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# CAFFEINE AND THEOPHYLLINE ANALOGUES: CORRELATION OF BEHAVIORAL EFFECTS WITH ACTIVITY AS ADENOSINE RECEPTOR ANTAGONISTS AND AS PHOSPHODIESTERASE INHIBITORS

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## Summary

The behavioral stimulant effects of xanthines, such as caffeine and theophylline, appear to involve blockade of central adenosine receptors. However, 3-isobutyl-1-methylxanthine (IBMX), a potent phosphodiesterase (PDE) inhibitor, produces behavioral depression. The effects of caffeine analogs on open field behavior of mice and potencies as antagonists of adenosine receptors and as inhibitors of three classes of brain PDE have been compared. 1,7-Dimethyl-3-propargylxanthine, 1,3,7-tripropargylxanthine, and 3,7-dimethyl-1-propargylxanthine, which have high affinity for adenosine receptors and weaker activity as PDE inhibitors, all increase behavioral activity. In contrast, 1,3,7-tripropylxanthine, a more potent inhibitor of the brain calcium-independent (Ca-indep) PDEs than 1,3,7-tripropargylxanthine, produces behavioral depression, even though both analogues are potent adenosine receptor antagonists. 7-Benzyl-IBMX, an active receptor antagonist and selective inhibitor of a brain calcium-dependent (Ca-dep) PDE, produces a slight behavioral activation. Xanthines that are potent adenosine receptor antagonists and relatively weak inhibitors of the Ca-indep PDEs reverse the depressant effects of $N^6$-cyclohexyladenosine, while xanthines, such as 1,3,7-tripropylxanthine, that are potent inhibitors of the Ca-indep PDEs, do not. The results suggest that the behavioral effects of xanthines may be determined primarily by relative activity as adenosine receptor antagonists and as inhibitors of brain Ca-indep PDEs.

Caffeine is the most widely used behavioral stimulant and mechanism of its action has been of considerable interest. Caffeine and other methylxanthines are classical antagonists at

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adenosine receptors (1,2,3). Two subtypes of cell-surface adenosine receptors ($A_1$ and $A_2$) are presently known in the brain tissue: $A_1$-receptors are inhibitory to adenylate cyclase and $A_2$-receptors are stimulatory to adenylate cyclase (4). Caffeine is equipotent at $A_1$- and $A_2$-receptors (2,5). Caffeine and other xanthines also inhibit PDEs (6) and thereby can increase concentrations of cyclic AMP in the brain. However, since caffeine and theophylline are very weak as PDE inhibitors, it is felt that the central stimulant effects of these methylxanthines are primarily due to blockade of adenosine receptors (2,7,8). Indeed, there is a good correlation between behavioral stimulant effects of xanthines and their antagonism at $A_1$-adenosine receptor sites (9). Furthermore, adenosine receptor agonists produce a behavioral sedation that can be reversed by xanthines. However, 3-isobutyl-1-methylxanthine (IBMX) is a potent adenosine receptor antagonist, but causes behavioral depression rather than stimulation (9). It was proposed that IBMX owed its behavioral depressant effects to the inhibition of phosphodiesterase (PDE). Other PDE inhibitors, such as rolipram, do cause severe sedation (10), presumably through the elevation of central levels of cyclic AMP. With respect to adenosine receptors, the role of the $A_1$- and $A_2$- subtypes in behavioral effects of xanthines remains unclear. Certainly, a selective $A_1$-receptor agonist, $N^6$-cyclohexyladenosine (CHA), can cause behavioral depression (9). However, the very potent $A_2$-receptor agonist, 5′-N-ethylcarboxamidoadenosine (NECA), which would be expected to increase cyclic AMP levels through activation of $A_2$-receptors, while concomitantly inhibiting formation of cyclic AMP through activation of $A_1$-receptors, is also profoundly depressant (11). It appears that adenosine-induced depression can be mediated through activation of either $A_1$- or $A_2$-receptors. The depressant action of both CHA and NECA are antagonized by caffeine and other xanthines (9,11).

In an attempt to clarify mechanisms involved in behavioral effects of xanthines, dose-dependency as antagonists of $A_1$- and $A_2$-adenosine receptors, as inhibitors of PDEs, and as modifiers of motor activity in control and CHA-treated mice has been compared for caffeine, theophylline, IBMX and seven caffeine analogues.

## Materials and Methods

Materials: [$^3$H]$N^6$-Phenylisopropyladenosine ([$^3$H]PIA, 38.5 Ci/mmol) and [2,8-$^3$H]cyclic AMP (36.1 Ci/mmol) were from New England Nuclear, Boston, MA. 1,7-Dimethyl-3-propargylxanthine, 1,3,7-tripropargylxanthine and enprofylline were provided by Dr. John Neumeyer, Research Biochemicals, Wayland, MA and were prepared under NIH grant SBIR 1R43AM37728-01. $N^6$-Cyclohexyladenosine and caffeine were from Sigma Chemical Co., St. Louis, MO; 3-isobutyl-1-methylxanthine from Aldrich Chemical Co., Milwaukee, WI; theophylline from Calbiochem, La Jolla, CA; and rolipram from Schering AG, Berlin. Synthesis of other xanthines has been described (7).

Assay of Activity of Adenosine Receptors: Inhibition of binding of 1 nM [$^3$H]PIA (38.5 Ci/mmol) to $A_1$-adenosine receptors in mouse brain membranes (male NIH, 20-35 g) was assayed as described for rat brain membranes (12). Whole mouse brain minus cerebellum and brain stem was used. Inhibition of binding by a range of concentrations of xanthine was determined in triplicate

in three experiments. Ki values ± SEM were calculated from $IC_{50}$ values using the Cheng and Prusoff equation (13) and a $K_D$ of 1.0 nM determined for binding of [$^3$H]PIA to mouse brain membranes. Data on affinity of xanthines for $A_2$-adenosine receptors in rat PC12 membranes were determined from concentration-response curves of 5′-N-ethylcarboxamidoadenosine (NECA) for the stimulation of adenylate cyclase of PC12 membranes in the presence and absence of the xanthines in three experiments (14). $K_B$ values for xanthines were calculated from the Schild equation (15) $K_B = C/(R-1)$, where C denotes the concentration of the xanthine and R denotes the ratio of the $EC_{50}$ values for NECA in the presence and absence of xanthine. Data are reported in Table 1 as means ± SEM (n = 3).

Assay of Inhibition of Phosphodiesterase Activity: Activity of xanthines as inhibitors of three classes of PDEs (soluble Ca-dep, soluble Ca-indep and membrane-bound Ca-indep PDEs) were determined. Male Sprague-Dawley rats (175-225 g) were sacrificed and a supernatant fraction and 105,000 x g pellet were obtained from washed brain after homogenization in 2 vol. of ice cold 20 mM Tris-HCl buffer (pH 7.5), containing 1 mM $MgCl_2$, 3 mM 2-mercaptoethanol and 0.1 mM EGTA, using a Brinkmann Polytron (setting 6, 15 sec) as described (16,17). A 1:10 and 1:20 dilution of the supernatant was used for assay of Ca-dep and Ca-indep PDE, respectively. For membrane-bound PDE, the 105,000 x g pellet was resuspended by vortexing in 2 vol. of the same buffer and then, centrifuged at 105,000 x g for 10 min. Centrifugation and resuspension were repeated 5 times and the final pellet was resuspended in 2 vol. of the same buffer. PDE activity was assayed essentially as previously described (17). The standard reaction mixture, in a final vol. of 0.1 ml, contained 0.5 µmol Tris-HCl buffer (pH 7.5), 0.5 µmol $MgCl_2$, and 10 µl of enzyme preparation. A concentration of 1 µM [$^3$H]cyclic AMP (36.1 Ci/mmol) was used for soluble Ca-indep and membrane PDEs and 50 µM [$^3$H]cyclic AMP for soluble Ca-dep PDE. For the assay of soluble Ca-indep and membrane PDE, 55 mM KCl and 0.1 mM EGTA were present in the reaction mixture to eliminate any contribution to activity from Ca-dep PDEs (17). For the assay of soluble Ca-dep PDE, 50 µM $CaCl_2$ and 150 µM 2-deoxy-cyclic AMP were present in the reaction mixture, the latter to eliminate any contribution to activity from Ca-indep PDE (17). Incubations were conducted at 37°C for 5 min and were terminated by heating at 100°C for 1 min. The product [$^3$H]5′-AMP was separated from remaining [$^3$H]cyclic AMP, using polyacrylamide-boronate gel affinity chromatography and assayed as described (18). $IC_{50}$ values are reported in Table 1 as means ± SEM (n = 3).

Locomotor Activity: Male NIH mice (National Cancer Institute, Bethesda, MD), weighing 20-35 g were housed in 15-20 per cage in 25 x 43 x 17 cm polypropylene cages and given food and water *ad libitum*. Mice were exposed to a 12-hr/12-hr light/dark cycle and allowed to adjust to their housing for at least 72 hrs before tested. Drugs were dissolved in an Emulphor and saline (1:4) mixture, and were then administered intraperitoneally (i.p.) in a volume of 5 ml/kg. The vehicle was injected to control animals. The mouse was immediately placed in the open field chamber. Locomotor activity measurements were started 10 min later. For xanthine reversal of CHA-induced depression, each xanthine was injected 1 min before CHA administration. The behavioral depressant effects of CHA in mice appear centrally

mediated in view of the lack of reversal by 8-p-sulfophenyl-theophylline, an adenosine receptor antagonist that does not penetrate into brain (see ref. 19). Locomotor activity was measured using an Opto-Varimax instrument, equipped with Apple IIe computer (Columbus Instruments, Columbus, Ohio). Data were analyzed, using one-way analysis of variance (ANOVA) and Student's t test was used for individual comparisons.

## Results

### Antagonistic Activity of Xanthines at Adenosine Receptors

Activities of a variety of theophylline and caffeine analogues as antagonists at adenosine receptor sites have been determined (5,14, and Table 1). All of these analogs are antagonists at $A_2$-adenosine receptors (Table 1) and a representative 10 of the 13 xanthines have been shown to be functional antagonists at fat cell $A_1$-adenosine receptors (see ref. 14). In general, substitution of theophylline and caffeine with propyl or propargyl substituents at the 1-, 3-, and/or 7- positions increases affinity of xanthine at both receptors (Table 1). Substitution of 1-methyl group of caffeine with propargyl produces selectivity for $A_2$-receptors. The affinity of xanthines for $A_1$-adenosine receptors are similar in membranes from mouse (Table 1) and rat brain (5), except for 1,3-dipropyl-7-methylxanthine and 3,7-dimethyl-1-propargylxanthine, which are about twice as potent in membranes from mouse brain than in membranes from rat brain.

### Inhibitory Effects of Xanthines on PDE activity

Potencies of caffeine and theophylline analogues in inhibiting rat brain PDEs (Table 1) are quite different from potencies in blocking adenosine receptors. Replacement of methyl groups of caffeine with n-propyl or propargyl results in more potent PDE inhibitors, as has been the case for the blockade of adenosine receptors. Substitution of methyl groups of caffeine with n-propyl groups gives rise to selectivity for Ca-indep PDEs, while substitution with propargyl groups does not. Selectivity for Ca-indep PDEs is greatest when all three methyl groups of caffeine are replaced with n-propyl groups (Table 1). 7-Benzyl-IBMX is the only analogue showing significant selectivity for Ca-dep PDEs.

### Effects of Xanthines on Mouse Locomotor Activity

CHA produces behavioral depression in a dose-dependent manner (Fig. 1). At 3 μmol/kg, mice are totally sedated. Depressant effects of CHA are reversed by theophylline (Fig. 2). The antagonism by theophylline is dose-dependent and with 10 μmol/kg of theophylline, distance traveled is not significantly different from control (Fig. 2). Treatment with theophylline alone results in a marked stimulation of locomotor activity.

Behavioral effects of 1,3,7-tripropargylxanthine are compared with those of 1,3,7-tripropylxanthine in Fig. 3. 1,3,7-Tripropargylxanthine, which is very potent adenosine receptor antagonist, produces marked stimulant effects and is the most potent in this regard among xanthines tested (Fig. 3A). However, 1,3,7-tripropylxanthine, which has similar potency as an adenosine receptor antagonist to the 1,3,7-tripropargylxanthine, produces behavioral depression instead of stimulation (Fig. 3B). 1,3,7-Tripropargylxanthine reverses the depressant effects of CHA, while 1,3,7-tripropylxanthine does not (Fig. 3). These two compounds

TABLE 1

Behavioral and Biochemical Activity of Xanthines.

| Xanthines (X) (Me = methyl) | Behavioral Effects (% of control)[a] | Affinity for Receptors Mouse $A_1$[b] (Ki, μM) | Rat $A_2$[c] ($K_B$, μM) | Rat PDE Activity ($IC_{50}$, μM) Soluble Ca-dep | Soluble Ca-indep | Membrane Ca-indep |
|---|---|---|---|---|---|---|
| Caffeine | 220 ± 29 | 59 ± 3.5 | 37 | 750 ± 87 | 480 ± 30 | 750 ± 84 |
| Theophylline | 280 ± 78 | 15 ± 2.3 | 17 | 350 ± 82 | 670 ± 140 | 550 ± 5.0 |
| IBMX | 22 ± 17 | 6.7 ± 0.5 | 3.2 ± 0.4 | 10 ± 0.9 | 16 ± 4.7 | 16 ± 4.0 |
| 1,3,7-Tripropargyl-X | 260 ± 160 | 3.8 ± 0.4 | 1.9 ± 0.5 | 46 ± 10 | 97 ± 10 | 120 ± 16 |
| 1,3,7-Tripropyl-X | 0 | 3.0 ± 0.09 | 6.5 | 89 ± 13 | 2.3 ± 0.3 | 5.9 ± 0.2 |
| 1,7-DiMe-3-propargyl-X | 250 ± 65 | 20 ± 2.3 | 21 ± 3.7 | 270 ± 20 | 220 ± 20 | 290 ± 21 |
| 3,7-DiMe-1-propargyl-X | 200 ± 130 | 22 ± 1.6 | 9.6 | 510 ± 190 | 200 ± 5.8 | 400 ± 30 |
| 7-Benzyl-IBMX | 130 ± 91 | 6.9 ± 0.4 | 7.5 ± 0.6 | 5.1 ± 0.55 | 41 ± 6.9 | 37 ± 11 |
| 1,3-Dipropyl-7-Me-X | 3.5 ± 2.7 | 3.2 ± 0.2 | 5.3 | 370 ± 90 | 9 ± 0.6 | 11 ± 0.8 |
| 1,7-DiMe-3-propyl-X | 81 ± 45 | 18 ± 2.8 | 15 | 230 ± 70 | 73 ± 9 | 110 ± 14 |
| 1,3-DiMe-7-propyl-X | 160 ± 17 | 31 ± 2.6 | 8.2 | 350 ± 50 | 280 ± 25 | 380 ± 33 |
| 1,3-Dipropyl-X | 3.6 ± 3.2 | 1.4 ± 0.2 | 5.4 | 130 ± 17 | 13 ± 2.1 | 11 ± 2.3 |
| Enprofylline (3-propylX) | 120 ± 49 | 55 ± 5.6 | 120 ± 17 | 560 ± 38 | 180 ± 30 | 340 ± 55 |

a. Xanthine dosage, 100 μmol/kg; b. Inhibition of [$^3$H]PIA binding to brain membranes (see Methods); c. Inhibition of NECA-stimulated PC12 cell adenylate cyclase (see Methods); single values are means from ref. 14.

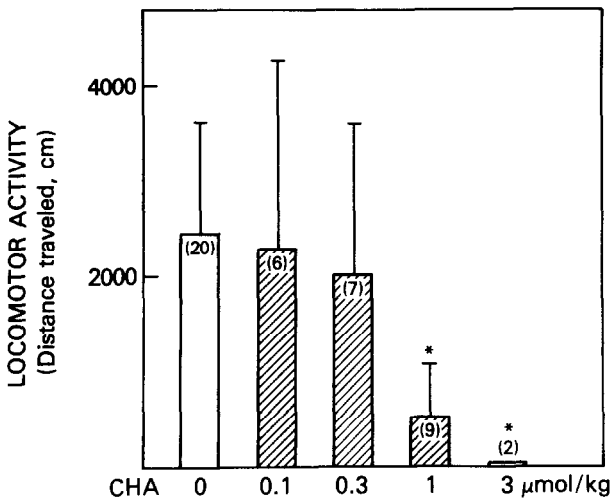


FIG. 1

Effects of CHA on locomotor activity of mice. Error bars indicate SD. The numbers of animals are in parentheses. *Significantly different from control, $p < 0.05$ by Student's t test.

differ in the effects on PDE activity (Table 1). 1,3,7-Tripropylxanthine is a selective inhibitor of Ca-indep PDEs. 1,3,7-Tripropargylxanthine is a much weaker inhibitor of Ca-indep PDEs than the 1,3,7-tripropylxanthine, while being only 2-fold less potent than 1,3,7-tripropylxanthine as an inhibitor of Ca-dep PDEs.

Behavioral effects of two other caffeine analogs are presented in Fig 4. 1,7-Dimethyl-3-propargylxanthine and 3,7-dimethyl-1-propargylxanthine, which are equipotent in receptor binding, are nearly equipotent in behavioral stimulant effects (Figs. 4A,B). Both xanthines reverse the depressant effects of CHA. Both of these compounds are relatively weak as PDE inhibitors (Table 1).

7-Benzyl IBMX, a potent adenosine receptor antagonist, is a weak behavioral stimulant and is relatively ineffective in reversing CHA depression (Fig 5). 7-Benzyl-IBMX is a somewhat selective inhibitor of Ca-dep PDE (Table 1).

Certain other xanthines, including IBMX and enprofylline, were tested for behavioral effects at a single dosage of 100 μmol/kg. IBMX (3-isobutyl-1-methylxanthine) was a behavioral depressant and was a potent inhibitor of both Ca-indep and Ca-dep PDEs (Table 1). Enprofylline (3-propylxanthine) had no significant behavioral effect and was relatively weak both in blocking adenosine receptors and as a inhibitor of PDEs (Table 1). Rolipram, a potent and specific inhibitor of Ca-indep PDE (18) was administered to mice at 10 μmol/kg and at this dose caused complete inhibition of behavioral activity. Rolipram was very weak as an inhibitor of mouse brain $A_1$-adenosine receptors (Ki > 250 μM) and of rat PC12 cell $A_2$-adenosine receptors ($K_B$ > 100 μM). It

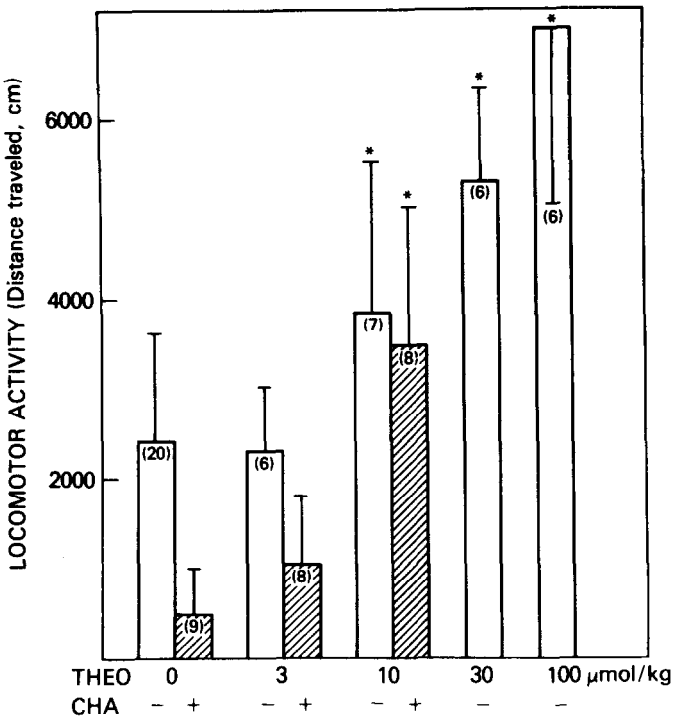


FIG. 2

Effects of theophylline in the absence (open bars) and the presence (hatched bars) of CHA on locomotor activity of mice. Error bars indicate SD. The numbers of animals are in parentheses. *Significantly different from control (open bars) or from CHA ( 1 μmol/kg) alone (hatched bars), $p < 0.05$ by Student's t test.

was a weak inhibitor of rat brain soluble Ca-dep PDE ($IC_{50}$, 340 ± 3.1 μM), but was a very potent inhibitor of soluble and membrane Ca-indep PDEs ($IC_{50}$ values, 1.1 ± 0.07 μM and 1.1 ± 0.2 μM, respectively).

## Discussion

Antagonism of central adenosine receptors has been proposed to be primarily responsible for the central stimulant effects of methylxanthines, such as caffeine and theophylline (9). Indeed, with the exception of IBMX, the potency of a various xanthines as antagonists of brain $A_1$-adenosine receptors correlates well with ability of xanthines to stimulate behavioral activity in mice (9). The anomalous behavioral depressant activity of IBMX was proposed to be due to its potent activity as a PDE inhibitor (9), which presumably predominated over its potent activity as an adenosine receptor antagonist. A variety of additional analogues of caffeine and theophylline have now been synthesized and their potency and selectivity towards $A_1$- and $A_2$-adenosine receptors have been determined. In particular, analogues of caffeine have been prepared in which the 1-, 3- and/or 7-methyl groups of caffeine have

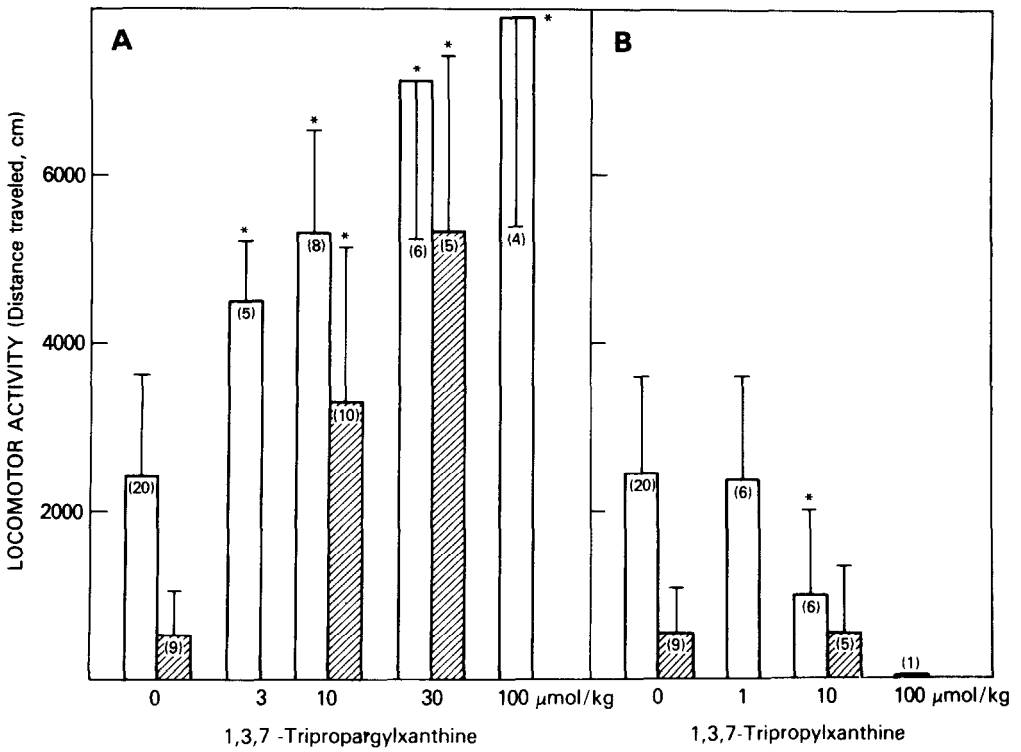


FIG. 3

Effects of 1,3,7-tripropargylxanthine (A) and 1,3,7-tripropylxanthine (B) in the absence (open bars) and the presence (hatched bars) of CHA on locomotor activity of mice. Error bars indicate SD. The numbers of animals are in parentheses. *Significantly different from control (open bars) or from CHA ( 1 μmol/kg) alone (hatched bars), $p < 0.05$ by Student's t test.

been replaced with n-propyl or propargyl groups (7). Such replacement significantly increases activity at both $A_1$- and $A_2$-receptors (5,14, see also Table 1). The effects of these caffeine analogues on behavior have not yet been systemically investigated. One analogue, 3,7-dimethyl-1-propargylxanthine is a potent behavioral stimulant and selectively reverses NECA-induced depression compared to CHA-induced depression (19). Such a selective reversal is consonant with a selectivity of this xanthine for $A_2$-receptors compared to $A_1$-receptors (5,14). NECA is a potent $A_2$-receptor agonist, while CHA is a potent and highly selective agonist for $A_1$-receptors (2). Another caffeine analogue, namely 1,3-dipropyl-7-methylxanthine had somewhat selective in vivo antagonistic effects on hypotensive responses due to activation of $A_2$-receptors by intravenous injection of adenosine analogues, while being somewhat less effective versus cardiac depressant responses due to activation of $A_1$-receptors (20). Nothing was known as to the activity of the caffeine analogues as inhibitors of PDEs.

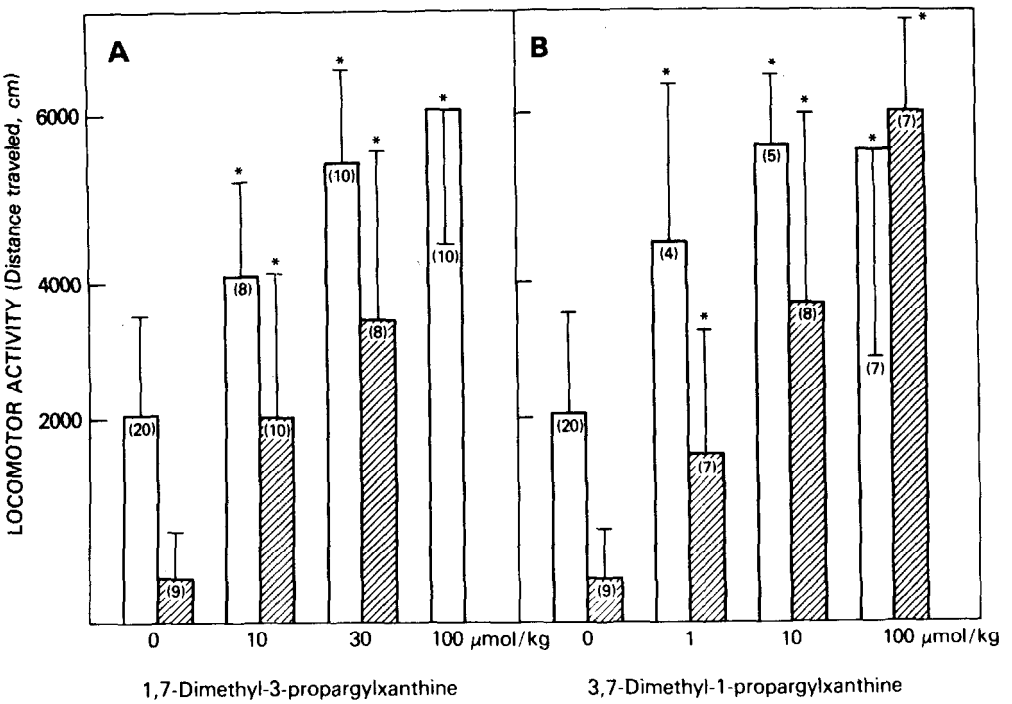


FIG. 4

Effects of 1,7-dimethyl-3-propargylxanthine (A) and 3,7-dimethyl-1-propargylxanthine (B) in the absence (open bars) and the presence (hatched bars) of CHA on locomotor activity of mice. Error bars indicate SD. The numbers of animals are in parentheses. *Significantly different from control (open bars) or from CHA ( 1 μmol/kg) alone (hatched bars), $p < 0.05$ by Student's t test.

Brain contains several different PDEs and it is unknown to what extent one or all of such enzymes are involved in behavioral depressant effects of PDE inhibitors. The current study measured effects on three such enzymes in rat brain, namely, soluble Ca-dep, soluble Ca-indep and membrane-bound Ca-indep PDEs. Structure-activity analysis of the inhibitory effects on PDEs of these alkylxanthines indicates that substitution of methyl groups of caffeine with either propyl or propargyl increases activity as PDE inhibitors (Table 1). However, propyl substitution gives rise to very large increases in potency as inhibitors of Ca-indep PDEs, while propargyl substitution has only modest effects on inhibitory potency for these PDEs. The propyl analogues are, unlike the propargyl analogues, relatively selective and very potent inhibitors of the Ca-indep PDEs. The propargyl analogues are, like caffeine and theophylline, relatively non-selective inhibitors. The 1,3-dipropyl analogue of theophylline is also selective for the Ca-indep PDEs. 7-Benzyl-IBMX is selective for the soluble Ca-dep PDE, while IBMX is relatively non-selective. Enprofylline, a xanthine with little or no central activity (21), was somewhat selective for the soluble Ca-indep PDE (Table 1), but was much less potent than the propyl analogues of caffeine or theophylline.

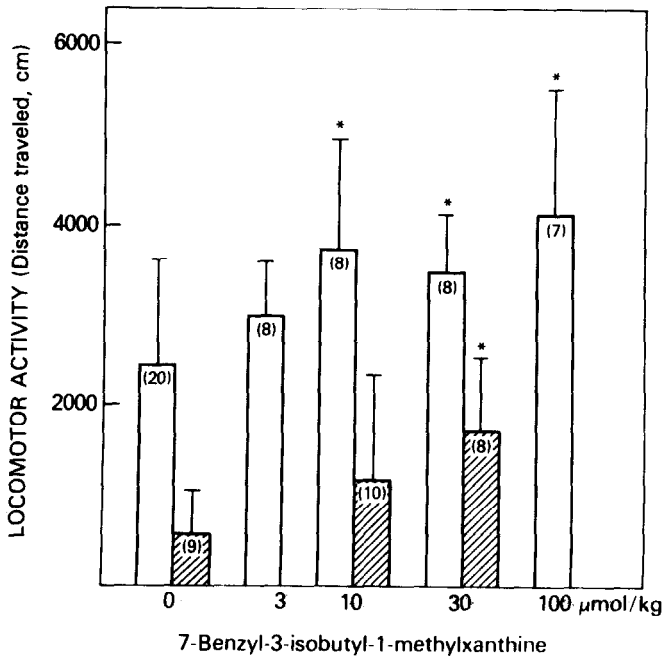


FIG. 5

Effects of 7-benzyl-3-isobutyl-1-methylxanthine in the absence (open bars) and the presence (hatched bars) of CHA on locomotor activity of mice. Error bars indicate SD. The numbers of animals are in parentheses. *Significantly different from control (open bars) or from CHA ( 1 μmol/kg) alone (hatched bars), $p < 0.05$ by Student's t test.

A subset of the xanthines were investigated for effects on open field locomotor activity, both alone and in combination with depressant dosages of the $A_1$-receptor agonist CHA. Theophylline, 1,3,7-tripropargylxanthine, 1,7-dimethyl-3-propargylxanthine and 3,7-dimethyl-1-propargylxanthine are all behavioral stimulants and all reverse CHA-elicited depression (Figs. 2,3A,4). All are relatively weak and non-selective inhibitors of PDE. In contrast, 1,3-dipropyl-7-methylxanthine and 1,3,7-tripropylxanthine are potent and selective inhibitors of Ca-indep PDE and both are central depressants (Table 1). 1,3,7-Tripropylxanthine does not reverse CHA-elicited depression (Fig. 3). Rolipram, a non-xanthine, very selective inhibitor of Ca-indep PDE (19, Table 1), is a behavioral depressant (10, Table 1). It appears that the behavioral depressant caffeine analogues are those that are selective or potent inhibitors of Ca-indep PDEs. The behavioral effects of 7-benzyl-IBMX, which is a potent selective inhibitor of the Ca-dep PDE, are consonant with this interpretation. 7-Benzyl-IBMX is not a behavioral depressant, but instead, has slight stimulant activity and partially reverses depressant effects of CHA (Fig. 5). The lack of behavioral activity of enprofylline compared to the stimulant properties of caffeine appears somewhat remarkable, since both xanthines are nearly equipotent in mouse brain $A_1$-adenosine receptors (Table 1). Enprofylline, however, is

many fold less potent at the $A_2$-adenosine receptor than caffeine. In addition, the potency of enprofylline at the $A_1$-adenosine receptors is only 3-fold greater than the potency as an inhibitor of the soluble Ca-indep PDE, while caffeine is 8-fold less potent. It should also be noted that enprofylline is very potent as an inhibitor of certain other PDE isozymes (22).

In summary, potencies of caffeine analogues as behavioral stimulants are consonant with their potency as antagonists at adenosine receptors except for xanthines that are potent inhibitors of PDEs. It would appear likely that the inhibition of PDEs, in particular Ca-indep PDEs, by xanthines results in a behavioral depression. The depressant 1,3,7-tripropylxanthine does not reverse CHA-induced behavioral depression, probably because its own depressant effects mask any effects of blockade of adenosine receptors. However, IBMX at low dosages has been reported to reverse depressant effects of CHA (9), an anomalous effect as yet unexplained. In a series of theophylline analogues, an increase in potency as inhibitors of PDEs has been shown to be associated with a shift from central stimulant properties in theophylline to depressant properties in 8-hexyltheophylline (23). The present results suggest that inhibition of Ca-indep PDE may be more involved in mechanisms leading to the depression that is induced by PDE inhibitors than is inhibition of Ca-dep PDE. Thus, selective inhibitors of Ca-indep PDE, such as rolipram and 1,3,7-tripropylxanthine, produce depression, while caffeine analogues, such as 1,3,7-tripropargylxanthine, that are weaker inhibitors of Ca-indep PDE produce behavioral stimulation. Further studies will be required to determine whether ratio of potencies as adenosine receptor antagonists and as selective inhibitors of PDEs may be predictive of the behavioral effects of xanthines and other adenosine receptor antagonists.

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