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Inherent Differences in Sensitivity to Methylxanthines Among Inbred Mice

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LOGAN, L., T. W. SEALE AND J. M. CARNEY. *Inherent differences in sensitivity to methylxanthines among inbred mice.* PHARMACOL BIOCHEM BEHAV **24**(5) 1281–1286, 1986.—The behavioral effects of caffeine, theophylline, paraxanthine, and theobromine on locomotor activity were analyzed in four strains of inbred mice that were previously shown to differ in their acute toxic responses to caffeine administered at high dosages. Dose response curves for the effects of caffeine, theophylline, paraxanthine and theobromine on locomotor activity were established in CBA/J, C57BL/6J, DBA/2J and SWR/J strains of inbred mice. Paraxanthine was the maximally effective methylxanthine in the CBA/J, DBA/2J and SWR/J strains, while in the C57BL/6J strain, caffeine was the maximally effective methylxanthine. Theophylline failed to stimulate locomotor activity in the C57BL/6J strain and theobromine failed to stimulate activity in all of the strains tested. Decreases in locomotor activity were seen at the 100 mg/kg dose of caffeine in the C57BL/6J mice and at the 100 mg/kg dose of theophylline in the C57BL/6J, DBA/2J and SWR/J strains. Theobromine produced decreases in locomotor activity in the C57BL/6J, DBA/2J and SWR/J strains of mice. In contrast to the other methylxanthines, paraxanthine failed to decrease activity across the range of doses tested (1.0–150 mg/kg). These data suggest that the methylxanthines have genetically specified multiple modes of action upon locomotor activity and that the use of genetically distinct strains of mice may have important value in the neurochemical and pharmacological dissection of methylxanthine-induced behavioral effects.

Methylxanthines Locomotor activity Inbred strains

CAFFEINE and the related methylxanthines, theophylline and theobromine, are probably the most widely consumed psychoactive compounds. In addition to being present in coffee and tea, caffeine and theophylline are used to treat apnea in preterm infants [1,32], and theophylline is widely prescribed in the treatment of asthma [24]. Caffeine is also present in a wide variety of non-prescription medications used for headache and dieting. Goldstein *et al.* [15] reported on individual differences in caffeine sensitivity with respect to onset of sleep in man. Such differences were suggested to be due to intrinsic differences at sites of action within the brain, rather than from differences in absorption, distribution or metabolism of caffeine. However, it remains to be established whether such differences in human responsiveness to caffeine are inherited or acquired and the mechanisms of such behavioral differences is unknown. In view of the widespread ingestion of these methylxanthines, we were interested in whether inherent variation in the responsiveness of the CNS to these substances might exist and whether such genetically determined variation might provide new insights into the mechanisms by which they induce behavioral effects.

In a recent study [28] the behavioral effects of one methylxanthine, caffeine, were determined at a range of doses in seven inbred strains of mice. Strain specific responses were identified for depression of locomotor activity, loss of righting ability, seizure induction, stress induced lethality, and lethal effects of caffeine. In this study, the SWR/J, C57BL/6J and DBA/2J strains showed a decrease in locomotor activity when given 125 mg/kg or 175 mg/kg caffeine while the CBA/J strain failed to exhibit a decrease in activity. The CBA/J strain was most sensitive to stress induced lethality following a 175 mg/kg dose of caffeine. A 200 mg/kg dose of caffeine was necessary for stress induced lethality to be exhibited in the DBA/2J strain while the C57BL/6J mice showed variable results at this dose of caffeine. The SWR/J strain was the least sensitive and required a dose of 250 mg/kg caffeine before stress induced death was observed. More than one caffeine induced behavioral change occurs in these strains of mice and sensitivity for each of these effects does not covary. For example, in contrast to relative supersensitivity to caffeine induced lethality, CBA/J mice fail to develop hypothermia when given 100 mg/kg caffeine while the SWR/J mice show a 4°C drop in body temperature when given this dose of caffeine [8]. Thus, these inbred strains of mice provide ideal experimental material for the genetic analysis of the mechanism(s) by which methylxanthines induce their behavioral effects.

Methylxanthines have been shown to exert a variety of effects on biological systems. Their biochemical actions include inhibition of cyclic nucleotide phosphodiesterases [2, 5, 9], inhibition of 5'nucleotidases [10,27] and mobilization of intracellular calcium [17, 18, 20]. Relatively high concentrations of methylxanthines are required to exert these effects in contrast to the levels necessary to bring about their actions mediated by adenosine receptors. Caffeine and theophylline have been shown to inhibit adenosine, cyclohexyladenosine and L-phenylisopropyladenosine binding

to adenosine receptors in rat brain membranes [10, 13, 26, 27, 29, 33] and to block adenosine-induced relaxation of myenteric plexus preparations [22]. Similarly, the adenosine-induced decreases in rat cerebral cortical neuron firing rate are antagonized by microiontophoretically applied methylxanthines [23]. Elevations in cyclic AMP levels mediated by the action of adenosine, 5'N-ethylcarboxamide adenosine or 2-chloroadenosine on the adenosine A-2 receptor are also antagonized by methylxanthines [4, 10, 13, 16]. It is unclear whether one or more than one of these neurochemical mechanisms underlies the behavioral effects elicited by methylxanthine administration.

Certain behavioral experiments indicate that the methylxanthines do have varying modes of action on the CNS. Stimulation of mouse locomotor activity has been reported to occur following the administration of caffeine, theophylline and the related dimethylxanthine, paraxanthine [28]. In contrast, theobromine failed to stimulate locomotor activity. In rats trained to respond under a variable interval operant schedule caffeine, theophylline and theobromine produced dose-dependent decreases in responding [6]. Caffeine and the related dimethylxanthines were shown to antagonize the behavioral effects of L-phenylisopropyladenosine in rats responding under a variable ratio schedule [21]. In rats trained to discriminate caffeine from saline, theophylline and paraxanthine generalized to the caffeine cue, while theobromine failed to generalize to caffeine [7]. Caffeine generalized to the theophylline cue in rats trained to discriminate theophylline from saline, while paraxanthine only partially generalized to the theophylline cue. These discrimination data would suggest that the methylxanthines can have different actions within the CNS.

In this present study we have extended our investigation of the behavioral action of caffeine on CBA/J, C57BL/6J, DBA/2J and SWR/J inbred mice to a different portion of the dose response curve and to a comparison of these effects of caffeine to the actions of several dimethylxanthines. Here we report that different strains of mice exhibit inherently different responses to individual methylxanthines.

METHOD

Twelve mice weighing 25–30 grams from each inbred strain (CBA/J, C57BL/6J, DBA/2J and SWR/J, obtained from Jackson Laboratories) were utilized in this study. The mice were housed in groups of six with hardwood bedding (Sani Chips, P. S. Murphy) and kept under a twelve hour light/dark cycle. Food and water were available ad lib.

Locomotor activity was monitored in eight activity chambers. Each activity chamber consisted of a 2 foot diameter circular arena, 10 in. high, equipped with two photocell detectors. Each detector was illuminated by a 25 W light bulb (General Electric, No. 25R14N) placed outside the arena with the light beam directed through a 1/2 in. hole in the side of the arena. To minimize background light, each bulb was housed 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 for each 1 hour activity session were recorded in six 10 minute interval periods. Activity sessions were conducted daily excluding weekends. Each mouse was placed in the activity chamber for one hour, five days a week for a period of 1–2 weeks before drug testing began. Data obtained during these habitation sessions demonstrated that mice had reached a steady state level of activity before drug testing. Vehicle sessions were conducted on the day preceding drug sessions. Drug trials were generally conducted on the fourth or fifth day of the week.

All drugs were administered via IP injection at a constant volume of 0.1 ml/10 g body weight. Both drug choice and dose were random among the groups of mice. Drug injections were made immediately prior to the mouse being placed in the activity chamber. Saline served as vehicle solution except for theobromine which was suspended in 0.5% methylcellulose. There was no difference between the effects of saline or methylcellulose on activity. Groups of 4–6 mice were used for each drug condition.

Data were analysed utilizing the SAS statistical program. Locomotor activity counts were subjected to a three factor (strain, drug, dose) analysis of variance. Two factor and one factor analyses of variance were conducted. *Post hoc* analyses were run where indicated. Control values for the four strains were further analyzed by Duncan's multiple range test. In all cases, a p-value <0.05 was considered significant.

RESULTS

Figure 1 shows the average control interval locomotor activity counts for the four strains. Activity was highest during the first ten minutes of the activity session and declined over the course of the 60 minute session. Initial activity was highest for the C57BL/6J strain. Activity of this strain declined to approximately 75% of the initial value by the end of the session. The CBA/J, DBA/2J and SWR/J strains were similar to each other in their interval activity counts and terminal activity was approximately 30% of the initial ten minute activity. Based upon the cumulative one hour activity counts the CBA/J, DBA/2J and SWR/J strains were statistically similar while the C57BL/6J strain had a significantly higher level of activity (Table 1).

The results of caffeine and dimethylxanthine administration is shown in Figs. 2–5. The points shown represent the one-hour cumulative locomotor activity counts. In the CBA/J mice, paraxanthine produced significant elevations in locomotor activity at all doses tested. At a dose of 32 mg/kg paraxanthine produced its maximal effect with activity being increased to 385% of baseline activity. At 32 mg/kg, theophylline produced its maximal effect upon activity. At this dose, activity was increased 306% above baseline. At the two lower doses, theophylline produced no significant effect upon activity. The 100 mg/kg dose of theophylline decreased activity to approximately 60% of baseline levels. The lowest dose of caffeine (3.2 mg/kg) was without effect on activity. The peak stimulation seen with caffeine occurred at a dose of 10 mg/kg. Locomotor activity was increased to 215% of the control value. The maximum amount of stimulation seen with caffeine was less than that observed for paraxanthine or theophylline. Higher doses of caffeine were without effect on locomotor activity. Theobromine produced no significant changes in activity doses up to 178 mg/kg.

In the C57BL/6J strain of mice, the 10 mg/kg dose of caffeine produced the largest amount of stimulation observed with any of the methylxanthines in this strain of mice. This stimulation amounted to a 215% increase in locomotor activity. The 32 mg/kg dose of caffeine was without significant effect on activity while the 100 mg/kg dose produced a significant decrease in the amount of activity observed. The low dose of paraxanthine was without effect on activity. The 10 mg/kg dose produced a significant increase in activity to

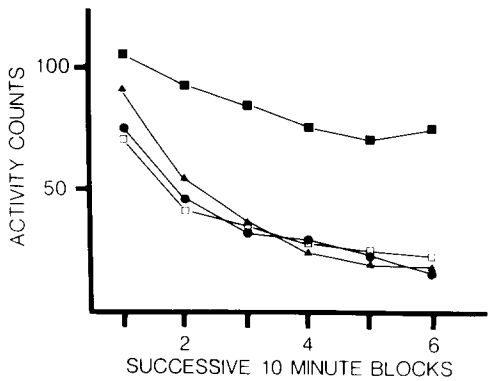

FIG. 1. Temporal pattern of control locomotor activity in 4 inbred strains of mice. Solid squares represent C57BL/6J mice. Triangles represent CBA/J mice. Circles represent DBA/2J mice and open squares represent SWR/J mice. Each point is the mean of 6 mice.

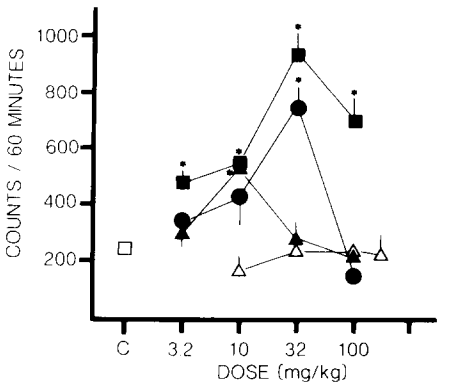

FIG. 2. Effects of caffeine and dimethylxanthines on locomotor activity in CBA/J mice. Values represent the average 60 minute cumulative activity counts for 4–6 mice. Solid triangles represent caffeine. Circles represent theophylline. Squares represent paraxanthine and open triangles represent theobromine. Vertical lines represent standard error of the mean. Asterisks denote significant difference from vehicle ($p<0.05$).

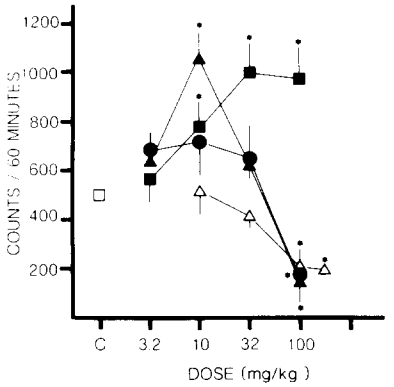

FIG. 3. Effects of caffeine and dimethylxanthines on locomotor activity in C57BL/6J mice. Values represent the average 60 minute cumulative activity counts for 4–6 mice. Symbols are the same as in Fig. 2.

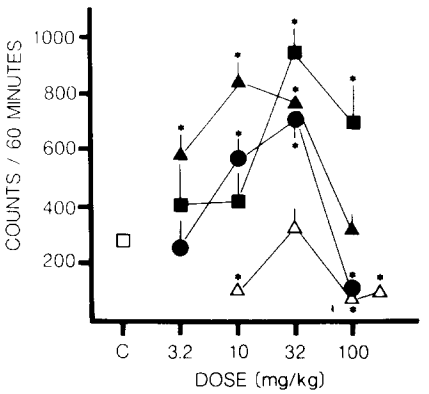

FIG. 4. Effects of caffeine and dimethylxanthines on locomotor activity in DBA/2J mice. Values represent the average 60 minute cumulative activity counts for 4–6 mice. Symbols are the same as in Fig. 2.

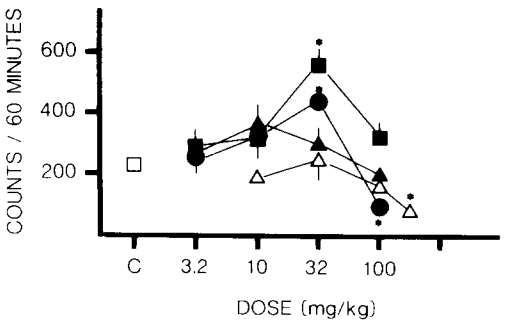

FIG. 5. Effects of caffeine and dimethylxanthines on locomotor activity in SWR/J mice. Values represent the average 60 minute cumulated activity counts for 4–6 mice. Symbols are the same as in Fig. 2.

TABLE 1

CONTROL LOCOMOTOR ACTIVITY COUNTS IN CBA/J, C57BL/6J, DBA/2J AND SWR/J STRAINS OF INBRED MICE

Strain	1 hr Cumulative Activity
C57BL/6J	496 ± 23 (83)*†
DBA/2J	277 ± 19 (83)‡
CBA/J	247 ± 13 (96)‡
SWR/J	231 ± 13 (93)‡

*Group mean ± standard error (number of observations).
*$p<0.05$ compared by Duncan's Multiple Range Test.
‡Statistically similar grouping by Duncan's Multiple range test.

TABLE 2

RANK ORDER OF METHYLXANTHINES IN PRODUCING MAXIMAL LOCOMOTOR ACTIVITY STIMULATION*

Strain	1	2	3	4
CBA/J	paraxanthine	theophylline	caffeine	theobromine‡
C57BL/6J	caffeine	paraxanthine	theophylline	theobromine§
DBA/2J	paraxanthine	caffeine	theophylline	theobromine§
SWR/J	paraxanthine	theophylline	caffeine†	theobromine§

*Based upon 1 hour cumulative locomotor activity counts. These data are summarized from Figs. 2–5.

†Stimulation was not statistically different from control.

‡No dose produced increases in behavior.

§Only decreases in activity.

approximately 150% of control. The maximal stimulation observed with paraxanthine was seen at the 32 mg/kg dose. At this dose, paraxanthine increased locomotor activity to a value of 203% above baseline levels. The highest dose of paraxanthine resulted in slightly less stimulation than the 32 mg/kg dose. In contrast to the CBA/J mice, theophylline did not produce significant stimulation at any of the doses tested. The 100 mg/kg dose of theophylline resulted in a significant decrease in locomotor activity. Theobromine failed to alter activity at the two lower doses, while at the 100 mg/kg and 178 mg/kg doses, activity was significantly decreased below control values. This is in contrast to the CBA/J mice in which theobromine failed to alter locomotor activity at any of the doses tested.

In the DBA/2J mice, the 32 mg/kg dose of paraxanthine resulted in the largest amount of stimulation seen with the methylxanthines in this strain. At this dose activity was increased to 345% above baseline levels. Thus the DBA/2J mice were similar to the CBA/J mice in that paraxanthine was the most effective methylxanthine in producing stimulation of locomotor activity. The 100 mg/kg dose of paraxanthine produced less stimulation than did the 32 mg/kg dose. In contrast to both the CBA/J and C57BL/6J mice the 3.2 mg/kg dose of caffeine produced a significant increase in locomotor activity. The 10 mg/kg dose of caffeine produced the maximal stimulation seen with this methylxanthine. Locomotor activity was increased to 304% of control values. As in the CBA/J and C57BL/6J strains, caffeine was more potent than the other methylxanthines. The 32 mg/kg dose of caffeine produced slightly less stimulation than the 10 mg/dose, while the 100 mg/kg dose was without significant effect on locomotor activity. The low dose of theophylline was without effect on activity while the 10 mg/kg and 32 mg/kg doses produced significant increases in activity. The largest stimulation seen with theophylline was 259% above baseline at the 32 mg/kg dose. Similar to the C57BL/6J mice, the 100 mg/kg dose of theophylline produced a significant decrease in locomotor activity. At the 100 mg/kg and 178% mg/kg dose, theobromine produced significant decreases in locomotor activity, an effect also seen in the C57BL/6J mice.

In the SWR/J mice, paraxanthine was the most effective methylxanthine in producing locomotor stimulation. The two lower doses of paraxanthine did not significantly alter activity. At the 32 mg/kg dose, paraxanthine significantly stimulated locomotor activity to 244% of control activity. In contrast to the other three strains, the 100 mg/kg dose of paraxanthine did not significantly increase activity. Theophylline was without effect on activity at the two lower doses. The 32 mg/kg dose of theophylline produced a 190% increase in locomotor activity, while the 100 mg/kg dose produced a significant decrease in the amount of activity observed. Thus, the SWR/J mice appear to be similar to the C57BL/6J and DBA/2J mice in that the highest dose of theophylline produces a decrease in locomotor activity. In contrast to the other three strains, caffeine failed to alter activity over the range of doses tested, although a small increase was seen at the 10 mg/kg dose. Theobromine also failed to stimulate locomotor activity while at the 178 mg/kg dose a significant decrease in activity was observed. As is the case with theophylline the SWR/J mice appear to be similar to the C57BL/6J and DBA/2J mice with respect to theobromine producing decreases in locomotor activity at the 178 mg/kg dose.

Three factor analysis of variance yielded significant overall results, $F(79,623)=15.91$, $p<0.01$. Significant strain, $F(3)=90.29$, $p<0.01$, drug, $F(3)=70.10$, $p<0.01$, and dose, $F(4)=68.97$, $p<0.01$, effects were obtained. Strain × drug, strain × dose, drug × dose, and strain × drug × dose interactions were significant at the $p<0.01$ level.

When analyzed by two factor analysis of variance, the CBA/J strain showed significant overall results, $F(19,166)=22.82$, $p<0.01$, with significant drug effects, $F(3)=36.79$, $p<0.01$, dose effects, $F(4)=42.77$, $p<0.01$, and drug × dose interaction, $F(12)=12.68$, $p<0.01$, being observed. In the C57BL/6J strain significant overall results were obtained, $F(19,145)=8.84$, $p<0.01$. Drug effects, $F(3)=14.89$, dose effects, $F(4)=10.04$, and drug × dose interaction, $F(12)=6.92$, were significant ($p<0.01$). The DBA/2J strain yielded significant overall results, $F(19,146)=14.35$, $p<0.01$, with significant drug and dose effects, $F(3)=27.80$ and $F(4)=25.29$, $p<0.01$, and drug × dose interaction, $F(12)=7.34$, $p<0.01$, being observed. Similarly the SWR/J strain showed significant overall results, $F(19,166)=7.21$, $p<0.01$. Drug effects, $F(3)=16.99$, dose effects, $F(4)=13.00$, and drug × dose interactions, $F(12)=2.84$, were significant ($p<0.01$). The contribution of genetic background to the rank order of maximal methylxanthine effects is demonstrated in Table 2. This ordering is uniquely associated with each of the inbred strains tested.

DISCUSSION

The results of the present study demonstrate that various inbred strains of mice have reliably different patterns of lo-

comotor activity when tested with compounds from a single chemical class. In this instance caffeine and its dimethylxanthine metabolites produced significantly different effects, depending on the strain of mouse tested. The SWR strain appeared to be hyporesponsive to the stimulant effects of the methylxanthines while within the other three strains the methylxanthines exhibited a differing rank order of maximal locomotor stimulation. This work extends the findings of Seale *et al.* [28] on the stimulant effects of caffeine to that of the other dimethylxanthines.

In an earlier study [30] caffeine maximally stimulated locomotor activity in rats at a dose of 20 mg/kg while theophylline exerted its maximal effects at 30 mg/kg. In addition theophylline appeared to produce a greater amount of locomotor stimulation than caffeine. In the present study theophylline produced a greater maximal stimulation of locomotor activity, compared to caffeine, in the CBA/J and SWR/J strains, while caffeine produced greater maximal effects in the C57BL/6J and DBA/2J strains. Snyder *et al.* [29] reported that theophylline was the most potent methylxanthine with regard to locomotor stimulation threshold at a dose of 10 μmoles/kg, while caffeine and paraxanthine produced their threshold stimulant effects at a dose of 30 μmoles/kg. However, paraxanthine appeared to be more potent when brain concentrations of the drug were taken into consideration. In their study the rank order of maximal stimulation of locomotor activity was caffeine>theophylline>paraxanthine. These data are opposite to the present study in which paraxanthine produced the greatest maximal effect in all strains except C57BL/6J. These reproducible, strain specific findings obtained in the present study suggest that different genes and, presumably, different gene products specify behavioral responsiveness to individual methylxanthines. Therefore, the differing rank orders of potency seen in the strains tested in this study and that reported by Snyder *et al.* [29] for outbred animals may be due to differences in the genome that code for those different systems that are sensitive to methylxanthines. An alternative explanation for the observed differences methylxanthine effects is that there are differences in metabolism and distribution. Differences in metabolism have been reported for some inbred strains [3]. However, a direct comparison of the caffeine plasma levels in CBA/J and SWR/J mice at a dose that stimulated CBA/J mice and decreased activity of SWR/J mice demonstrated identical plasma levels [7].

The stimulant effects of methylxanthines are generally considered to be a result of their ability to antagonize endogenous adenosine binding to adenosine receptors [3, 9, 11, 19, 29]. Adenosine is believed to exert its effects at two types of receptors, the A_1 adenosine receptor which is inhibitory to adenylate cyclase and the A_2 adenosine receptor which is stimulatory to adenylate cyclase [10]. With the exception of theobromine, the methylxanthines appear to be nonselective in their ability to antagonize A_1 and A_2 type responses [10]. The observed differences in maximal locomotor stimulant activity produced by caffeine, theophylline and paraxanthine in the inbred strains could be due to differences in brain adenosine content or to differing proportions of A_1 and A_2 receptors across the various strains. At the present time, no data are available on adenosine content, adenosine turnover or adenosine receptor differences in inbred mice. Alternatively, such observed differences may be related to varying degrees of phosphodiesterase inhibition since strain differences in phosphodiesterase levels have been reported [25]. It is possible that interstrain differences in adenosine receptor morphology may affect the relative affinities of the methylxanthines for the receptor and account for differences in maximal locomotor stimulation across strains.

The results of the present study raise some important questions about the physiology and pharmacology of adenosine. Are the stimulant effects of methylxanthines due to blockade of CNS adenosine receptors? If this is so, are mice that show little or no stimulation lacking one or more of the adenosine receptor systems? In a recent study we found that SWR/J mice were sensitive to decreases in activity produced by the adenosine receptor agonists N-cyclohexyladenosine and 5'-N-ethyl carboxamide adenosine (unpublished results). These effects were blocked by doses of caffeine that were effective adenosine antagonist in other strains. Thus, the differences in stimulant effects of caffeine in the various mouse strains cannot be due to an absence of adenosine sensitive systems. An alternative hypothesis is that the observed differences in the qualitative and quantitative effects of xanthines are due to differences in the activity of CNS purinergic neurons. Fredholm *et al.* [11, 14, 19] and others have characterized the *in vitro* release of adenosine from rat hippocampal slices. The present data suggest that adenosine is not tonically released in those mice that are refractory to caffeine and that it is tonically released in caffeine sensitive mouse strains. Thus, the observed differences in caffeine sensitivity may provide the opportunity to study the inheritance purinergic nervous system function.

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