced by amphetamine appears to be a peripheral effect resulting from the oxidation of free fatty acids which are mobilized when norepinephrine is released by d-amphetamine from sympathetic nerve endings in adipose tissue [4, 7, 15]. Propranolol blocks this effect [7,14], a finding which implicates a *beta*-adrenergic mechanism.

Two inbred strains of mice, C57BL/6 and DBA/2, are known to be inherently different from one another in their number of central dopamine receptors [3]. These strains also differ from one another in their central response to dopamine agonists as measured by locomotor activity changes and stereotyped behavior following drug administration [16,18]. Behaviors determined by different brain regions are differentially affected [16]. It was therefore of interest to determine whether the thermomodulatory effects of d-amphetamine would differ significantly between the two strains. The relative deficit of central dopamine receptors in DBA/2 might be expected to cause these mice to be refractory to amphetamine-induced hypothermia. Recently we observed that DBA/2 mice are refractory to caffeine-induced hypothermia when compared to C57BL/6 mice [17]. We now report that an intrinsic difference in d-amphetamine-induced modulation of core temperature does occur between these strains, but that this difference resides in the reduced magnitude of the *hyperthermia* occurring in C57BL/6, not in a decrease in the hypothermia induced in DBA/2. By the use of recombinant inbred lines, the inheritance of d-amphetamine responsiveness is shown to differ from the inheritance of susceptibility to caffeine-induced hypothermia in this pair of strains [17] and from previously described differences in d-amphetamine response differences between BALB/c and C3H inbred mice [10].

METHOD

Subjects

Male mice of inbred strains C57BL/6J, DBA/2J, their F_1 hybrid B6D2F1, and recombinant inbred strains BXD-9, 11, 15, 19, 10, 21, 23, 27, 18 and 30 (Jackson Laboratory, Bar Harbor ME) were housed in groups of 6 animals per cage on a continuous 12 hr light-dark cycle under constant temperature and humidity. Animals were approximately 8 weeks of age. Animals were used only once, i.e., for a single administration of d-amphetamine or carrier. The litter was kiln dried

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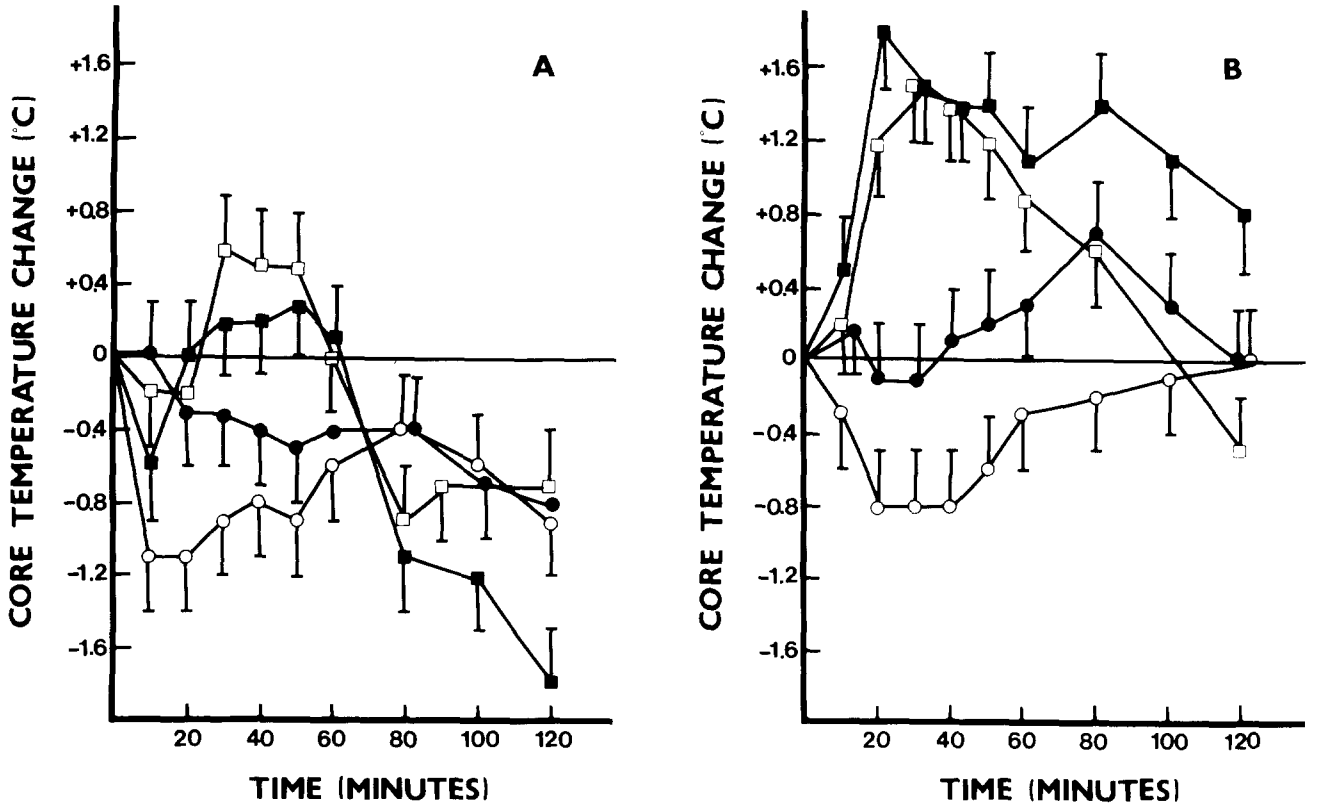

FIG. 1. Time and dosage dependence of d-amphetamine-induced alteration in core temperature in two strains of inbred mice. A. Response of C57BL/6 mice. B. Response of DBA/2 mice. Individual animals (n=6 for each dosage) were self compared. Values represent mean core temperature±S.E.M. (●) 0.5 mg/kg IP) (○) 2.0 mg/kg IP; (□) 10.0 mg/kg IP; (■) 20.0 mg/kg IP. Response at 5 mg/kg IP is not shown to reduce the complexity of the figure.

aspen wood chips (Sani-Chips, P. J. Murphey). Free access to a standard rodent pellet feed (Lab/Blox, Wayne) and water was given.

Procedure and Apparatus

Amphetamine-induced changes in core temperature were determined in the following way. Unrestrained animals were put singly into standard plastic mouse cages (29×18 cm) without litter for 30 minutes prior to d-amphetamine administration. Room temperature was maintained at 21.5±0.5°C during the course of the experiments. Rectal temperatures were determined immediately prior to drug administration to establish basal values and at 10 or 20 minute intervals following drug injection. A freshly prepared d-amphetamine solution (d-amphetamine sulfate, NIDA Psychomimetics Committee) in physiological saline was administered IP in a fixed volume to body weight ratio (0.2 ml/20 g) regardless of the dosage administered. For each experimental and control determination, six mice were used. Rectal temperature was measured with a YSI-44TA tele-thermometer (Yellow Springs Instrument Co.). The termister probe (YSI-402), lubricated with vaseline, was inserted 2.6 cm into the rectum, and temperature was recorded when a stable value was achieved (i.e., about 30 seconds after insertion of the probe).

RESULTS AND DISCUSSION

Dosage and Time Dependence of Amphetamine-Induced Changes in Core Temperature

Figure 1 shows in both C57BL/6 and DBA/2 strains of mice that the time course and dosage dependence of d-amphetamine-induced alteration of core temperature are complex. At low doses (2 mg/kg IP) C57BL/6 mice become hypothermic within 10 minutes after drug administration and remain so for 60 minutes or longer (Fig. 1A). Hypothermia induced by the 2 mg/kg dose is statistically different ($p<0.01$) from the saline control for 60 minutes whereas the lower dose (0.5 mg/kg IP) was not. In DBA/2 (Fig. 1B) the effect of the 2 mg/kg IP dose was similar to that found in C57BL/6 mice, although the magnitude of the maximal hypothermia was somewhat less (−0.8°C versus −1.1°C, respectively).

In DBA/2 mice (as in C57BL/6 mice), 0.5 mg/kg IP had no significant effect compared to the saline-injected animals (saline-injected control data not shown). At 5 mg/kg IP, DBA/2 mice became slightly hyperthermic (+0.7±0.3°C) compared to controls but C57BL/6 mice remained hypothermic (−1.3±0.3°C). These data can be interpreted in two ways: (1) the *hypothermic* response of DBA/2 is blunted compared to C57BL/6, or (2) the *hyperthermic* response of C57BL/6 is reduced compared to that of DBA/2.

At even higher amphetamine doses (10 or 20 mg/kg IP) the

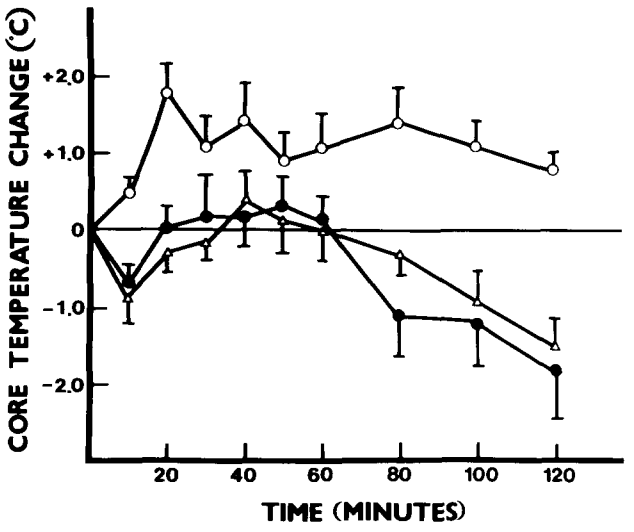

FIG. 2. Comparison of d-amphetamine-induced changes in core temperature in strains DBA/2, C57BL/6 and their F_1 hybrid strain B6D2F1. d-Amphetamine (20 mg/kg IP) was administered at t_0 and core temperature was determined over a 2 hour interval. Individual animals (n=6 for each strain) were self-compared. Values represent mean core temperature±S.E.M. (○) DBA/2 mice; (●) C57BL/6 mice; (△) B6D2F1 mice.

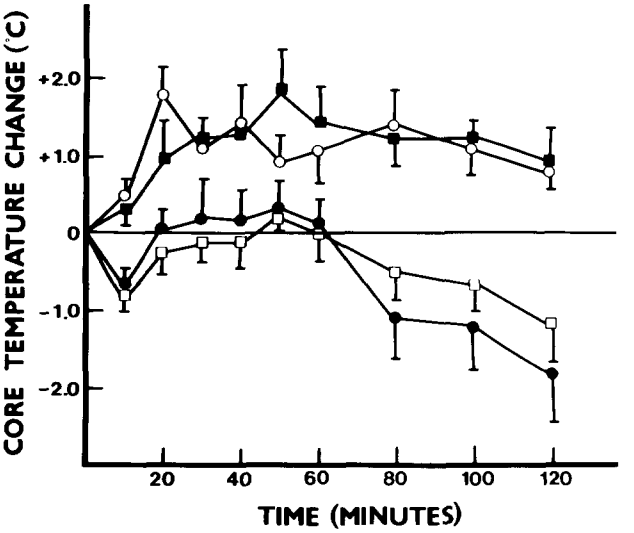

FIG. 3. Comparison of d-amphetamine-induced changes in core temperature in strains DBA/2, C57BL/6 and two recombinant inbred lines, BXD-30 and BXD-21. d-Amphetamine (20 mg/kg IP) was administered at t_0 and core temperature was determined over a 2 hour interval. The two BXD lines depicted here are representative of the responses of the other 8 recombinant inbred lined derived from crosses of the DBA/2 and C57BL/6 progenitor strains. Individual animals were self-compared. Values represent mean core temperature±S.E.M. (○) DBA/2 mice; (●) C57BL/6 mice; (■) BXD-30 mice; (□) BXD-21 mice.

thermoregulatory responses of the two strains are still readily distinguishable from one another. At both doses DBA/2 mice become significantly hyperthermic ($p<0.01$) with a maximum temperature increase (+1.5 and +1.8°C respectively) being achieved 20 to 30 minutes post drug administration. In contrast, C57BL/6 mice become significantly hypothermic ($p<0.05$ compared to control; $p<0.01$ compared to DBA/2) 10 minutes after a dose of 20 mg/kg IP and subsequently become only modestly hyperthermic (+0.2–+0.6°C) 20 to 30 minutes after these doses. Significant hyperthermia is maintained for at least 80 minutes post injection in DBA/2 but the core temperature of C57BL/6 mice returns to basal level by 60 minutes. Thus, the amphetamine-induced hyperthermic response is blunted in C57BL/6 mice compared to DBA/2 mice, and its duration appears to be reduced. Amphetamine effects that we observed in DBA/2 mice are similar to those seen in other mouse strains (e.g., in Swiss Webster CF-1 [15], NMRI [5] and BALB/c [10] mice) while C57BL/6 more closely resembles the hyporesponsiveness reported to occur in C3H [5,10].

Core Temperature Changes Induced by d-Amphetamine in the F_1 Hybrid Strain, B6D2F1, and Recombinant Inbred Strains Derived from C57BL/6 and DBA/2 Strains

Figure 2 compares the effect of d-amphetamine administration (20 mg/kg IP) on core temperature in F_1 hybrid mice (strain B6D2F1) to the effect of this dose of d-amphetamine on its two parental strains. The time course and magnitude of change in core temperature of the F_1 hybrid mice are indistinguishable from their C57BL/6 parent but clearly distinguishable from their DBA/2 parent at this dose. This result clearly demonstrates that the blunted hyperthermic response of C57BL/6 is dominant to the more profound hyperthermic response elicited in DBA/2 mice.

To determine whether the difference in the magnitude of the response to d-amphetamine-induced hyperthermia between C57BL/6 and DBA/2 is under the control of a single gene with two different alleles or is determined by more complex (polygenic) mechanisms, we examined the effect of d-amphetamine administration (20 mg/kg IP) on core temperature in 10 recombinant inbred lines. Recombinant inbred lines are derived by line breeding, initiated from F_1 hybrid individuals, for at least 20 generations. If a phenotypic trait is determined by more than one gene, this breeding regimen allows recombination of the various contributing genes into new, but obligately homozygous, configurations. Thus, new and/or intermediate phenotypes can arise from newly derived genetic combinations of two or more underlying gene differences. If the phenotypic differences of the progenitor strains reflect a single gene difference encoded by a single pair of alleles, only the two parental phenotypes will occur among the recombinant inbred lines.

The two types of responses found in the BXD recombinant inbred lines are exemplified by two lines, BXD-30 and BXD-21, shown in Fig. 3. The response of one line, BXD-30, closely resembles the thermoregulatory effect of d-amphetamine on its DBA/2 progenitor while that of BXD-21 is similar to its C57BL/6 progenitor. Maximal hyperthermia induced within 1 hour following d-amphetamine administration in each of the 10 BXD lines is summarized in Fig. 4 and is compared to C57BL/6 and DBA/2 responses. Only two phenotypic classes emerge, one in which the temperature response of each BXD line is statistically indistinguishable from its DBA/2 progenitor (composed of lines, BXD-9, 17,

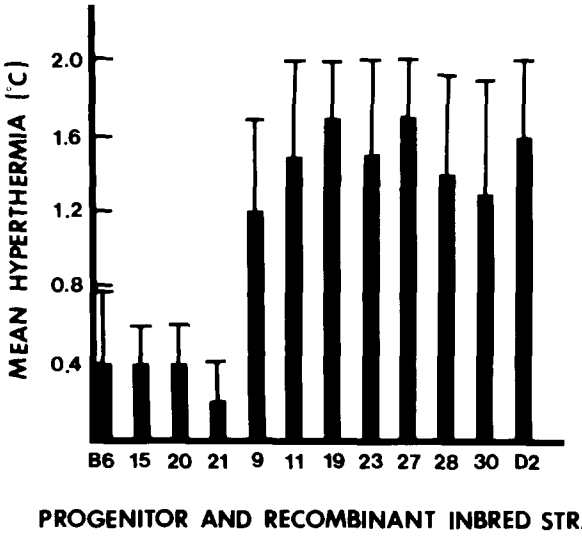

FIG. 4. Changes in core temperature in response to d-amphetamine (20 mg/kg IP) administration in DBA/2 and C57BL/6 progenitor strains compared to 10 recombinant inbred (BXD) derivative lines. B6=C57BL/6; D2=DBA/2; the numbers represent individual recombinant inbred lines, i.e., 30=recombinant inbred line BXD-30. Values represent the mean maximal increase in core temperature±S.E.M. within a 60 minute period post dosing.

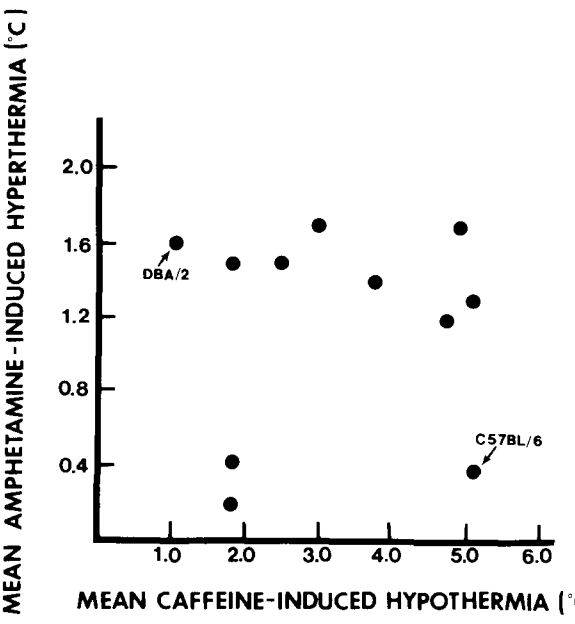

FIG. 5. Lack of correlation of amphetamine-induced maximal hyperthermia with caffeine-induced maximal hypothermia in C57BL/6, DBA/2 and 9 derived recombinant inbred (BXD) lines. Each point represents a strain shown in Fig. 4. One BXD strain has been omitted because its susceptibility to caffeine was not examined. Data on caffeine responsiveness of these strains was taken from Seale *et al.* [14]. r=−0.05.

19, 23, 27, 28, 30] and a second, the response of which is not statistically different from its C57BL/6 progenitor (composed of *3* lines, BXD-15, 20 and 21). The two classes are significantly different from one another ($p<0.01$). No obviously intermediate phenotypic responses were found. We interpret these data to mean that the difference in d-amphetamine-induced hyperthermia between C57BL/6 and DBA/2 mice is controlled by a single gene.

Our data suggest that DBA/2 and C57BL/6 mice differ from one another by a simply determined inherited difference in some component of the thermoregulatory mechanisms affected by d-amphetamine. Because the hyperthermic arm of the amphetamine-induced effects seem differentially reduced in C57BL/6, this difference (amphetamine hyporesponsive) probably results from a reduction in the efficacy of the peripheral, norepinephrine-related thermogenic mechanism [4]. Alternatively, a difference in compartmentation or catabolism of amphetamine might account for the difference in responsiveness between C57BL/6 and DBA/2. The observation that d-amphetamine-induced hyperthermia in C57BL/6 is comparable in magnitude and duration to that in DBA/2 at low doses argues against increased catabolism of d-amphetamine in C57BL/6. These two strains of mice also are reported to differ in their response to d-amphetamine-induced enhancement of locomotor activity. DBA/2 mice are stimulated to a much greater extent than are C57BL/6 mice [1]. The mechanism(s) underlying this latter difference between strains is unknown, and it remains to be established whether such differences are correlated phenotypically, genotypically and mechanistically with the differences in d-amphetamine-induced thermomodulation.

Another pair of inbred mouse strains, BALB/c and C3H, also differ from one another in d-amphetamine-induced changes in core temperature [10]. The hyporesponsiveness of C3H does not result in a difference in compartmentation or catabolism of d-amphetamine in this strain [9]. A conventional genetic analysis examining responsiveness in F_1, F_2 and backcross generations [10] indicated that the sensitivity to d-amphetamine-induced hyperthermia was controlled by several genes. This finding suggests that the d-amphetamine hyporesponsiveness of C3H and C57BL/6 mouse strains is determined by different genes.

It is of interest that DBA/2 and C57BL/6 mice also differ in the effect of caffeine administration on core temperature [17]. High doses of caffeine (50 mg/kg IP) induce marked hypothermia in C57BL/6 but have little effect on DBA/2 mice. Caffeine induced hypothermia in strain B6D2F1, hybrid. This finding indicates that susceptibility to caffeine-induced depression of core temperature is dominant to non-responsiveness. Similarly, B6D2F1 shows that susceptibility of d-amphetamine-induced hypothermia (at a high dose) is dominant to the capacity of this drug to induce hyperthermia. This similarity in phenotypic dominance might suggest a common genetically altered mechanism underlying the pharmacologically-induced thermoregulatory differences between DBA/2 and C57BL/6. However, our data suggest that the d-amphetamine-induced effects on core temperature are determined by a genetically distinct mechanism from that underlying the caffeine effects. A single gene appears to determine the difference in temperature response to d-amphetamine in these two strains, but the differences in caffeine-induced alteration of core temperature are determined in a genetically more complex manner [17]. The corre-

lation of the maximal hyperthermic responses induced by d-amphetamine with the maximal hypothermia induced by caffeine in the 10 BXD lines and their progenitors is examined in Fig. 5. Inspection of Fig. 5 suggests that there is no obvious relationship between the genetic determinants of susceptibility to caffeine-induced hypothermia and d-amphetamine hyperthermia. The correlation coefficient was low ($r = -.05$).

Further studies are needed to establish the neurochemical and physiological mechanisms underlying the inherited difference in amphetamine responsiveness between C57BL/6 and DBA/2 mice which we have identified in this report.

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