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Complex Genetic Determinants of Susceptibility to Methylxanthine-Induced Locomotor Activity Changes

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SEALE, T. W., T. H. RODERICK, P. JOHNSON, L. LOGAN, O. M. RENNERT AND J. M. CARNEY. *Complex genetic determinants of susceptibility to methylxanthine-induced locomotor activity changes.* PHARMACOL BIOCHEM BEHAV **24**(5) 1333–1341, 1986.—The intent of this study was to investigate the role of inheritance in the determination of susceptibility to methylxanthine-induced behavioral changes. Two strains of inbred mice, SWR and CBA, which differ significantly in their response to caffeine- and theophylline-induced stimulation of locomotor activity, were used in classical genetic crosses to produce reciprocal F_1 hybrids, reciprocal backcross progeny and F_2 progeny. Theophylline dose reponse curves in the reciprocal F_1 hybrid strains were identical to each other and to their methylxanthine-responsive (CBA) parent. These results indicated that theophylline responsiveness behaved as a simple autosomal dominant trait. Behavioral responses of these F_1 hybrid strains to caffeine showed the same maximal enhancement of locomotor activity as their CBA progenitor at a dose 10 mg/kg IP, but locomotor activity stimulation also occurred at 32 mg/kg IP, a dose which inhibited their CBA parent. These data suggest that the genes specifying caffeine responsiveness differ from those encoding theophylline responsiveness. For both caffeine and theophylline, behavioral phenotypes and their expected frequencies of occurrence among backcross and F_2 progeny differed significantly from the segregation ratios expected for a trait determined by a single gene. These non-Mendelian segregation ratios suggest that locomotor activity stimulation by both of these methylxanthines is polygenically determined. It was anticipated that the same genetically encoded neurochemical mechanism would underlie the difference in behavioral response to the two methylxanthines. However, no significant correlation between caffeine-induced and theophylline-induced stimulation of locomotor activity was observed among progeny derived from backcrosses or F_1 self-crosses. These data establish that the behavioral effects of methylxanthines on locomotor activity levels are inherited in a complex manner and that, at least in these two strains of inbred mice, different genetic or genetically encoded neurochemical mechanisms underlie the behavioral effects of caffeine and theophylline.

Caffeine Theophylline Locomotor activity Inbred mice Behavior genetics Polygenic inheritance

THE methylxanthines, caffeine (1,3,7-trimethylxanthine) and theophylline (1,3-dimethylxanthine) are two of the most widely consumed and clincially employed psychoactive compounds. The dosage-dependent ability of these compounds to stimulate the activity of the central nervous system results in a variety of behavioral changes in animals including stimulation or inhibition of locomotor activity [19, 36, 41], alteration of differential reinforcement of low rate responding (DRL) performance [43], changes in core temperature [6, 33, 35], loss of righting ability, clonic seizures, tonic seizures and death [36,37]. In addition to their bronchodilating and antisoporific effects in man, caffeine and theophylline have other potent central nervous system (CNS) and peripheral effects and can induce diuresis, diarrhea, tachycardia, cardiac arrhythmias, insomnia, withdrawal headaches, anxiety, seizures and death [1, 11, 30, 31, 42, 45]. These manifestations of methylxanthine intake vary markedly among individuals [7, 11, 14, 15, 39, 42]. While various medical and environmental conditions as well as ethnic origin contribute to inter-individual variation in pharmacodynamics of caffeine and theophylline [16, 22, 26, 28], the variation in plasma levels frequently does not explain the heterogeneity of individual responses to these drugs [6, 11, 18, 39, 40]. It remains to be established whether such differences in man reflect intrinsic alterations in neuropharmacological responsiveness of the central and peripheral nervous systems and whether such differences, if they occur, are acquired or inherited.

To investigate the genetic determinants responsible for variation in responsiveness of the CNS to methylxanthines,

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we have used inbred mouse strains as a model for neuropharmacogenetic studies of these compounds in mammals. When several different screening procedures expected to detect qualitatively distinct effects of methylxanthines on the CNS were carried out on seven inbred strains of mice, marked variations in responsiveness to caffeine were identified among these strains [36]. This variation in behavioral responsiveness to caffeine extends to other methylxanthine including paraxanthine and theophylline [24]. Two strains of inbred mice, SWR and CBA, hold special interest for these studies because they differ significantly from one another in their susceptibility to several methylxanthine-induced behavioral changes including alteration of locomotor activity, induction of hypothermia, seizure induction and stress potentiated lethality [6, 24, 36, 37]. For several reasons these differences in response to methylxanthines cannot be simply explained by pharmacodynamic changes in methylxanthine handling between the strains: (1) no differences in blood levels of caffeine occur between the strains at doses which elicit marked behavioral differences between them [6]; (2) no differences in theophylline excretion has been identified between them [4]; (3) the relative dose-dependent response of one behavioral trait does not predict the response of a second trait, e.g., CBA mice are hyposensitive to caffeine-induced hypothermia compared to the SWR strain [6] but significantly more sensitive than SWR mice to caffeine-induced seizures [36,37]. The relative susceptibility to caffeine-induced lethality, a behavioral marker related to tonic seizure induction, appears to be determined by an autosomal dominant single gene difference between these strains [37].

In the present study we have extended this genetic investigation of the determinants of behavioral sensitivity to methylxanthines in CBA and SWR inbred mice to the following questions: (1) What is the mode of inheritance of the difference in susceptibility to theophylline-induced and caffeine-induced enhancement of locomotor activity levels? (2) Are identical genetic determinants responsible for coincident differences in responsiveness to caffeine and theophylline? Here we report that different genes encode sensitivity to methylxanthine-induced lethality and stimulation of locomotor activity. Further, the lack of correlation between caffeine and theophylline responsiveness in backcross and F_2 progeny suggests that different genes encode the relative behavioral susceptibility to these two closely structurally related methylxanthines.

METHOD

Animals

Ten to fifteen week old male mice of strain CBA/J and SWR/J, obtained from Jackson Laboratory, Bar Harbor, ME, were housed in groups of six animals per cage on a continuous 12 hr light-dart cycle under constant temperature (21–23°C). Throughout this study we examined the effects of methylxanthines only in male animals. Because of the lack of recombinant inbred strains from these two progenitor strains, conventional crosses were used for the genetic analysis. Progeny of crosses were tested for behavioral responsiveness at the same age as used to test their CBA and SWR progenitors. $CBSWF_1$ and $SWCBF_1$ hybrid strains were obtained from reciprocal crosses of the two progenitor strains. Male F_2 progeny were obtained from self-crosses of F_1 hybrid females to F_1 hybrid males. Backcross progeny were obtained by crossing F_1 hybrid females to parental males from each strain. The litter used was kiln dried hardwood (Sani-Chips, P. J. Murphey). Free access to a standard rodent pellet food (Lab/Blox, Wayne) and water were given.

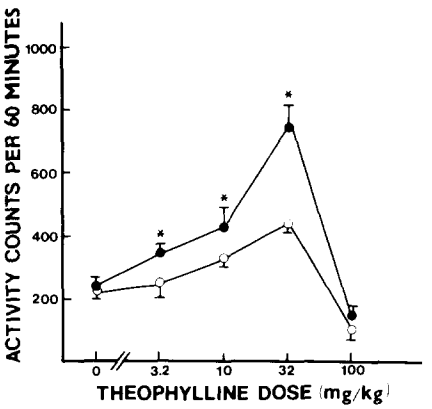

FIG. 1. Dose dependence of theophylline-induced stimulation of locomotor activity in CBA and SWR inbred mice. Animals (n=6 for each dose) received an IP injection of drug at the time that measurement of locomotor activity was initiated. Each point represents the response of an individual animal activity. Values presented in this and subsequent figures are total activity values post dosing. (○) SWR, (●) CBA, responses to theophylline differ significantly from one another in the two strains at dose indicated by (*).

Drug Administration

Methylxanthine dosages and time courses used in these studies were determined empirically in preliminary experiments based on our previous experience with the effects of these drugs in inbred mice [1, 2, 3]. A fixed volume (0.1 ml/10 g) of a freshly prepared caffeine or theophylline (Sigma Chemical Co.) solution dissolved in physiological saline containing approximately 0.2 mM NaOH was administered by intraperitoneal injection immediately prior to the beginning of behavioral measurements. Six to 12 mice of the CBA, SWR, $CBSWF_1$ and $SWCBF_1$ strains were used to evaluate the behavioral effects of each methylxanthine dose tested. Screening of behavioral responses of the backcross and F_2 progeny was carried out at the dose of drug that evoked the maximum difference in behavioral effect between the two parental strains. These doses were 32 mg/kg IP for theophylline-induced and 10 mg/kg IP for caffeine-induced effects on locomotor activity.

In certain instances, such as backcross and F_2 progeny testing, animals were tested for their responses more than once and received more than one dose of caffeine or theophylline. To insure that the multiple testing did not alter the intrinsic responsiveness of these animals to the methylxanthines, control behavior tests were carried out on both parental strains and F_1 progeny. Multiple behavioral assessments and methylxanthine injections were carried out at weekly intervals. When the results of repeated testing of the behavioral effects were compared to single tests on drug naive animals, no significant difference in the effect of the methylxanthines on locomotor activity was found between the animals treated with the different regimens. Therefore, under the conditions used, multiple testing of methylxanthine responsiveness in individual animals gave reliable results.

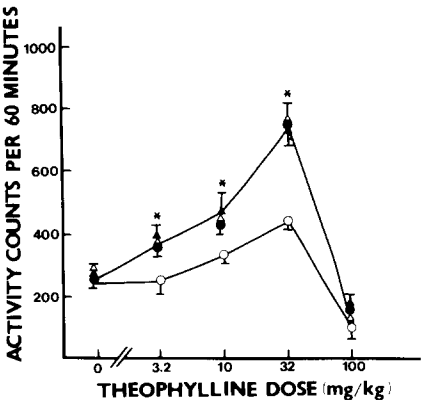

FIG. 2. Dose dependence of theophylline-induced stimulation of locomotor activity in $SWCBF_1$ and $CBSWF_1$ hybrid strains compared to their parental strains. Animals (n=6–10 for each dose) received an IP injection of drug at the time that measurement of locomotor activity was initiated. Each point represents the response of an individual animal activity. Values presented in this and subsequent figures are total activity values post dosing. (○) SWR; (●) CBA; (△) $SWCBF_1$ hybrid; (▲) $CBSWF_1$ hybrid; (*) responses to theophylline differ signficantly from the SWR strain.

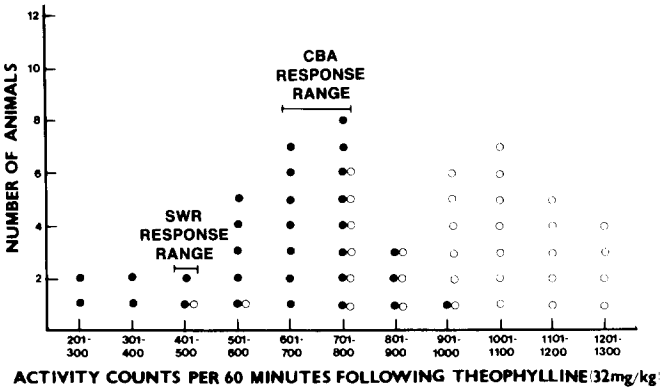

FIG. 3. Segregation of theophylline-induced changes in locomotor activity in progeny from reciprocal backcrosses of F_1 hybrids to SWR and CBA parental strains. F_1 hybrid females were backcrossed to SWR males or CBA males and progeny (n=30 from F_1 × SWR (○) and n=33 from F_1 × CBA) (●)) were screened for the effect of theophylline (32 mg/kg IP) on locomotor activity. Each point represents the response of an individual animal activity. Values presented in this and subsequent figures are total activity values post dosing. Basal values have not been subtracted since there was no significant change in basal activity of backcross of F_2 progeny compared to their CBA and SWR progenitors.

Locomotor Activity Testing

Locomotor activity was monitored in eight activity chambers. Each activity chamber consisted of a 2 foot diameter circular arena, 10 inches high, equipped with two photocell detectors. Each detector was illuminated by a 25 W light bulb (General Electric, #25R14N) placed outside the arena with the light beam directed through a 1/2 inch hole in the side of the arena. To minimize background light, each bulb was shielded in a metal box. The two bulbs were the only source of lighting within the chamber. A Rockwell AIM 65 microprocessor system was used for data acquisition. Data recorded for each 1 hr activity session consisted of 10 min interval counts and cummulative total counts for the 60 min period. Activity sessions were conducted daily excluding week-ends from 0800 to 1530 hours. The mice were allowed to habituate to the activity chambers for a period of 1–2 weeks before drug testing began. Vehicle sessions were conducted on the day preceding drug sessions. Drug trials were generally conducted on the fourth or fifth day of the week.

RESULTS

Theophylline-Induced Changes in Locomotor Activity of CBA and SWR Inbred Mice and Their Reciprocal F_1 Hybrid Derivatives, $SWCBF_1$ and $CBSWF_1$

The different susceptibilities of SWR and CBA inbred mice to the dose-dependent stimulation of locomotor activity by theophylline are shown in Fig. 1. Doses of 3.2–32 mg/kg IP significantly stimulation activity in CBA mice compared to uninjected or vehicle injected control animals. In contrast, SWR mice were significantly stimulated only at a dose of 32 mg/kg IP. Higher doses of theophylline (data not shown) had no further stimulating effect on locomotor activity of SWR mice. A dose of 100 mg/kg IP inhibited both strains of mice to about the same extent compared to basal activity levels under these conditions. Thus, although the potency of theophylline in the two strains of mice is similar (ED_{50}=24 mg/kg IP), the efficacy of this methylxanthine is significantly lower in the SWR strain.

Under the conditions used in these experiments, the basal locomotor activity levels of the parental strains were not significantly different from one another. This observation is important because it removes a potential behavioral difference which might confound the genetic analysis of the drug-induced behavioral effects. If inherited determinants for both basal locomotor activity and for methylxanthine responsiveness were segregating in the crosses to be analyzed, differential effects of methylxanthines on locomotor activity might result from genetically determined differences in the relative contribution of various mechanisms to overall locomotor activity, rather than inherent differences in sensitivity of these neural inputs to stimulation by methylxanthines.

To determine the dominance characteristics of this difference in sensitivity to theophylline-induced stimulation of activity, the dose dependent effects of this methylxanthine were characterized in F_1 hybrid progeny derived from reciprocal crosses of the two parental strains (CBA × SWR; SWR × CBA). At all doses, the effect of theophylline on each of the two F_1 hybrid lines was indistinguishable from the CBA strain of mice and significantly different from the SWR parent strain (Fig. 2). These data indicate that the high level of responsiveness to locomotor activity stimulation by theophylline is completely dominant to hyporesponsiveness. Since the reciprocal F_1 hybrid strains give identical results, the gene(s) determining this trait are autosomal rather than sex linked. Basal activities of the two hybrid strains were not significantly different from their two parental strains, a further indication that no genetically determined cryptic differences affecting basal locomotor activity occur between the SWR and CBA strains.

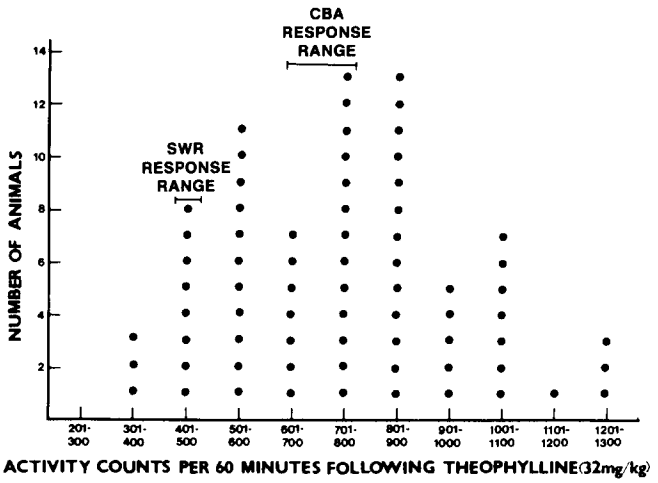

FIG. 4. Segregation of theophylline-induced changes in locomotor activity in F_2 progeny derived from F_1 hybrid self-crosses. Progeny (n=71) were screened for the effect of theophylline (32 mg/kg IP) administration on locomotor activity. Animals received an IP injection of the drug at the time that measurement of locomotor activity was initiated. Each point represents the response of an individual animal.

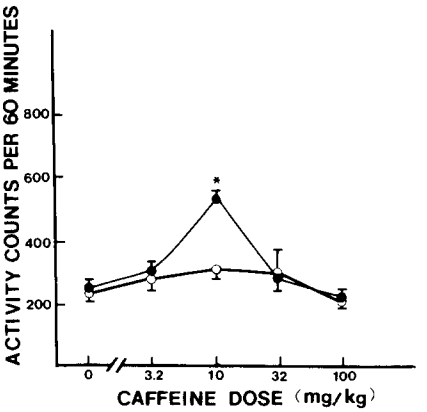

FIG. 5. Dose dependence of caffeine-induced stimulation of locomotor activity in CBA and SWR inbred mice. Animals (n=6–12 for each dose) received an IP injection of drug at the time that measurement of locomotor activity was initiated. (○) SWR; (●) CBA; (*) responses to caffeine differ significantly from one another in the two strains.

Segregation of Responsiveness to Theophylline-Induced Stimulation of Locomotor Activity in Backcross Progeny

To determine if this difference in theophylline responsiveness between SWR and CBA inbred mice was controlled by a single gene or in a more complex manner, segregation analysis of theophylline responsiveness was carried out on progeny from reciprocal backcrosses (F_1 hybrid females crossed either to CBA or SWR males). Since the reciprocal F_1 hybrid strains have a theophylline response which is phenotypically indistinguishable from their CBA (responsive) parent, the expected segregation ratios for phenotypic classes derived from these crosses are simply calculated for a single gene determinant. If a single pair of alleles determines the difference in the relative ability of theophylline to increase locomotor activity levels, the backcross of an F_1 hybrid to CBA should yield only one phenotypic class in which all progeny resemble their CBA parent. For the reciprocal cross, $F_1 \times$ SWR, two phenotypic classes occurring in a 1:1 ratio are expected. One of these phenotypic classes should be indistinguishable from its CBA parent, and the other is expected to be hyporesponsive like the SWR parent. Significant departures from these expectations for phenotypic characteristics, either in the number of phenotypic classes or in the number of occurrences of progeny in each class, indicate that a simple Mendelian explanation for the inheritance of theophylline responsiveness does not apply and that this trait is determined in a polygenic or multifactorial manner.

Figure 3 shows the results of the behavioral analysis carried out on 63 progeny from the two reciprocal backcrosses. Basal locomotor activities of these progeny were also indistinguishable from their progenitors (data not shown). From the backcross to CBA mice, the progeny do not fall into a single response class resembling their CBA parent. In fact, 2/33 progeny were clearly hyporesponsive compared to CBA. Most animals (25/33) were significantly more responsive to theophylline than was the CBA parent. In the reciprocal cross, two phenotypic classes were expected, one CBA-like and identical to the progeny of the other backcross, and a second class, which is SWR-like in response and equal in number to the higher responders. Figure 3 shows that both hyporesponders (stimulated to a lesser extent than SWR mice) and supersensitives (response significantly greater than CBA mice) occurred among progeny of this backcross. Further, the magnitude of the theophylline response among responders was distinctly different between the two backcrosses. Finally, if the progeny of the backcrosses to SWR are arbitrarily grouped into two categories, CBA-like (activity levels >501/hr) and SWR-like (activity levels <501/hr), the ratio of progeny in the two classes is significantly different ($p=0.01$) from the expected value of 1:1. Taken together, these data indicate that the difference between the efficacy of theophylline-stimulated locomotor activity in CBA and SWR inbred mice is inherited in a complex, apparently polygenic manner.

Segregation of Locomotor Activity Responsiveness to Theophylline in F_2 Progeny

A second approach to the genetic analysis of theophylline responsiveness is the characterization of theophylline effects on progeny derived from self-crossing of F_1 hybrid animals. Since the CBA-like response to theophylline is dominant, a 3:1 ratio of CBA-like to SWR-like responses is expected among progeny of this cross if a single pair of alleles determines the response to this methylxanthine. Among the 71 F_2 progeny screened for their response to a theophylline dose of 32 mg/kg IP, 18 low responders and 53 high responders were expected. Figure 4 shows the range of responses of these self-cross progeny and their failure to fall into the two discrete phenotypic classes expected to occur if a single gene determined theophylline sensitivity. Of these 71 F_2 progeny, 29 responded to theophylline to a greater extent than did their CBA progenitor, 11 were intermediate to both parental responses and 3 were quite hyporesponsive compared to their SWR progenitor. The range of responses was very similar to that found for the backcross progeny (Fig. 3). These data lend further support to the contention that the differ-

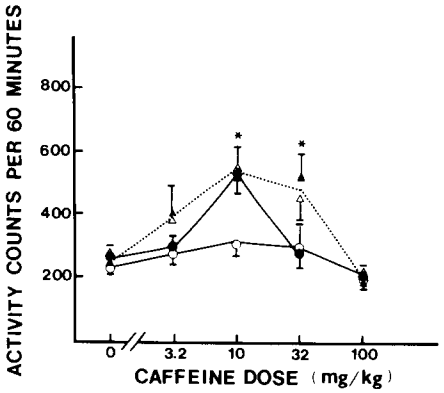

FIG. 6. Dose dependence of caffeine-induced stimulation of locomotor activity in $SWCBF_1$ and $CBSWF_1$ hybrid strains compared to their parental strains. Animals (n=6–12 for each dose) received an IP injection of drug at the time that measurement of locomotor activity was initiated. (○) SWR; (●) CBA; (△) $SWCBF_1$ and $CBSWF_1$ (▲) hybrid strains differ significantly from SWR at the 10 mg/kg IP dose and from both parental strains (but not from each other) at the 32 mg/kg IP dose.

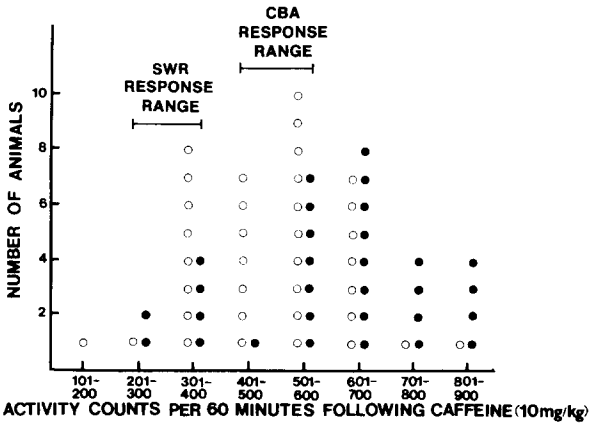

FIG. 7. Segregation of caffeine-induced changes in locomotor activity in progeny from reciprocal backcrosses of F_1 hybrids to SWR and CBA parental strains. F_1 hybrid females were backcrossed to SWR males or CBA males and the progeny (n=38 from F_1 × SWR (○) and n=30 from F_1 × CBA (●)) were screened for the effect of caffeine (10 mg/kg IP) on locomotor activity.

ence in the effect of theophylline on locomotor activity in the CBA and SWR strains is determined in a genetically complex manner.

Caffeine-Induced Changes in Locomotor Activity of SWR and CBA Inbred Mice and Their Reciprocal F_1 Hybrid Derivatives, Strains $SWCBF_1$ and $CBSWF_1$

The different susceptibilities of SWR and CBA inbred mice to the dose-dependent stimulation of locomotor activity by caffeine are shown in Fig. 5. A dose of 3.2 mg/kg IP caused a small but statistically insignificant increase in locomotor activity of both strains. CBA mice were significantly stimulated by a dose of 10 mg/kg IP compared to controls or compared to SWR mice. Although this dose of caffeine produced the maximal behavioral response on the CBA strain, the magnitude of the incubation of locomotor activity was significantly less than that achieved with theophylline. Neither strain showed significantly increased locomotor activity (compared to basal levels) at the higher dosages employed. Activity of SWR mice were not significantly stimulated at any of these doses nor additional ones (data not shown) tested. Thus, the SWR strain is hyporesponsive compared to CBA strain to the locomotor activity stimulating effects of both caffeine and theophylline as we had previously reported [24].

When the dominance characteristic of this trait was examined in reciprocal F_1 hybrid strains (Fig. 6), both strains responded to the same extent as their CBA parent at 10 mg/kg IP, the dose producing the maximal effect in the CBA strain. This observation suggested that caffeine responsiveness, like theophylline responsiveness, showed simple dominance to hyporesponsiveness. However, examination of the dose response curve of the hybrid strains in Fig. 6 reveals that both strains show somewhat increased (although not statistically significant) locomotor activity at a dose of 3.2 mg/kg IP compared to their parents and significantly increased ($p<0.05$) locomotor activity at 32 mg/kg IP. The latter dose was reexamined three times on 12 mice, and each experiment gave the same result. Although the maximal effect of caffeine occurred at the same dose as in the CBA strain, it appears that the inhibitory effects of higher caffeine doses (e.g., ≥32 mg/kg IP in CBA) were less significant in the hybrid strains.

Segregation of Locomotor Activity Responsiveness to Caffeine in Backcross Progeny

To determine if susceptibility to caffeine-induced enhancement of locomotor activity was encoded by a single gene, a segregation analysis of the behavior of progeny from reciprocal backcrosses of F_1 hybrids to the CBA or SWR strain was carried out as we had done for theophylline responsiveness. A single screening dose of 10 mg/kg IP was chosen because it produced the maximal difference in behavioral response between SWR and CBA and because both obligately heterozygous F_1 hybrid strains responded in a manner indistinguishable from their CBA parent at this dosage. The predicted phenotypic classes based upon a single gene hypothesis are the same as those previously described for theophylline responsiveness.

Figure 7 shows the results of this analysis. Among the progeny (n=38) of the backcross of the F_1 hybrid to SWR, two classes of progeny (CBA-like in behavior or hyporesponsive like SWR) in a 1:1 ratio were expected on a single gene hypothesis. Two findings obviate this hypothesis. A significant number of animals (9/38) were found to have locomotor activities significantly higher than their CBA parent after this caffeine dose. Such a new, qualitatively different behavioral class is not expected on the basis of a single gene model for the determination of caffeine sensitivity. Quantitatively considered, data from this backcross also disagrees with this model. If progeny from this cross are simply divided into hyporesponders (SWR-like) and responders (≥CBA activity levels), the observed ratio is respectively 11:27 compared to the expected ratio of 19:19. The observed ratio is significantly ($p<0.01$) different from this expectation.

Progeny from the reciprocal cross of F_1 hybrids to their CBA parent strain are all expected to be CBA-like in re-

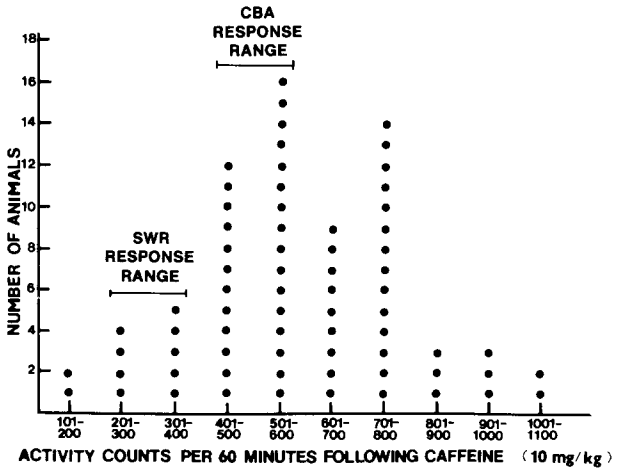

FIG. 8. Segregation of caffeine-induced change in locomotor activity in F_2 progeny derived from hybrid self-crosses. Progeny (n=70) were screened for the effect of caffeine administration (10 mg/kg IP) on locomotor activity. Animals received an IP injection of the drug at the time that measurement of locomotor activity was initiated.

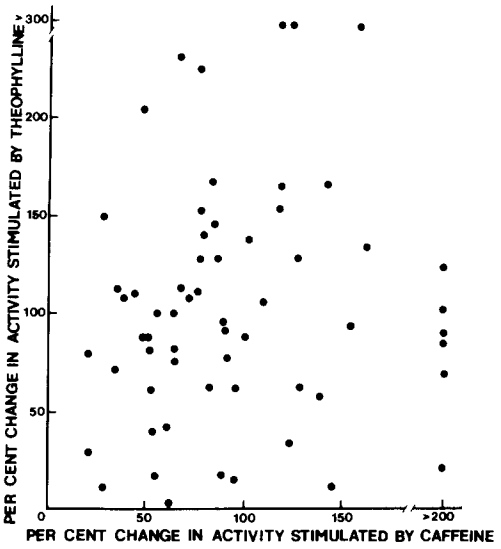

FIG. 9. Lack of correlation between theophylline and caffeine responsiveness among F_2 progeny mice. The data is from the same mice (n=67) shown in Figs. 4 and 8. Each point represents the response of an individual mouse whose response to both methylxanthines was tested. There was no significant correlation (r=0.097) between the behavioral action of caffeine and theophylline on individual mice from this self-cross.

sponse to caffeine. However, both caffeine sensitive progeny (17/31) with locomotor activities outside of the range of the CBA parent and mice with an SWR-like phenotype (6/31 progeny) were identified in this backcross. These two behavioral classes are unexpected if the segregation of a single pair of allels were responsible for the difference in caffeine susceptibility. Taken together, the data from both backcrosses suggest the existence of complex genetic determination of this behavioral response to caffeine.

Segregation of Locomotor Activity Responsiveness to Caffeine in F_2 Progeny

The locomotor activity range following a 10 mg/kg IP dose of caffeine was also determined in F_2 progeny (n=70) derived from self-crossing F_1 hybrid strains. Two phenotypic classes of behavioral response, one SWR-like and one CBA-like, occurring in a 1:3 ratio are expected if a single pair of alleles determines caffeine responsiveness. Locomotor activity following caffeine administration is shown in individual F_2 progeny in Fig. 8. Among these progeny, 33/70 had activity levels outside of those expected from parental phenotypes. Although the ratio of hyporesponders to responders was not significantly different ($p=0.1$) from the hypothetical value of 3:1 when individual mice were arbitrarily categorized into two groups (SWR-like and responders with ≥CBA activity levels), the large number of animals in phenotypic classes other than parental ones again strongly suggests that this effect is determined in a genetically complex manner.

The Relationship Between the Genetic Determinants of Susceptibility to the Effects of Caffeine and Theophylline

If the genetic determinants of susceptibility to the behavioral effects of caffeine and theophylline are identical, then relative responsiveness to these two methylxanthines should co-segregate in backcross and self-cross (F_2) progeny. This question can be evaluated from the data shown in Figs. 9 and 10. Comparison of the relative change in locomotor activity following caffeine or theophylline administration in 67 F_2 progeny tested individually with both methylxanthines is shown in Fig. 9. No correlation (r=0.097) existed between the effects of caffeine and theophylline on locomotor activity in individual animals. [If absolute activity levels after drug administration instead of relative changes (data not shown) were compared for the same, low correlation was observed.] A similarly low correlation (r=0.12) between the effects of the two methylxanthines on locomotor activity in individual animals also was observed in progeny (n=41) obtained from the reciprocal backcrosses of F_1 hybrids to their SWR or CBA progenitors (Fig. 10). Since the CBA strain was responsive to both theophylline and to caffeine and the SWR was hyporesponsive to both, it appears that genetic recombination can lead to the separation of the inherited determinants of susceptibility to caffeine from genes which determine susceptibility to theophylline. These separable, complex genetic determinants of susceptibility to the behavioral effects on locomotor activity of the two methylxanthines can then assort to produce new phenotypes (response levels) which differ from the parental strains.

DISCUSSION

The present findings indicate that the differential effects of methylxanthines on locomotor activity in CBA and SWR strains of inbred mice are not inherited in a simple Mendelian fashion. The complex pattern of behavioral-pharmacological phenotypes found among the progeny derived from both backcrosses of F_1 hybrids to their progenitor strains and from self-crosses of these F_1 hybrids do not fit the expecta-

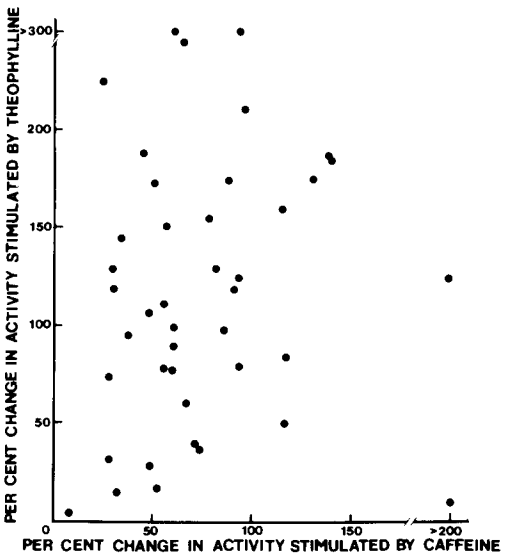

FIG. 10. Lack of correlation between theophylline and caffeine responsiveness among progeny of the reciprocal backcrosses. The data is from the same mice (n=41) shown in Figs. 3 and 7 whose response to both methylxanthines was tested. Each point represents the response of an individual mouse. There was no significant correlation (r=0.012) between the behavioral action of caffeine and theophylline on individual mice from these backcrosses of F_1 hybrids to either their SWR or CBA parent.

tions for the segregation of a simple Mendelian trait. These data suggest that caffeine and theophylline responsiveness are both polygenically determined. We have previously shown that the marked difference in caffeine-induced hypothermia found between C57Bl/6 and DBA/2 inbred mouse strains also is polygenically determined [10]. The difference in efficacy but not potency of theophylline-induced stimulation of locomotor activity between CBA and SWR mice might suggest that the number of target sites for the action of this methylxanthine differ between the two strains. If the genetically specified difference in methylxanthine responsiveness related solely to the number of targets and the genes specifying these targets were simply additive, then the phenotype of F_1 hybrids should be intermediate to the two progenitor strains. The pharmacological responsiveness of the reciprocal F_1 hybrids was not intermediate, but indistinguishable from their CBA progenitor (at the dose eliciting maximal response). This finding suggests that the genes determining methylxanthine responsiveness do not interact in a simple additive fashion or that the threshold for a behavioral response equivalent to the CBA progenitor can occur when only half of the CBA genetic determinants of this response are present.

Recognizing this complexity, the behavior of individual animals with pharmacological responses which differ from both parental phenotypes (e.g., F_2 mice which were less responsive to theophylline than their hyporesponsive SWR progenitor, or others which were stimulated to a greater extent than their CBA progenitor) presumably results from reassortment of several genes, some of which affect stimulatory responses to methylxanthines while other genes specify inhibitory responses or are null alleles. By this interpretation, the genotype of supersensitives (i.e., progeny mice whose reponse significanly exceeds their CBA progenitor) includes more genes encoding the stimulation of activity levels by the methylxanthine than does its CBA progenitor. Alternatively, fewer genes encoding responses inhibitory to the stimulating effects of a methylxanthine, or fewer genes encoding no response to methylxanthine administration (null alleles) may reside in the genotype of hyperresponding progeny than in the genome of their CBA progenitor strain. Progeny which are hyporesponsive compared to their SWR progenitor are expected to have fewer genes specifying targets upon which a methylxanthine can act to elicit increased activity levels. They also may include in their genome more genes which act in an inhibitory manner opposing these responses. Behavioral phenotypes intermediate to the two progenitor strains, thus, would possess a number of genes, the summation of whose interactions lead to an intermediate behavioral response.

The lack of concordant segregation of behavioral responsiveness to caffeine and theophylline in these progeny shows that a significant fraction of the genetic determinants encoding the response to these two methylxanthines are physically distinct and separable from one another. In contrast, susceptibility to caffeine-induced, stress-potentiated lethality, which also differs between the CBA and SWR strains, appears to be specified in a single gene fashion [37]. Therefore, even within a single pair of inbred mouse strains, differential responses to the various behavioral effects of one pharmacological agent or to different analogs of one pharmacological class can arise by separate, genetically distinct mechanisms. This observation points out the potential complexity of pharmacologically-induced behavioral changes and their genetic control. Although some genetically-specified differences in pharmacological responsiveness may simultaneously affect several behavioral traits, others may be behavior-specific (see [12, 27, 28] for additional discussion).

The neurochemical mechanisms which underlie the observed pharmacogenetic differences in behavioral response of these two strains of inbred mice to methylxanthines are unclear at present. At high doses, methylxanthines can elicit the release of brain catecholamines [3], inhibit cyclic nucleotide phosphodiesterases [2, 5, 8], inhibit 5'-nucleotidases [10,35], alter intracellular calcium compartmentation [20,23] and interact with central type benzodiazepine receptors [25]. At lower doses, methylxanthines antagonize adenosine-mediated decreases in cerebral cortical firing rate [29], block the binding of specific ligands to adenosine receptors [10, 13, 34, 41], and antagonize the elevation of cyclic AMP levels stimulated by binding of adenosine agonists to A-2 adenosine receptors [10, 13, 17].

Because of the dosages of caffeine and theophylline used in our study, it is tempting to assume that the enhancement of locomotor activity by these methylxanthines results from their blockade of the neuromodulatory effect of endogenous adenosine [9, 10, 31, 44]. The potencies of a series of methylxanthines in stimulating locomotor activity have been correlated with their ability to compete with radioligand binding to brain adenosine receptors in one mouse strain [41]. Theophylline is usually found to be slightly more potent than caffeine in the displacement of adenosine ligands from their receptors [10,41], and methylxanthines are potent inhibitors of ligand binding to both adenosine A-1 and A-2 receptors in all brain regions [9]. One possible explanation for the inherited differences in methylxanthine responsiveness between SWR and CBA inbred mice is that their CNS contains different numbers of adenosine receptors. If this simple explanation were correct, then the relative behavioral

affect of caffeine and theophylline should be correlated in progeny with different numbers of genetically-specified adenosine receptors. This correlation was not found in the present study in F_1 hybrids (at higher doses), or in F_2 and backcross progeny, nor was if found in a previous study when relative responses to four methylxanthines were assessed among different strains of inbred mice [3]. Further, preliminary data on adenosine receptor quantitation in forebrain membranes reveal no substantial change in total receptor number between these two strains of mice (Seale, unpublished results). The present in vivo behavioral data suggest to us that more than one neurochemical mechanism is involved in the behavioral response difference to methylxanthines between SWR and CBA mice. This contention is further supported by the different mode of inheritance of susceptibility to caffeine-induced lethality [4] in these same strains. Perhaps the number or function of adenosine receptor subtypes is physiologically altered as a consequence of subtle, inherited changes in the regional distribution of adenosine levels, adenylcyclase levels, or phosphodiesterase activities in the brains of the two inbred strains [10, 21, 32, 44].

Whatever the exact neurochemical mechanism(s) by which methylxanthine effects on locomotor are elicited, the data presented here clearly establish that inherited variation in behavioral responsiveness to these compounds does occur. Complex genetic mechanisms are implicated in specifying at least certain of the differences in methylxanthine-mediated behavioral responses between mouse strains. The diversity of inherent variation in strain-specific behavioral responses to this class of pharmacological agents which we have already identified suggests that significant inherited variation in central responsiveness to these compounds might be anticipated in other mammalian species. Within inbred mouse strains there may exist additional, mechanistically distinct inherited lesions which alter behavioral responses to these drugs in other ways. By further characterizing these behavioral variants and combining their mutations in new genetic combinations, these inbred mouse should provide a powerful new analytical tool to obtain new insights into the central and peripheral mechanism(s) of action of methylxanthines.

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