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# Hyperuricemia and Locomotor Activity in Developing Rats

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BARRERA, C. M., R. E. HUNTER AND W. P. DUNLAP. *Hyperuricemia and locomotor activity in developing rats*. PHARMACOL BIOCHEM BEHAV 33(2) 367–369, 1989.—This research was motivated by the previous finding that serum uric acid levels correlate with symptoms of hyperactivity in normal children. We attempted, therefore, to investigate this relationship in an animal model. Dose and time relationships between allantoxanamide-induced heightened serum uric acid and locomotor activity were investigated. A dose- and time-dependent relationship was shown between serum uric acid levels and allantoxanamide. Those doses of allantoxanamide which elevated serum uric acid produced time-dependent changes in locomotor activity. In the first two hours following injection, activity increased relative to controls, in the next two-hour block activity decreased, only to rise again above control levels in the third two-hour period. The possible role of uric acid and allantoxanamide are discussed in relation to these complex changes in activity.

Uric acid Allantoxanamide Uricase inhibitor Hyperactivity

URIC acid levels were significantly correlated with hyperactivity, impulsivity, and approached significance with short attention span in a group of normal preschool children (1). Because a correlation does not imply causality we were left with the question of whether activity levels raise uric acid or elevated uric acid raises motor activity. This present study attempts to test the hypothesis that during early brain development elevated serum uric acid raises motor activity in rats.

A problem with hyperuricemic animal models is that most nonprimate animals possess the ability, through the enzyme uricase, to rapidly break down uric acid to allantoin. Instead of using direct injections of uric acid, therefore, the uricase inhibitor allantoxanamide was used to block the action of uricase and thus elevate serum uric acid in our rats (5).

Uric acid, because it contains a xanthine ring like caffeine and theophylline, has been implicated to be a mild cortical stimulant in man (9). Other behaviors which are known to correlate with uric acid include: Improved learning and memory in rats, even after electroconvulsive treatment (2); intelligence, drive, and achievement in college professors (8); motivation and over-achievement as well as marks and extracurricular activities in high school students (6,7); dominance (leadership scale) achievement motivation composite test in hyperuricemics versus normouricemics, and leadership and achievement behavior, i.e., activity-oriented behavior, in adults, but not with hyperuricemia acquired later in adult life (3).

This project had three phases: The first phase reproduced previous findings that allantoxanamide increases uric acid in the rat. In the second phase, control and experimental groups were weaned and acclimatized to an activity-measuring chamber and their activities were measured over a 24-hour period. In the third phase the experimental group was taken off allantoxanamide one week after the end of phase two. The activities of the experimental and control subjects were again measured as in phase two.

## METHOD

### *Subjects*

Female Sprague-Dawley rats (n = 14) mated to a criterion of three ejaculations produced pups which formed the subject pool. All rats were maintained on a 12:12 (2 a.m. onset:2 p.m. offset) light:dark cycle with ad lib food (Purina Lab Chow) and water. The pregnant females were individually housed in 20-gallon glass terrariums, and pups were housed with their mothers until weaned at age 27 days.

### *Apparatus*

Injections were done using 0.5 ml Hamilton microsyringes until Day 14, after which one ml tuberculin syringes were used. Decapitations were performed with a pair of stainless steel scissors. B-D Microtainer Brand Capillary Blood-Serum Separators were used to collect the blood and store the serum. The serum was separated at 1000 × g for 25 minutes.

Activity tests were conducted in 30 cm diameter by 31 cm in height enclosed cylinders with two intersecting photoelectric beams placed at 90° angles. Floor and ceiling were 1.27 cm mesh hardware cloth. Interruption of a beam resulted in a score of one being recorded on an electromechanical printing counter set to print half-hourly. Data were grouped into two-hour blocks.

### *Injectates*

Allantoxanamide (Sigma Chemical Co.) and its solvent 0.25% methyl cellulose, were maintained in the dark at 5°C throughout the study. The 100 mg/kg group were given allantoxanamide

injections in a concentration of 10 mg/ml and the 70 mg/kg group were given injections in a concentration of 7 mg/ml. Volume delivered was dependent on the weight of the rat, therefore the volume delivered increased as the rat gained weight.

*Serum Uric Acid Assays*

The female pups were used to validate the efficacy of allantoxanamide at the chosen concentrations of 70 and 100 mg/kg, and they were sacrificed at a time at which a sufficient amount of blood could be obtained. On the day of birth (Day 1) 77 female pups were randomly assigned within litters to one of three treatment groups and were marked subcutaneously with India ink to designate group membership. Beginning on Day 4 the females began their daily SC injections of vehicle, 70 mg/kg, or 100 mg/kg allantoxanamide suspended in 0.25% methyl cellulose solution in their dorsum. Daily weights were recorded to insure proper dosing. All injections were given at the onset of the dark cycle and continued through Day 10 (the seventh day of injections).

On Day 10 the females were decapitated at 4, 8, 12, and 24 hours postinjection, so that there were at least six females from each dosage group sacrificed at each time. Each female was injected with 500 units of heparin IP, two minutes before decapitation, to facilitate blood collection. Following decapitation, the blood was collected from the torso, centrifuged and the serum refrigerated at 5°C. The serum samples were analyzed by a uricase method (4) at Smith-Kline Bioscience Laboratories.

*Activity Tests*

On Day 1, 42 male pups were marked and randomly assigned to the groups in the same manner as previously done for the females. Beginning on Day 4 the males were injected following the same procedure as used on the females (vehicle, 70 mg/kg, 100 mg/kg of allantoxanamide suspended in 0.25% methyl cellulose solution). Daily weights were recorded to insure proper dosing and the injections continued through Day 28. On Day 10 the males were earpunched for identification purposes.

On Day 27 the males were weaned and adapted to the activity chambers (1 rat/chamber) over a 24-hour period while receiving ad lib food and water. On Day 28, following their injections at the onset of the dark cycle, the males were returned to the activity chambers and their activity was recorded over a 24-hour period. The tests were conducted with the males matched by litter. Following the end of the 24-hour testing period the males were returned to their 20-gallon glass terrariums with their other litter mates (3 rats/terrarium) with ad lib food and water. On Day 34 the males were returned to the activity chambers to adapt for the next test. On Day 35 the males were tested again over a 24-hour period in order to determine if the effects of the increased serum uric acid levels were permanent.

## RESULTS

Uric acid titers of control, 70 mg/kg and 100 mg/kg allantoxanamide subjects as functions of time since last injection are shown in Table 1. As can be seen, uric acid levels are elevated for at least 8 hours in the 70 mg/kg group, and for at least 12 hours in the 100 mg/kg group. When analyzed by Analysis of Variance (ANOVA) the main effects and interaction of dose and time since last injection were all highly significant (see bottom of Table 1). Subsequent Newman-Keuls range tests revealed that the differences described above were significant at or beyond the 0.05 level (depicted by letters in Table 1). In order to control for possible impacts of the daily injections of allantoxanamide on the general health of these animals, daily weights were recorded for all animals. These data, when analyzed by ANOVA, revealed no significant drug effects on body weight. Activity levels of control, 70 mg/kg and 100 mg/kg groups during the first 12 hours following injection, at the onset of the dark cycle, are shown in Fig. 1. As can be seen, the allantoxanamide-treated subjects were initially more active than the vehicle during the first two hours, but then activity of these two experimental groups dropped below that of the controls during the second two-hour block, then rose again above control levels in the third two-hour block. This complex pattern of variability in activity levels with respect to time was supported by a significant groups by time interaction, $F(10, 130) = 3.45$, $p = 0.0005$. Means were compared by the Newman-Keuls test within individual blocks, which revealed that the 100 mg/kg group significantly exceeded the control in Block 1; both the 70 and 100 mg/kg groups were less active than the control in Block 2, and the 100 mg/kg group again was significantly more active than the control in the third block of time (hour 4–6).

TABLE 1

URIC ACID LEVELS IN VEHICLE, 70 mg/kg, AND 100 mg/kg OF ALLANTOXANAMIDE GROUPS AT 4, 8, 12, 24 HOURS POSTINJECTION FOR FEMALE RATS INJECTED DAILY FROM DAY 4 TO DAY 10

| Time (hours) Post-injection | Vehicle Mean (S.D.) (n) | 70 mg/kg Mean (S.D.) (n) | 100 mg/kg Mean (S.D.) (n) |
|---|---|---|---|
| injection | | | |
| 4 | 3.62*† (1.07)(6) | 6.17§ (0.59)(6) | 6.48§ (0.57)(6) |
| 8 | 3.90*† (0.41)(6) | 5.02‡ (0.53)(6) | 6.02§ (0.89)(6) |
| 12 | 3.43* (0.42)(7) | 3.66*† (0.37)(7) | 4.63†‡ (0.93)(7) |
| 24 | 3.53*† (0.49)(7) | 4.06*† (0.79)(7) | 3.60*† (0.54)(6) |

N.B. Means sharing a common symbol do not differ significantly.
Dose, $F(2,65) = 36.94$, $p<0.0001$; Time, $F(3,65) = 28.84$, $p<0.0001$; Dose × Time, $F(6,65) = 7.00$, $p<0.0001$. n refers to number of rats.

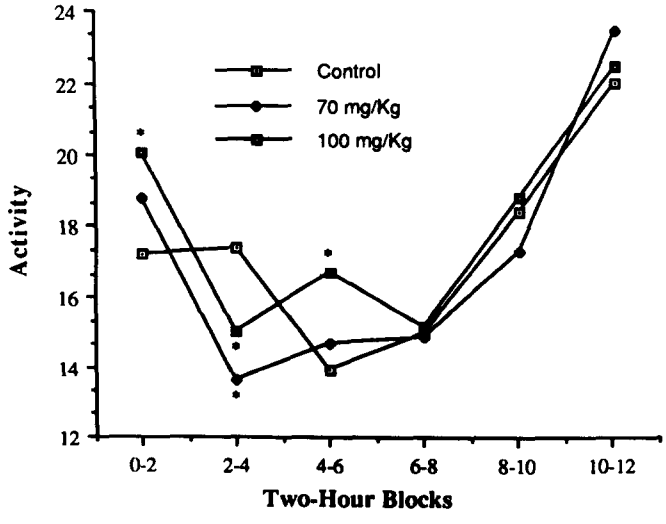


FIG. 1. Activity of male rats injected with vehicle, 70 mg/kg, and 100 mg/kg of allantoxanamide. All groups had 14 subjects measured during the dark cycle. *Indicates statistical significance compared to control.

Analysis of the subsequent 12-hour period, during the light part of the cycle, revealed no significant differences between the groups. All subjects were discontinued from their daily injections for one week and were again tested for activity to look for possible residual effects or rebound from their previous drug treatment. Analysis of these later data showed no significant residual effects of previous drug treatment.

## DISCUSSION

Although uric acid can affect learning behavior by interfering with habit reversal (2), locomotor activity failed to increase with direct injections of uric acid (2). The study by Essman (2), however, had two methodological deficiencies: Acute as opposed to chronic effects of uric acid were studied, and animals' activities were measured for only a 90-minute period in a closed field to which they had not been acclimatized; this small amount of time given to unacclimatized animals may be insufficient to detect subtle changes in activity. The rats in the present study were examined for acute and chronic effects of allantoxanamide-induced hyperuricemia over a period of 24 hr, and were given 24 hr to acclimate to their surroundings before any measurement of activity was performed.

In the present study we see a dose-dependent elevation of uric acid (Table 1) and activity (Fig. 1) after injection of allantoxanamide. The dose-dependent rise in activity has two peaks. The second peak in activity seems to coincide with the peak in serum uric acid between 4–6 hours (Fig. 1). Although we did not measure serum uric acid level at six hours, the literature has shown that at six hours the allantoxanamide doses of 70 and 100 mg/kg serum uric acid levels are sustained or slightly higher to those at 4 hours (5). It would be reasonable, therefore, to assume that in our experimental groups, serum uric acid levels at six hours would be similar to serum uric acid levels at four hours. The first peak in activity may perhaps be explained by the initial injection of allantoxanamide. Although the molecular structure of allantoxanamide is dissimilar to that of uric acid it is derived from the alkaline oxidation of uric acid (10) and may therefore retain some of the proposed biological effects of uric acid.

Not all the data can be accounted for by the explanation that as serum uric acid levels peak activity does also. The 100 mg/kg experimental group had elevated serum uric acid levels which were not significantly lower at eight hours than the four hour serum uric acid level, and yet the activity of both experimental groups at the 6–8-hour block were not significantly different from the activity of the control group. This discrepancy might be ascribed to serum uric acid being measured in a group that differed in gender and age from the group in which activity is measured. We feel that gender is not a likely factor since both groups were prepubertal and thus any hormonal differences should be negligible. Age differences were also not felt to be of great importance because of the similar curves, at the dosages of allantoxanamide given, between our rats and older rats in another study (5). It has been noted that rats show two hourly intervals of activity and rest superimposed on circadian related locomotor activity because of dark/light cycling (11). Perhaps the effects of allantoxanamide and the subsequent rise in serum uric acid may enhance these natural cycles of activity and rest and activity levels do not necessarily coincide strictly with serum uric acid and serum allantoxanamide levels alone. Alternate explanations are possible, for example, other xanthine-containing compounds, e.g., caffeine and theophylline, have been noted to give a biphasic response in activity. After high doses of caffeine or theophylline were injected in adult rats (13), activity initially dropped then rose as serum levels of caffeine or theophylline fell. This scenario, although it may demonstrate that multiphase responses in activity are possible, would not directly account for our present data.

Allantoxanamide could also act as a peripheral irritant. It has been noted that allantoxanamide used in doses much higher than used here is toxic to several different organ systems in the rat (12,14). Although it is difficult to disprove irritation on the part of the animals, our data show no difference in weights between experimental and control groups, and no local inflammatory response was noted at the site of injection of allantoxanamide.

It is clear from this study that rat locomotor activity is increased after injection of the uricase inhibitor allantoxanamide and subsequent rise in serum uric acid. The change in activity is complex and therefore difficult to explain in a simple cause and effect manner. These results, although provocative, are as yet preliminary and further studies regarding the behavioral and pharmacological effects of uric acid will need to be done.

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