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Interstrain Variation in Acute Toxic Response to Caffeine Among Inbred Mice[1]

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SEALE, T. W., P. JOHNSON, J. M. CARNEY AND O. M. RENNERT. *Interstrain variation in acute toxic response to caffeine among inbred mice.* PHARMACOL BIOCHEM BEHAV 20(4) 567–573, 1984.—Acute toxic dosage-dependent behavioral effects of caffeine were compared in adult males from seven inbred mouse strains (A/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J, SWR/J). C57BL/6J, chosen as a "prototypic" mouse strain, was used to determine behavioral responses to a broad range (5–500 mg/kg) of caffeine doses. Five phenotypic characteristics—locomotor activity, righting ability, clonic seizure induction, stress-induced lethality, death without external stress—were scored at various caffeine doses in drug-naive animals under empirically optimized, rigidly constant experimental conditions. Mice (n=12 for each point) received single IP injections of a fixed volume/g body weight of physiological saline carrier with or without caffeine in doses ranging from 125–500 mg/kg. Loss of righting ability was scored at 1, 3, 5 min post dosing and at 5 min intervals thereafter for 20 min. In the same animals the occurrence of clonic seizures was scored as to time of onset and severity for 20 min after drug administration. When these proceeded to tonic seizures, deach occurred in less than 20 min. Animals surviving for 20 min were immediately stressed by a swim test in 25°C water, and death-producing tonic seizures were scored for 2 min. In other animals locomotor activity was measured 15 or 60 min after caffeine administration. By any single behavioral criterion or a combination of these criteria, marked differences in response to toxic caffeine doses were observed between strains. These results indicate that behavioral toxicity testing of alkylxanthines in a single mouse strain may be misleading and suggest that toxic responses of the central nervous system to this class of compounds are genetically influenced in mammals.

Caffeine Inbred mice Central nervous system Drug-induced seizures Motor activity Behavioral toxicology Behavioral genetics Stress

CAFFEINE is the most widely used drug in man which affects the central nervous system (CNS) [7]. Goldstein *et al.* [8,9] reported the occurrence of marked individual variation in behavioral response to caffeine in man. Somatic manifestations involving the CNS, cardiovascular system, gastrointestinal system and genitourinary system are cognitively identified by many users in high and low consumption subgroups and may predict consumption levels or abstinence [21]. However, it remains to be established whether such differences in responsiveness are CNS-specific and genetically or environmentally determined. The biochemical effects of methylxanthines have been studied extensively, and several distinct mechanisms of action have been identified. (1) Caffeine is a competitive antagonist of brain adenosine receptors [6] and may modulate adenylate cyclase activity, thereby altering adenosine 3',5'-cyclicmonophosphate (cAMP) levels. (2) Caffeine inhibits cAMP phosphodiesterase and thereby leads to the accumulation of brain cAMP [5]. (3) Caffeine can cause the release of brain catecholamines which in turn stimulate adrenergic receptor-adenylate cyclase complexes to increase cAMP synthesis [1]. (4) Caffeine also may alter calcium-mediated effects in some tissues [11]. Since these biochemical effects occur at different dosages of caffeine, behavioral effects at a specific caffeine dose may reflect one or more of the recognized biochemical mechanisms. Thus, if genetic variability in susceptibility to the behavioral effects of caffeine does occur between individuals, such variation in behavioral expression might result from any of several mechanistically distinct biochemical genetic changes.

To establish whether, among different animals of the same species, reproducible, inherent differences in behavioral response to caffeine could be demonstrated under well-defined conditions, we used normal inbred mice as a neuropharmacogenetic model system to investigate intrastrain and interstrain variation in dose-dependent caffeine susceptibility. Several distinct screening procedures expected to detect qualitatively distinct effects were used. Marked inherent differences in acute toxic caffeine-induced behavioral changes were observed among the seven common inbred strains of normal mice investigated in this study.

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METHOD

Subjects

Adult male mice of inbred strains A/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J and SWR/J (Jackson Laboratory, Bar Harbor, ME) weighing approximately 20 g were housed in groups of 6 animals per cage on a continuous 12 hr light-dark cycle under constant humidity and temperature (21–23°C). It should be recognized that both age and weight can potentially influence pharmacological responsiveness, and in this study we elected to keep weight constant. Typically, ages of animals we used varied from 7–30 weeks. Aimals were drug naive and were used only a single time after drug administration. The litter used was aspen wood chips (Sani-chips, P. J. Murphey). Free access to a standard rodent pellet food (Lab/Blox, Wayne) and water was given.

Behavioral Testing

Caffeine administration. Drug dosage and time courses used in these studies were determined empirically by the behavioral responses of strain C57BL/6, and subsequently other strains were screened to determine their response at dosages giving distinctly different behavioral effects in C57BL/6. A fixed volume (0.5 ml/20 g) of freshly prepared caffeine (Sigma) dissolved in physiological saline containing approximately 0.2 mM NaOH were administered by IP injection. In the initial experiments with C57BL/6, caffeine was administered in 20 mg/kg increments from control (0 caffeine) to 120 mg/kg doses, and the behavioral phenotypes described by the tests outlined below. Higher dosages (200–500 mg/kg) were determined initially in 100 mg/kg increments. Thresholds for C57BL/6 for each of the effects were then determined. From these values, other strains were screened for their response to dosages which were most informative for C57BL/6 behavioral changes. When hypo- and hyper-responding strains for a given phenotype were identified, several different additional dosages of caffeine were usually used to try to better define the dose causing the appropriate behavioral end point in that particular strain.

Locomotor activity suppression. Locomotor activity of individual animals (n=12 for each strain) was measured in an automatic activity monitoring device (Digiscan, Omnitech Electronics) which used an infrared detection system. It was determined empirically that a 7 min period of activity measurement begun immediately after an animal was placed in the monitor maximized absolute activity values. In certain strains, e.g., DBA/2, previous experience in the activity monitor led to marked decreases in activity levels. To remove this variable which could be confused with the actual effects of caffeine administration, prior to caffeine administration animals from each strain were conditioned by repetitious exposure to the activity monitor (usually twice a day for 5 days) until their activity reached a constant rate upon repeated testing. At either 15 or 60 min following IP caffeine injection, locomotor activity was measured and compared to baseline activity determined in duplicate 1 hr prior to injection.

Loss of righting ability. A different group of animals (n=6–12 for each dose) than that used for locomotor activity was used to determine the loss of righting ability, seizure activity and lethality. Each of these latter behavioral effects of caffeine were simultaneouly evaluated in the same animal. Righting activity following manual placement of the animal on its side was determined in each strain before and at various times (1, 3, 5 min and at 5 min intervals thereafter) following caffeine administration. Loss of righting activity scored by two observers was said to occur when a significant increase in the time to right was noted (typically greater than 2 sec).

Seizure activity and lethality. The occurrence of clonic and tonic seizures was scored according to the description of Seyfried [17]. The initiation of caffeine-induced seizures is not invariably associated with the onset of an explosive burst of wild running as it is typically in audiogenic seizures. Animals within a single strain may make exaggerated running movements or be quiescent before the onset of seizures. A clonic seizure occurs when the mouse falls over on its side and displays violent pulsating-kicking movements. The tonic seizure phase begins as all four legs are rigidly extended to the rear. Respiratory arrest usually follows the occurrence of a tonic seizure induced by caffeine treatment. Clonic seizure activity was scored as positive when their occurrence lasted for more than 1 min. Typically, once clonic seizures were initiated, they were continuous or nearly so. When tonic seizures followed clonic seizures, the animal usually died within 2 min of the occurrence of the first tonic seizure. Death following caffeine administration but with no additional extra stress was scored as positive if it occurred within 20 min following injection. Animals that survived this period usually survived for at least 4 hr.

Stress-induced death. Early in the course of these studies we noted that some animals, ones which gave no evidence of tonic seizures after caffeine administration and sometimes did not show clonic seizures, would progress rapidly to tonic seizures and death after stress. The type of stress-loud noise, aggressive handling by the investigator, aggression by cage mates—did not seem to matter. Because a rapid test for stress-induced tonic seizures and death after caffeine treatment offered an additional behavioral parameter, we developed a simple, inexpensive, reliable and quantitative method to achieve this effect. A swim stress, achieved by gently placing individual animals in a large beaker of water, gave reproducible results which could be quantitated in terms of time of onset of tonic seizures and death. Twenty min after caffeine administration, mice were subjected to a swimming stress by placing individual animals in a 2 liter beaker containing water at 25°C. Untreated or non-responding animals swam actively for greater than 2 min and did not have tonic seizures nor die. Inactive animals simply floated. Induction of tonic seizures and death occurred in less than 2 min in responding strains. Animals did not appear to drown but to die of respiratory arrest prior to submersion of their heads. The test was scored as positive if seizures and death occurred in less than 2 min.

RESULTS

Characterization of Dosage-Dependent Caffeine-Induced Effects in a Prototypic Inbred Strain

Behavior of C57BL/6. Initially we undertook to establish a baseline of dosage-dependent caffeine-induced behavioral effects in a single "prototypic" inbred strain. Data gathered on this strain served to determine dosages to be used initially to screen for hypo- and hyper-responsiveness in other inbred mouse strains. We chose C57BL/6 as our prototypic strain for the following reasons: (1) it is a widely studied, "typical" mouse strain; (2) a wide spectrum of neuropharmacological data is available for this strain; (3) it is especially suitable for genetic experiments, e.g., the availability of recombinant in-

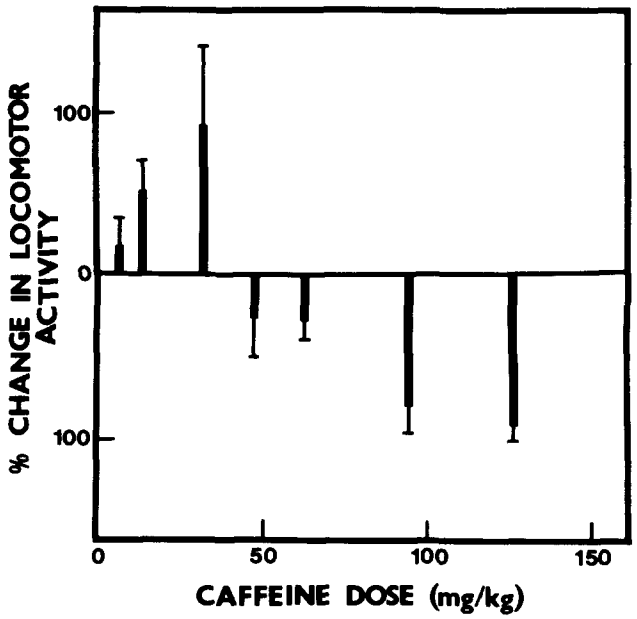

FIG. 1. Dosage-dependent effects of caffeine on locomotor activity of C57BL/6 mice. Activity of animals (n=12) for each dose 15 min after injection of caffeine was self-compared to pretreatment activity and the results of individual animals were averaged. Values are the mean percentage change ±SD in locomotor activity. No change of activity level from the basal rate would be indicated by a value of 0. Values above the zero line indicate stimulation of locomotor activity; values below the zero line indicate inhibition of locomotor activity.

bred strains derived from crosses of this strain to others. Figure 1 shows the dosage dependent effects of caffeine on locomotor activity in C57BL/6. Values represent the percentage change in activity levels of treated animals self-compared to their pretreated activity levels. No change in activity level after caffeine treatment would be indicated in Fig. 1 by a value of 0. Control animals, in which the saline carrier alone was injected, showed no significant change (0±15%) in locomotor activity. As the dose of caffeine was increased above 10 mg/kg, locomotor was significantly stimulated in a dose-dependent manner until it increased to an average of about two times the basal rate at a dose of 30 mg/kg. At higher dosages the locomotor activity declined toward the basal level. The effect of caffeine on locomotor activity was sharply biphasic since, at doses above 40 mg/kg, significant inhibition of locomotor activity occurred in a dose-dependent fashion. Maximal reduction of locomotor activity to values of 5–10% of basal levels occurred at doses of ≥100 mg/kg. At caffeine doses above 10 mg/kg, the qualitative responses of individual animals were homogeneous at a given dose, i.e., all animals receiving 30 mg/kg caffeine showed significantly enhanced locomotor activity compared to their basal rates and all animals receiving a dose of 125 mg/kg had significantly reduced locomotor activity.

At doses of caffeine up to 125 mg/kg, the only acute toxic effect noted in strain C57BL/6J was the marked reduction in locomotor activity. Significantly higher doses of caffeine were required to reproducibly alter righting ability, to induce seizures, to produce stress-induced tonic seizures and death, and to cause rapid lethality (Table 1). Representative doses given in Table 1 indicate that these five behavioral markers were responsive to different caffeine doses and that homogeneity of behavioral response for a given dose was high. The threshold dose for the rapid loss of righting ability for this strain was about 175 mg/kg, significantly higher than the dose required for 90% inhibition of locomotor activity (100 mg/kg). Clonic seizures occurred in all animals at a dose of 200 mg/kg but were detected in 0/12 mice given 187 mg/kg and scored for 20 min. As the dose of caffeine was further increased above 200 mg/kg, the mean latency of onset of seizures decreased from about 12 min at 200 mg/kg to about 3.5 min at 250 mg/kg. At doses of caffeine up to about 350 mg/kg we observed no tonic seizures. Once animals had initiated clonic seizures, they seized continuously or for extended periods with brief non-seizing intervals for the test period of 20 min.

We observed that a simple, easily controlled and reproducible environmental stress—a swim test (see the Method section)—could cause susceptible treated mice to rapidly undergo tonic seizures which led to apparent respiratory arrest and death in <1 min. For example, at 250 mg/kg all treated C57BL/6 mice had clonic seizures but none progressed spontaneously to tonic seizures and death. However, when these mice pre-treated with 250 mg/kg were subjected to the stress of a forced swim, all had tonic seizures, arrested and died within 0.3 min. Uninjected controls, saline injected controls and mice receiving a caffeine dose of less than 200 mg/kg did not have tonic seizures when subjected to swimming induced stress and survived the test period. Although stress induced seizures and death did not occur below 200 mg/kg, C57BL/6 mice showed greatly reduced swimming activity at 175 mg/kg and most did not swim but essentially floated for the entire test period at 187 mg/kg. At still higher caffeine dosages, death occurred rapidly. 500 mg/kg caused 100% lethality in under 2.5 min in otherwise non-stressed animals.

Age at the time of caffeine administration might significantly alter caffeine-induced behavioral changes if intrinsic CNS sensitivity or metabolism of the drug changes as a function of age. To determine whether this was a significant concern, we compared the responses of "young" (3.5 weeks old) and "old" (50 weeks old) C57BL/6 male mice to caffeine-induced effects just described in our standard young adult C57BL/6 mice. An entire dose response curve (data not shown) over the range of 100–500 mg/kg caffeine was conducted in the recently weaned "young" mice (n=6–12 for each dose), and the toxic behavioral responses were found to be identical to those shown in Table 1 for young adults. Three groups (n=6 each) of old mice also were tested, one at 175 mg/kg, one at 187 mg/kg and one at 250 mg/kg. They responded identically to their younger counterparts (see Table 1). Therefore, over the age range of 3.5–50 weeks there was no significant change in sensitivity or behavioral manifestations elicited by toxic levels of caffeine in this strain.

Screening for Strain Specific Differences in Behavioral Response to Toxic Doses of Caffeine

The previous data taken together suggested to us that these five caffeine-induced behavioral effects might be usefully applied to screening for differences in acute toxic responses among inbred mouse strains. The behavioral effects elicited by caffeine were sharply defined ones which were produced in a reproducible, dose-dependent manner. Thus, single doses could be chosen to screen for hyporesponsiveness or supersensitivity among strains. A second important

TABLE 1

DOSAGE DEPENDENCE OF ACUTE CAFFEINE TOXIC EFFECTS IN C57BL/6J MICE

Caffeine Dosage (mg/kg)	Locomotor Activity Depression	Righting Ability Loss	Seizure Induction	Stress-Induced Tonic Seizures/Death	Death Without Additional Stress
100	+ (90 ± 5%)	−	−	−	−
125	+ (92 ± 5%)	−	−	−	−
175	+ (≥95%)	+ (≤5 min)	−	− (greatly reduced swimming)	
187	+ (≥95%)	+ (<5 min)	−	− (float, no swimming)	−
200	+ (≥95%)	+ (<5min)	+ (clonic; <20 min)	± (8/12 seize and die)	
250	+ (≥95%)	+(<3 min)	+ (clonic; <5 min)	+ (tonic, death <0.3 min)	−
500	ND	+ (<1 min)	+ (tonic; <2 min)	ND	+ (<2.5 min)

(−) No animal gave a positive response; (±) intrastrain variability in behavioral response; (+) all animals responded in the same positive manner; (ND) not determined because of rapid death of animal. (n=12) For each dose. Time values indicate maximal latency of onset after dose was administered.

TABLE 2

INHIBITION OF LOCOMOTOR ACTIVITY BY HIGH DOSAGE CAFFEINE ADMINISTERED TO SEVEN INBRED MOUSE STRAINS

	Inbred Strain						
Caffeine Dosage (mg/kg)	C57BL/6	SWR	DBA/2	A	CBA	C3H/He	BALB/c
94	91 ± 5	85 ± 6	82 ± 6	85 ± 7	0 (no effect or stimulated)	18 ± 15	16 ± 10
125	92 ± 5	87 ± 15	81 ± 12	80 ± 15	0 (no effect or stimulated)	43 ± 20	20 ± 10
175	>95	>95	88 ± 10	>95	61 ± 17	60 ± 15	80 ± 4
187	>95	>95	85 ± 6	>95	66 ± 15	68 ± 15	85 ± 10
200	>95	>95	89 ± 5	>95	55 ± 21	47 ± 28	89 ± 15

Values expressed in this table are percent inhibition of locomotor activity calculated by comparing activity in the same animal before and after caffeine administration (n=12 for each dose).

Saline treated controls from each strain were found not to be significantly different in basal activity from untreated controls.

feature of these data was the demonstration of substantial differences in caffeine dose required to elicit each specific behavior. This observation suggested that separate mechanisms with differential susceptibility to the toxic effects of caffeine might underlie these behavioral traits. Each might in principle be under separate genetic control and, therefore, each might show phenotypic and genotypic variation independently from the other caffeine-sensitive traits. Finally, intrastrain variation was low for each of these behavioral traits. Groups of animals responded in a qualitatively identical and quantitatively similar fashion to a given dose of caffeine. No variation in response was observed between different batches of C57BL/6 mice when the batches were obtained and tested several months apart. Based on our initial results, age-dependent changes did not appear to pose a significant technical problem for these screening experiments either. Having established a phenotypic profile of the toxic effects of caffeine on strain C57BL/6, we screened six other inbred mouse strain at the critical doses of caffeine shown in Table 1 to determine their behavioral responses.

Locomotor activity. Table 2 shows the inhibitory effect of high dosage caffeine or locomotor activity in the 7 inbred mouse strains we investigated. Locomotor activity was determined in animals (n=12) for each strain just prior to and 15 min after caffeine administration, and the percentage change in activity was calculated. The effect of administration of the saline carrier was found to be negligible in each strain. Quantitatively and qualitatively similar effects to those seen in C57BL/6 were observed in strains SWR, DBA/2 and A at each of the caffeine dosages tested, i.e., locomotor activity was severely inhibited. In contrast, the C3H/He, BALB/c and CBA strains differed from these 4 strains and from one another. Activity of C3H/He was significantly reduced by the administration of 125 mg/kg caffeine

TABLE 3

SUMMARY OF PHENOTYPIC DIFFERENCES IN ACUTE TOXIC EFFECTS OF HIGH DOSAGE CAFFEINE AMONG INBRED MOUSE STRAINS

Phenotype	Inbred Strain SWR	C57BL/6	DBA/2	BALB/c	C3H/HeJ	A	CBA
Locomotor Activity							
decreased ≥80% at 125 mg/kg	+	+	+	−	−	+	−
decreased ≥80% at 175 mg/kg	+	+	+	+	+	+	−
Loss of Righting Ability							
at 175 mg/kg	+	+	+	−	+	+	+
Seizure Induction							
clonic at 175 mg/kg	−	−	−	−	−	−	+
clonic at 187 mg/kg	−	−	−	−	−	±	+
clonic at 200 mg/kg	±	+	+	±	+	+	+
Stress-Induced Lethality							
at 175 mg/kg	−	−	−	−	−	±	+
at 187 mg/kg	−	−	±	±	−	+	+
at 200 mg/kg	−	±	+	+	+	+	+
Death (in<20 min)							
at 250 mg/kg	−	−	−	−	+	−	−

(−) No animals gave a positive response; (±) intrastrain variability in response, >1/4 of the animals responded positively; (+) all animals responded in the same positive manner.

but to a lesser extent (43±20% versus 92±5% reduction) than C57BL/6. BALB/c activity was only marginally suppressed (20±10%) at this dose. In contrast, CBA animals given 125 mg/kg were not inhibited but were either unaffected or greatly stimulated (as much as 400% in some animals). This intrastrain heterogeniety of caffeine-induced changes in locomotor activity was observed only in CBA, and the within strain variability was especially evident at 125 mg/kg. At higher doses, e.g., 175 mg/kg, activity of all CBA animals was suppressed, but even at the highest doses examined, this strain retained considerably higher locomotor activity levels than did any strain other than C3H. No significant difference in caffeine-induced response (compared to the behavior after 15 min) was detected in any strain when animals were scored 60 min after caffeine administration instead of 15 min after the dose was given. The data in Table 2 show that dose-dependent, strain specific differences in response to toxic levels of caffeine do occur among inbred mouse strains and that relative inhibition of locomotor activity is a useful marker for detection of such differences. These data are summarized in Table 3 and compared to other behavioral response differences which occurred among these strains when toxic levels of caffeine were administered to each.

Righting ability. Based upon the response of C57BL/6, loss of righting ability was the behavior next altered after locomotor activity by increasing the dose of caffeine (Table 1). Occurrence and the latency of the loss of righting ability were scored for a 20 min interval following the IP administration of 125–500 mg/kg caffeine. No loss of righting ability was observed at 125 mg/kg in any of the strains. Reproducible loss of righting occurred at 175 mg/kg in all strains except BALB/c (Table 3). Between these two doses, no caffeine dose was found which would produce a differential effect upon any of the 7 strains. Within a strain all animals lost their ability to right themselves at about the same time. The latency ranged from ≤2.6 min in CBA to ≤7 min in DBA. The overlap between the 6 responding strains with regard to the latency for loss of righting ability was too great to be useful as a discriminant phenotypic variable. When the dose of caffeine was increased to 187 mg/kg, BALB/c lost righting activity with a latency of ≤5 min. At this dose it could not be distinguished from the other strains. Thus, righting ability seems to be a less sensitive index to discriminate caffeine sensitivity differences among strains than is locomotor activity. However, as a phenotypic test it may identify a different genetically controlled underlying target/mechanism for caffeine action than does the locomotor assay (compare BALB/c to CBA).

Seizure induction. At still higher doses of caffeine, clonic seizures were induced in C57BL/6 (Table 1). Based upon these findings we assessed seizure induction in the other strains. The occurrence of clonic seizures was examined for 20 min after administration of caffeine (125–500 mg/kg). Seizure induction did not occur in any strain except CBA at dosages below 175 mg/kg (Table 3). This dose induced clonic seizures in each CBA animal tested within 5 min. Strain CBA was, thus, markedly more sensitive to caffeine-induced seizures than other strains. Even at 187 mg/kg only CBA mice consistently seized, although one other strain was partially affected at this dose. At 187 mg/kg one-third of the A animals had clonic seizures within 7 min of drug administration. At 200 mg/kg all animals tested from DBA/2, C3H/He, A and CBA showed significant clonic seizure activity. The latter 3 strain had latencies of seizure onset of ≤5 min. Three-fourths of C57BL/6 animals had clonic seizures at 200 mg/kg. C57BL/6 had a more variable latency period ranging from 3–20 min, and more than half of the affected animals had latencies >5 min. One half of BALB/c animals and 3/4 of SWR animals were more resistant to the induction of clonic seizures by caffeine and showed no seizure activity at 200 mg/kg. At higher doses, e.g., 250 mg/kg, caffeine induced clonic seizures in all animals in all strains tested. There was a significant overlap in latency of onset (about 5 min) among all strains at these higher dosages. These data indicate that

from a screening point of view, supersensitivity to caffeine-induced seizures is best detected at dosages of about 175 mg/kg. To carefully characterize strain-specific differences care must be taken in this assay to recognize intrastrain heterogeniety of phenotypic response.

The clonic seizures persisted intermittently or continuously, once they occurred, for the duration of the 20 min test period. At caffeine doses ≤250 mg/kg, these clonic seizures rarely progressed to tonic seizures. In only one strain, C3H/He, did progression to tonic seizures regularly occur (Table 3). In C3H/He every animal receiving 250 mg/kg progressed from clonic seizures to tonic seizures which were almost immediately followed by death. The latency for tonic seizures and death in C3H/He was ≤19 min. This finding suggests that either susceptibility to caffeine-induced tonic seizures or susceptibility to stress-induced death may vary independently of the susceptibility to caffeine-induced clonic seizures.

Stress-induced lethality. We observed that rapid changes in position, loud noise or other startle-producing stimuli sometimes initiated the onset of tonic seizures and death in a clonically seizing animal. In C57BL/6 mice swim-stress-induced death first was detected at a caffeine dose of 200 mg/kg. To search for strain differences in stress-induced tonic seizures after caffeine administration, we subjected animals to a simple controlled stress by demanding that they swim 2 min in 25°C water. All strains tested after receiving injections of saline were found to swim vigorously for >2 min and did not tonically seize nor did they die. At caffeine doses below 175 mg/kg no effects on swimming behavior were noted in any of the strains. At 175 mg/kg all CBA animals had tonic seizures and died in <2 min upon swim testing (Table 3). Strain A mice also seized and died at this dose but intrastrain variability was marked (3/12 having tonic seizures and dying in <2 min) in contrast to CBA mice. This dose did not induce tonic seizures and death in the other strains. At both 175 and 187 mg/kg, C57BL/6 animals did not seize and die but neither did they swim. These animals simply floated for most of the 2 min test period at this caffeine dose. This behavior was not observed in any other strain at any caffeine dose. All A and CBA mice had tonic seizures and died at 187 mg/kg after stress in ≤1.3 min. This dose of 187 mg/kg was partially discriminative because 2 additional strains, DBA/2 and BALB/c, now responded but 3 strains still did not. About half of DBA/2 and BALB/c animals had tonic seizures under these conditions at 187 mg/kg. SWR, C57BL/6 and C3H/He were unaffected and survived this dose. At 200 mg/kg all animals which responded had tonic seizures and died with an even shorter latency (<1 min). At 200 mg/kg, 5 out of 6 of the responding strains (DBA/2, BALB/c, C3H/He, A and CBA) were homogeneous in their responses, i.e., all treated mice seized and died after the stress. Three fourths of the C57BL/6 animals had tonic seizures and died when swim stressed 20 min after being given a dose of 200 mg/kg. In contrast, the SWR strain was refractory to tonic seizure induction and death induced by stress after 200 mg/kg caffeine. However, at 250 mg/kg SWR was affected in a manner identical to that of the other 5 strains tested (C3H/He tonically seized and died prior to the swim stress, see Table 3). Twenty min after the 250 mg/kg caffeine dose, all animals from these 6 strains had tonic seizures and died in <0.3 min after exposure to the stress of swimming. These data indicate that susceptibility to stress induced tonic seizures and death (due apparently to respiratory arrest which accompanies the tonic seizures) after toxic doses of caffeine varies markedly among these 7 strains. Several distinct classes of phenotypic response can be established based upon the dose dependence of the behavioral effect. The intrastrain homogeniety of the response makes this an attractive phenotypic marker for genetic analysis of this caffeine-mediated response.

DISCUSSION

The availability of inbred mouse strains has provided a powerful tool for the biochemical genetic dissection of complex biological phenomena [2, 16, 17, 18]. Inherent strain-specific differences in behavioral responses to centrally active compounds, e.g., apomorphine [20], morphine [12] pentylene-tetrazol [16], are among the many traits which vary from one strain to another. There have been few studies which have investigated the occurrence of strain-specific differences in susceptibility to caffeine and other alkylxanthines. Such studies are of importance, not only because of the insight that they may provide into the range of pharmacogenetic variation and its underlying molecular determinants, but also because these findings may have important implications for the practical concerns of toxicological testing. For example, chronic caffeine administration in CBA/USC mice intensifies stress-induced hormonal and pathophysiological changes in a manner which has implications for human health [10]. However, the data which we presented suggest that our closely related CBA strain may differ markedly from other mouse strains with regard to caffeine responsiveness. Thus, the generalizability of the implications of chronic caffeine studies in a single strain may be questioned. One brief report by Bushnel and Lehmann [3] indicated differences in caffeine-induced seizure activity between inbred strains. The effects of pre- and post-trial low dosage caffeine administration on simultaneous visual discrimination also was found to differ in three strains of mice [4]. At the biochemical level, Sattin [15] observed that adenosine stimulated the increased accumulation of cAMP in chopped cerebral cortex tissue to a greater extent in two inbred strains, SEC/1Re and DBA/2, than in C57BL/6. A possible explanation of these differences was that the latter strain appeared to have higher phosphodiesterase levels.

To initiate our systematic characterization of the neuropharmacogenetics of caffeine, we chose seven inbred mouse strains based upon their known behavioral differences (e.g., [19]), their known genetic diversity [14] and the limited data available on sensitivity to alkylxanthines which indicated strain differences. The data which we have presented indicate that different inbred strains do have intrinsically different susceptibilities to the dosage dependent acute toxic effects of caffeine. Among strains, the relative susceptibility to caffeine determined by one phenotypic criterion is not necessarily related to the relative susceptibility determined by another of these criteria. These dissimilarities between a strain's response to caffeine, when more than one phenotypic criterion is considered, imply that different underlying mechanisms cause the several behavioral effects of caffeine. For example, the observed difference in sensitivity to caffeine-induced reduction in locomotor activity might be due to an increase in intracellular brain calcium, whereas seizure activity might be due to inhibition of particular phosphodiesterases. Alternatively, the observed differences in sensitivity may be due to differences in absorbtion, distribution or metabolism of caffeine. However, this appears unlikely since there were several different phenotypic re-

sponses to caffeine which cannot be simply explained by variation in only one of these possible underlying mechanisms. Because the traits we examined appear to be able to vary independently of one another (i.e., all strains are not like either SWR or CBA) they appear to be under separable genetic control.

In man caffeine can produce potent CNS, cardiovascular and peripheral effects, either alone or in combination [13,21]. Our data obtained from inbred mice suggest that both the qualitative and quantitative aspects of caffeine-induced effects may be under separable genetic control and that inherited variation in response to caffeine and related compounds may be common among normal mice and perhaps other mammals. More comprehensive genetic, pharmacological and biochemical studies are needed to establish the exact mechanisms for these differences.

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