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Effects of Uric Acid and Caffeine on A_1 Adenosine Receptor Binding in Developing Rat Brain

ROBERT E. HUNTER, CARLOS M. BARRERA,*[1] GARY P. DOHANICH AND WILLIAM P. DUNLAP

*Department of Psychology, Tulane University and *Veterans Administration Medical Center, New Orleans, LA*

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HUNTER, R. E., C. M. BARRERA, G. P. DOHANICH AND W. P. DUNLAP. *Effects of uric acid and caffeine on A_1 adenosine receptor binding in developing rat brain.* PHARMACOL BIOCHEM BEHAV **35**(4) 791–795, 1990.— Previous studies have demonstrated that elevated levels of serum uric acid or caffeine are associated with increased locomotor activity in rats and humans. Since uric acid and caffeine are structurally similar, it was hypothesized that these compounds alter locomotor activity through a common neural mechanism, specifically by acting as receptor antagonists at adenosine A_1 binding sites. In vitro competition of caffeine and uric acid against the A_1 agonist, [^{3}H] cyclohexyladenosine ([^{3}H]CHA), was conducted using homogenates of adult rat forebrain. Caffeine effectively competed for the A_1 binding site as previously reported (IC_{50} = 107 μM), but uric acid failed to compete with [^{3}H]CHA binding at concentrations within a relevant physiological range. Nevertheless, in vivo experiments indicated that chronic elevation of uric acid following allantoxanamide treatment of male rats on days 4–27 of life significantly decreased A_1 receptor binding in the striatum, a region traditionally implicated in mammalian locomotion. In contrast, chronic caffeine treatment on days 4–27 of life caused an increase in A_1 receptor binding in the cortex similar to increases reported previously in whole brain. These changes in A_1 receptor binding following chronic elevation of uric acid or caffeine did not persist in rats that had been withdrawn from allantoxanamide or caffeine treatment for 14 days. Results from in vitro and in vivo experiments indicate that despite a similar molecular structure uric acid does not act by the same mechanism as caffeine to increase locomotor activity in rats.

Adenosine Caffeine Uric acid Allantoxanamide Locomotor activity

IT has been suggested for over a century that elevated serum uric acid levels are associated with higher intelligence and achievement (3). Correlations between serum uric acid and intelligence measures, however, have been rather modest (31), while college grades have shown a U-shaped relation to serum uric acid level (32). A more reliable relationship is the correlation of serum uric acid level with general activity and goal-oriented behavior (7, 12, 19, 20, 29). Barrera, Ruiz and Dunlap (2) reported serum uric acid levels to be positively correlated to symptoms of hyperactivity in children. Evidence for a similar increase of urinary uric acid level in hyperactives has been shown (16).

The structural similarity of uric acid to caffeine and other methylxanthines with behavioral actions suggests that these compounds may influence behavior through a common mechanism (26). The behavioral effects of caffeine have been shown to be mediated through its ability to block adenosine receptors in the brain (9). Chronic caffeine consumption has been shown to increase adenosine A_1 receptor number in rats by up-regulation of receptors (4). Because both caffeine and uric acid elevate activity levels, as well as share a similar molecular structure, we examined whether uric acid, like caffeine, interacts with adenosine A_1 receptors in various areas of rat brain under in vitro and in vivo conditions.

Hyperuricemia models in the rat are complicated by the fact that rats have high levels of the enzyme, uricase, which rapidly metabolizes uric acid to allantoin. In order to produce hyperuricemia in rats in the present study, the compound allantoxanamide was administered to block uricase and cause the accumulation of endogenous uric acid (18). Barrera, Hunter and Dunlap (3) found that daily allantoxanamide treatment in developing rats increased both serum uric acid levels and locomotor activity.

In the first experiment, in vitro competition of caffeine, uric acid, and allantoxanamide for adenosine A_1 receptors was determined in forebrain preparations from adult male rats. In the second and third experiments, the effects of chronic elevation of uric acid by daily injections of allantoxanamide on adenosine A_1 receptor binding was investigated in various brain regions of young male rats. It was hypothesized that uric acid, like caffeine, would compete for A_1 receptor sites under in vitro conditions and induce up-regulation of A_1 receptor sites following chronic elevation

[1]Requests for reprints should be addressed to Carlos M. Barrera, M.D., VA Medical Center/Research 151, 1601 Perdido St., New Orleans, LA 70146.

under in vivo conditions.

EXPERIMENT 1

In Experiment 1, the effects of uric acid, allantoxanamide, and caffeine on adenosine A_1 receptor binding were examined in adult rat forebrain tissue under in vitro conditions to determine if these compounds interact directly with A_1 binding sites.

Method

Ten adult male rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) were maintained on a 12:12 reversed light-dark cycle with free access to food and water. The rats (250–350 g) were sacrificed by decapitation 2–3 hr after onset of the dark phase. The brain of each animal, minus the cerebellum, was prepared following the procedure of an earlier study (4). Tissues were homogenized in 20 volumes of cold 50 mM Tris-HCl buffer (pH = 7.4) in a glass vessel with a Teflon pestle. Homogenates were centrifuged at 30,000 × g for 20 min at 4°C. After discarding the supernatant, the pellet was resuspended in buffer and centrifuged again at 30,000 × g for 20 min at 4°C. The resuspended pellet was then incubated with adenosine deaminase (2 units/ml of tissue) at room temperature for 30 min to remove endogenous adenosine. The preparation was centrifuged again at 30,000 × g for 20 min at 4°C. After discarding the supernatant, the pellet was resuspended in buffer and stored at −60°C. Freezing did not alter binding.

Competition assays were performed using the stable adenosine analog N^6-cyclohexyl [^{3}H] adenosine ([^{3}H]CHA, 27 Ci/mmol, NEN Products, Boston, MA) at a concentration of 3 nM. Each assay was performed in triplicate by incubating 200 μg of membrane suspension with [^{3}H]CHA and the competitor (caffeine, uric acid, or allantoxanamide) in 50 mM Tris-HCl (pH 7.4) at a final volume of 500 μl for 120 min at room temperature. Stock dilutions of competitor were prepared in 50 mM Tris-HCl (pH = 7.4) to obtain final incubation concentrations for caffeine (10^{-8} M to 0.05 M), uric acid (2.0×10^{-4} M to 5.3×10^{-3} M), and allantoxanamide (2.0×10^{-4} M to 5.3×10^{-3} M). Uric acid was dissolved in 50 mM Tris base and the pH adjusted to 7.4 with 4 N HCl. Competition with [^{3}H]CHA by uric acid in the presence of 5.3×10^{-3} M allantoxanamide was also examined.

Nonspecific binding was determined in the presence of 5 μM unlabeled CHA. Incubations were terminated by vacuum filtration through Whatman GF/B glass fiber filters. The filters were air dried and counted in 5 ml of Liquiscint scintillation fluid (National Diagnostics, Manville, NJ). Protein content was determined by the Bradford (6) method using bovine serum albumin for standards. Unless otherwise specified all chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

Results

Competition data were transformed to a logit plot in order to calculate the IC_{50} by linear regression. Caffeine competed with [^{3}H]CHA (3 nM) at an IC_{50} of 107 μM in agreement with previous reports (8, 24, 30). In addition, the slope of the regression line was −2.3 which suggests competition at a single binding site. Uric acid also competed with [^{3}H]CHA (3 nM), although its competition curve is markedly shifted towards the right compared to that of caffeine (Fig. 1). Allantoxanamide did not inhibit binding at any of the concentrations used. An IC_{50} was not calculated for the uric acid competition curve due to insufficient data points. No difference was found between uric acid competition alone and uric acid competition in the presence of a high concentration of allantoxanamide.

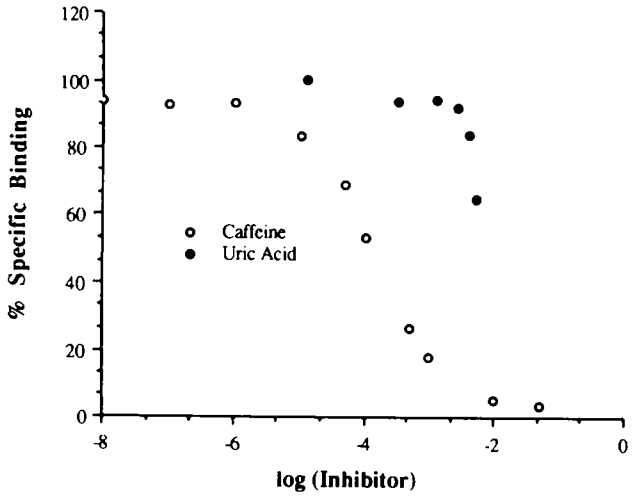

FIG. 1. Inhibition of [^{3}H]CHA binding by caffeine and uric acid.

EXPERIMENT 2

In Experiment 2, the in vivo effects of chronic administration of caffeine and allantoxanamide on A_1 adenosine receptors binding were examined in various brain areas from young male rats in order to determine if repeated exposure to these compounds caused a similar up-regulation of A_1 receptors.

Method

Twelve adult female Sprague-Dawley rats were mated to a criterion of three ejaculations by adult male rats. The 11 litters produced from these matings containing a minimum of three male pups formed the subject pool. The pups were housed with their mothers until weaning at 27 days of age, after which they were separated from their mothers and housed 3 per cage. On the day of birth (Day 1), three male pups from each litter were randomly assigned to one of three treatment groups (control, caffeine, allantoxanamide; n = 10) and were marked subcutaneously with india ink to denote group membership. Beginning on Day 4, the male pups received daily subcutaneous injections of either caffeine (50 mg/kg) or allantoxanamide (100 mg/kg) suspended in a vehicle of 0.25% methyl cellulose or vehicle alone (control). Daily weights were recorded to maintain proper dosing. Injections continued through Day 27. On Day 28, the rats were decapitated, the brains were removed, and the cortex, striatum, and thalamus of each brain were dissected (14).

The brain areas were homogenized individually by area and by rat in 1 ml of cold Tris-HCl buffer (pH = 7.4) in glass vessels with a Teflon pestle. The homogenates were prepared as described in Experiment 1 and stored at −60°C. Binding was determined at a concentration 3 nM [^{3}H]CHA. Each assay was performed in triplicate, as described in Experiment 1, by incubating the membrane suspension (150 μg protein) and the ligand in 50 mM Tris-HCl (pH 7.4) at a final volume of 500 μl for 120 min at room temperature.

Results

Small decreases in body weight were recorded in the allantoxanamide (80 g) and caffeine groups (76 g) compared to control males (84 g). There were no other apparent adverse health effects due to the treatment and none of the animals died.

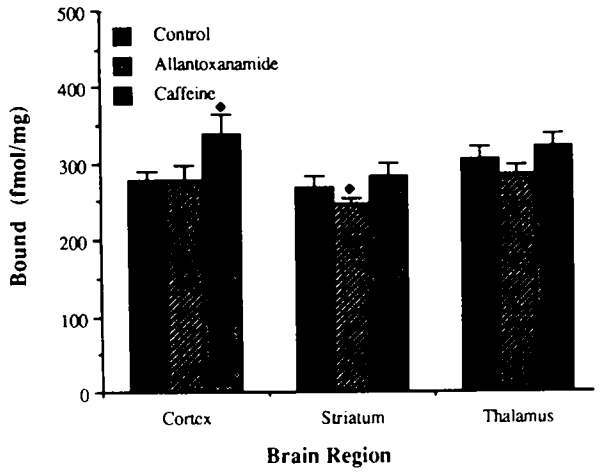

FIG. 2. Regional distribution with standard error bars of [^{3}H]CHA binding in the brains of 28-day-old male rats (n = 11) injected daily with control, allantoxanamide, or caffeine. (◇ = significantly different from control.)

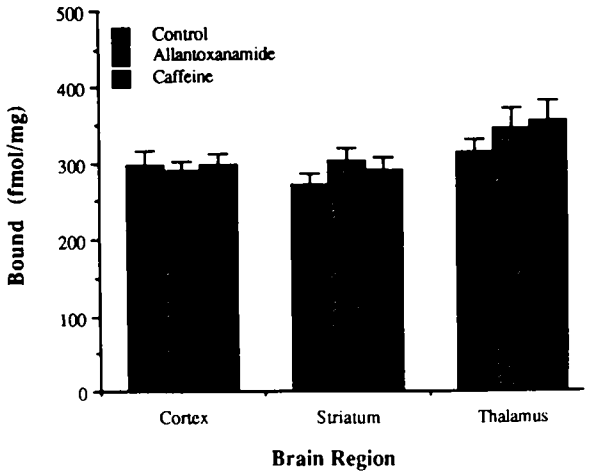

FIG. 3. Regional distribution with standard error bars of [^{3}H]CHA binding in the brains of 42-day-old rats (n = 7) injected daily with control, allantoxanamide, or caffeine for 28 days then stopped till day 42.

A matched subjects (litter mates) analysis of variance (ANOVA) with the factorial variables treatment and brain area was conducted on the data from the single point assays. Significant effects were further analyzed by the Newman-Keuls procedure. [^{3}H]CHA binding (Fig. 2) showed both significant treatment effects in response to chronic caffeine or allantoxanamide treatment, $F(2,20) = 5.09$, $p = 0.0163$, and brain region differences, $F(2,20) = 4.48$, $p = 0.0247$. Newman-Keuls comparisons indicated that caffeine-treated rats had significantly increased binding over controls ($p = 0.0462$). The allantoxanamide-treated rats displayed less binding than the controls, although not significantly less.

There was also a significant treatment by brain area interaction, $F(4,40) = 3.05$, $p = 0.0278$. Chronic caffeine administration produced a significant increase in binding in the cortex ($p < 0.0001$) compared to controls. There was a nonsignificant trend towards increased binding in the thalamus and the striatum following chronic caffeine treatment. Chronic allantoxanamide treatment produced a significant decrease in [^{3}H]CHA binding compared to controls in the striatum ($p = 0.0383$). There was also a nonsignificant trend towards decreased binding in the thalamus ($p = 0.0812$). No significant difference in binding between allantoxanamide and controls was observed in the cortex.

EXPERIMENT 3

Experiment 3 was similar to Experiment 2 except that chronic caffeine and allantoxanamide treatments were suspended for two weeks prior to assay in order to examine the permanence of treatment effects on A_1 binding.

Method

Seven of the 11 litters from Experiment 2 contained at least three extra males which were used as the subjects in Experiment 3. The three extra males from each of these litters were randomly assigned to three treatment groups (control, caffeine, or allantoxanamide). These pups received the same treatments as the pups in Experiment 2 and were given their last injections on Day 27 followed by a two-week period without treatment. On Day 42, the pups were decapitated, the brains were removed, and the cortex, striatum, and thalamus of each brain were prepared as described in Experiment 2. Binding was determined at a concentration of 3 nM of [^{3}H]CHA as previously described.

Results

ANOVA of [^{3}H]CHA binding 14 days after withdrawal of the treatments (Fig. 3) revealed significant brain region differences in binding, $F(2,12) = 10.31$, $p = 0.0025$, however, neither the treatment effect, $F(2,12) = 2.07$, $p = 0.1685$, nor the treatment by brain region interaction was significant. Therefore, there is little evidence of permanent effects of caffeine or allantoxanamide on A_1 adenosine receptor binding.

DISCUSSION

In Experiment 1, in vitro inhibition of [^{3}H]CHA binding by caffeine ($IC_{50} = 107$ μM) was consistent with in vitro effects reported in previous studies in which [^{3}H]CHA concentrations of 1–3 nM were inhibited at IC_{50} values of 50–100 μM (8, 24, 30). Allantoxanamide did not inhibit [^{3}H]CHA binding throughout the range of concentrations tested, while uric acid produced mild inhibition of [^{3}H]CHA binding. However, the concentrations at which in vitro inhibition of [^{3}H]CHA by uric acid was achieved do not correspond to serum uric acid levels normally found in rat plasma. Therefore, it is unlikely that uric acid produces its behavioral effects (3) through a direct action on A_1 adenosine receptors.

The possibility that uric acid might be enzymatically degraded during incubation by endogenous uricase also was examined in Experiment 1. Since allantoxanamide does not compete with [^{3}H]CHA binding but is known to interfere with the degradation of uric acid by uricase, uric acid was competed against [^{3}H]CHA in the presence of a high concentration of allantoxanamide to determine if degradation of uric acid influenced uric acid competition. No significant change in [^{3}H]CHA binding was found when uric acid competition experiments were performed in the presence of allantoxanamide. It was concluded that endogenous uricase does not alter uric acid concentrations during the two-hour incubation period.

The distribution of adenosine receptors follows the pattern of binding found in previous studies (15, 27, 33). The highest density of sites occurred in the thalamus with lower densities in the cortex and striatum. Chronic caffeine exposure produced a significant

increase in binding in the cortex and a trend towards an increase in binding in the striatum and thalamus. The up-regulation was similar to the increases seen in previous ontogenetic studies in which whole brains were used (5,25). The highly significant increases of binding sites in the cortex seems to have accounted for the majority of this effect. These findings suggest that cortical adenosine receptors are more sensitive to the effects of chronic caffeine during development than striatal and thalamic receptors. The increase in binding seen in the cortex after chronic caffeine exposure may explain the rapid development of tolerance to the ability of caffeine to stimulate locomotor behavior (17). Hyperuricemia induced by chronic allantoxanamide treatment did not produce a caffeine-like increase in adenosine binding. The single point assay, instead, indicated a statistically significant reduction in A_1 binding in the striatum in response to hyperuricemia. It has been suggested that purines have a neuromodulatory role in the brain (34). Uric acid has been found in considerable concentrations in the striatum of the rat (35) and, therefore, it may not be surprising that this brain area is most affected by a rise in serum uric acid levels. Indeed, microdialysis techniques have demonstrated that uric acid is released in various areas of the sheep brain (21–23) in response to behavioral activities.

The effects of chronically elevated serum uric acid levels were not consistent with the caffeine model for up-regulating adenosine receptor binding. Furthermore, the weak in vitro inhibition of [^{3}H]CHA binding by uric acid suggests that uric acid does not interact directly with A_1 adenosine binding sites in vivo. Behavioral effects of other methylxanthines parallel their affinities for adenosine receptors (1, 4, 30). Thus, it would seem unlikely that uric acid or allantoxanamide would produce significant in vivo effects through direct interaction with A_1 adenosine receptors. The data suggest that the action of uric acid and caffeine are through different neural mechanisms. Such findings run parallel to differential pharmacological effects of these compounds on another neurotransmitter system. Uric acid has been shown to decrease serotonin levels in the rat brain by affecting its synthesis and degradation (10), while caffeine raises brain serotonin levels (28) but not through effects on synthesis (11).

In conclusion, this study shows that the action of uric acid (8-oxy-xanthine) does not parallel the biochemical actions of caffeine (1,3,7-trimethyl-xanthine) with regard to A_1 adenosine receptor binding despite similar molecular structure. Uric acid does not inhibit A_1 adenosine receptors at physiological levels. A decrease in striatal A_1 adenosine binding in the chronically elevated serum uric acid group is inconsistent with the finding that uric acid failed to affect in vitro binding of the A_1 agonist [^{3}H]CHA. Our data indicate that although previous research demonstrated that elevated uric acid is associated with increased locomotor activity, this behavioral effect is not mediated by a direct interaction with A_1 adenosine receptors.

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