LESCH-NYHAN SYNDROME-LIKE BEHAVIOR IN RATS FROM CAFFEINE INGESTION

Changes in HGPRTase activity, urea and some nitrogen metabolism enzymes

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1. Introduction

The methylxanthines caffeine, theophylline and theobromine are present in such widely consumed food-products as coffee, chocolate and tea. They have been used in medicines as respiratory and cardiac stimulants as well as diuretics [1,2].

These compounds are potent mutagens in *Escherichia coli* and other lower organisms and have been shown to enhance the effect of other mutagenic agents such as ultraviolet light [3–6]. Mammals, however, do not seem to be susceptible to such activity possibly because they can demethylate these compounds. Methylxanthines may induce chromosomal aberrations in cell cultures and have an antimitotic activity in some cellular systems. Caffeine has been shown to inhibit DNA repair mechanisms, thus allowing sublethal damage to chromosomes to become lethal [7–11].

Rats and rabbits receiving high doses of caffeine mimic the behavior of Lesch–Nyhan patients [12,13]. The Lesch–Nyhan syndrome is a neurologic X-linked disorder, characterized by mental retardation and an aggressive attitude with self-destructive biting, and which is associated with a loss of hypoxanthine guanine phosphoribosyltransferase activity [14–16].

Tissues from children suffering from Lesch–Nyhan disease have an increased activity of some enzymes of the biosynthetic pathway for pyrimidines [17,18]. However, although a number of parameters have been studied in mammals treated with caffeine [12,19], there have been no reports concerning its effect on nitrogen metabolism and biosynthesis of purines. We show here a decrease of hypoxanthine guanine phosphoribosyltransferase (HGPRTase) and aspartate transcarbamylase (ATCase) activities and an increase in ammonia-dependent carbamyl phosphate synthetase (CPS I) and ornithine transcarbamylase (OTCase) activities in rats ingesting high concentrations of caffeine and theophylline, as well as increased blood levels of urea, and their correlation with self-mutilation.

2. Materials and methods

Groups of adult albino rats (200 g body wt) were maintained on an 18% protein diet and given 2, 4 and 8 g/l of caffeine or 4 g/l of theophylline in their drinking water for 50 days; 100 g/l of sucrose were added to mask the bitter taste of caffeine solutions.

Levels of urea in blood taken from the tail were measured every 2 days in the first week and once a week thereafter. Body weight, liquid and food intake were recorded throughout the experiment.

Activities of HGPRTase in erythrocytes, ATCase in erythrocytes, liver and brain, CPS I and OTCase in liver and acetylcholinesterase in erythrocytes and brain were measured.

Hypoxanthine guanine phosphoribosyltransferase in erythrocytes was measured by the method in [20] as modified [17].

Aspartate transcarbamylase in erythrocytes was assayed by a modification of the method in [17], with the addition of 0.007 IU of urease to the assay mixture. The carbamyl aspartate formed was estimated colorimetrically [21].

Liver, in 0.154 M KCl (1:9) and brain, in water (1:4), were homogenized in an Ultraturrax and ATCase was determined as described for erythrocytes.

Carbamyl phosphate synthetase I and ornithine transcarbamylase were measured in liver homogenate (1:20). The assay for carbamyl phosphate synthetase

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I included: 13.75 μmol ammonium bicarbonate, 2.75 μmol magnesium sulfate, 1.375 μmol ATP (pH 7.0), 1.375 μmol *N*-acetylglutamate, 1.4 μg pyruvate kinase and 25 μl homogenate in a final volume of 275 μl. Before the addition of pyruvate kinase the mixture was bubbled with CO_2. The reaction was incubated 15 min at 37°C and stopped with 1 ml 15% $HClO_4$; the citrulline formed was measured colorimetrically [21]. For ornithine transcarbamylase the assay included 30 μmol glycylglycine (pH 8.0), 20 μmol carbamyl phosphate, disodium salt, 15 μmol L-ornithine, water and 25 μl homogenate in a final volume of 1.0 ml. After 15 min at 37°C, the reaction was stopped with 2.5 ml 15% $HClO_4$ and the citrulline was measured.

Acetylcholinesterase in erythrocytes and brain homogenate was assayed as in [22].

3. Results

Rats imbibing daily high amounts of a caffeine solution (8 g/l) developed symptoms of self-biting before 15 days (80% of animals); a progressive loss of body weight was associated with these symptoms and most of the rats finally died. The animals that did not develop symptoms by approximately the 20th day remained apparently unaffected for the duration of the experiment (2 months). Females were 3-times more susceptible than males. The body weight, a week after the onset of the disorder, was decreased by 40% of control values (experimental mean value = 133 ± 30 g; control mean value = 225 ± 20 g). The animals in the other two groups, given 2 and 4 g/l caffeine to drink, did not develop symptoms, although they showed a loss of body weight of 10% and 30%, respectively. The mean consumption of caffeine (males and females) was ~200 mg . kg^{-1} . day^{-1} for the 2 groups with the higher concentrations of caffeine in their drinking fluid and 120 mg . kg^{-1} . day^{-1} for the lower dose.

A decrease in food and liquid intake, proportional to the concentration of caffeine in the drinking fluid, was observed at the beginning of the experiments. When caffeine administration was stopped, rats recovered their appetite and ate and drank as the control animals; increasing the concentration of caffeine in the drinking fluid again caused a decreased intake (fig.1a,b).

Blood urea increased in all experimental rats from 6.3 ± 0.6 μmol/ml blood to 8.7 ± 1.2, 10.3 ± 1.4 and 11.2 ± 1.3 μmol/ml blood for groups receiving 2, 4 and 8 g/l of caffeine, respectively. After several days of treatment, these levels diminished to nearly steady values proportional to the intake (fig.2). On caffeine withdrawal blood urea decreased to normal values, while increasing the concentration of caffeine caused an elevation of urea in blood.

The activity of HGPRTase in erythrocytes of rats treated with caffeine and with symptoms of self-biting was decreased to levels ranging from non-detectable to 2.95 nmol hypoxanthine . h^{-1} . mg $protein^{-1}$. As illustrated in table 1, the mean value for controls was 6.0 ± 0.84 nmol . h^{-1} . mg $protein^{-1}$, while for the experimental animals it was 1.4 ± 1.2 nmol . h^{-1} . mg $protein^{-1}$. It is evident that control values for rats are markedly lower than those observed in humans [17].

In table 1 we also show that the activity of ATCase in erythrocytes of animals with autodestructive behavior fell by >95%, that of liver and brain was

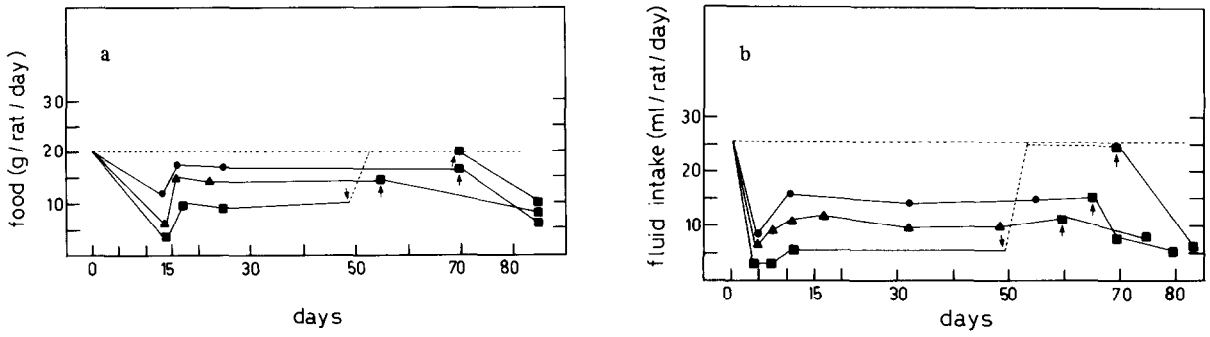

Fig.1. Daily food (a) and fluid (b) intake of rats treated with (•) 2 g/l caffeine, (▲) 4 g/l caffeine and (■) 8 g/l caffeine, (– – –) mean of controls; (↓) withdrawal of, and (↑) increased dosage of caffeine.

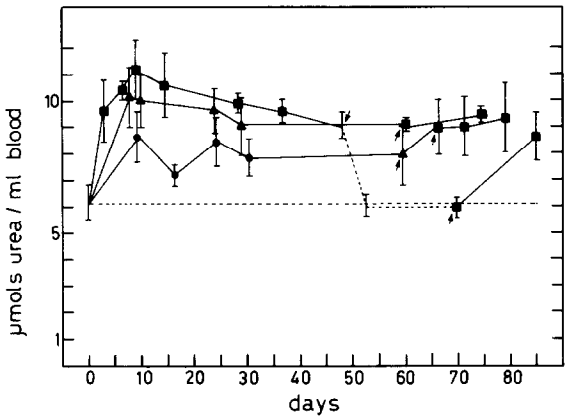

Fig.2. Blood urea levels during treatment with (●) 2 g/l caffeine, (▲) 4 g/l caffeine and (■) 8 g/l caffeine. Urea was measured as in [18]; values are expressed as mean ± SD; (– – –) mean of controls; (↓) withdrawal of, and (↑) increased dosage of caffeine.

reduced to 38% and 36% of controls, respectively. CPS I and OTCase activities showed increases of up to 66% and 30% of controls, respectively.

There were no significant changes in the activities of any of the enzymes tested in animals receiving 2 and 4 g/l caffeine, nor in those receiving 8 g/l caffeine which showed no autodestructive behavior.

Rats ingesting 4 g/l theophylline showed symptoms similar to those receiving the highest dosage of caffeine (8 g/l); blood urea levels were enhanced and the activity of erythrocyte ATCase was diminished to 5% that of controls.

The incidence of self-biting in rats as a result of caffeine intake seems to be related more to the initial drug concentration than to the daily dose. Although the dose . kg body wt^{-1} . day^{-1} was greater when animals were drinking 4 g/l caffeine (211 mg caffeine . kg^{-1} . day^{-1}), symptoms occurred only in animals receiving 8 g/l caffeine. In addition, the sensitivity to the treatment was apparently not related to habituation, since the severity of symptoms was similar when the increase in dose was gradual and when the higher dose was given directly. Theophylline has proved to be more effective than caffeine in inducing auto-mutilation, since only half the concentration was needed. However, other animals which received theobromine and xanthine (in their food, because of low solubility in water), showed only a slight loss of weight.

Concerning the activity of acetylcholinesterase, the measurements in erythrocytes and brain of rats ingesting 8 g/l caffeine and with self-biting were found to be similar to those of controls (mean control values = 6.6 ± 0.7 nmol thiocholine . min^{-1} . mg $protein^{-1}$ for erythrocytes and 139 ± 9 nmol . min^{-1} . mg $protein^{-1}$ for brain; mean experimental values = 11.0 ± 7.1 nmol thiocholine . min^{-1} . mg $protein^{-1}$ for erythrocytes and 121 ± 18.9 nmol . min^{-1} . mg $protein^{-1}$ for brain).

Table 1
Hypoxanthine-guanine phosphoribosyltransferase, aspartate transcarbamylase, carbamylphosphate synthetase I and ornithine transcarbamylase activities in tissues of rats treated with caffeine and with behavioral symptoms

Enzyme tested	Caffeine in drinking fluid		*P*
	0 g/l mean ± SD	8 g/l mean ± SD	
Hypoxanthine guanine phosphoribosyltransferase			
from erythrocytes	6.0 ± 0.8 (7)	1.4 ± 1.2 (9)	<0.001
Aspartate transcarbamylase			
from erythrocytes	9.2 ± 2.5 (10)	0.4 ± 0.4 (5)	<0.001
from liver	143.0 ± 42.0 (11)	87.7 ± 23.0 (3)	<0.05
from brain	34.7 ± 7.8 (9)	22.2 ± 12.4 (4)	<0.05
Carbamyl phosphate synthetase I			
from liver	146.6 ± 17.0 (17)	247.7 ± 22.0 (4)	<0.001
Ornithine transcarbamylase			
from liver	11 619 ± 1548 (7)	15 017 ± 779 (4)	<0.05

HGPRTase and ATCase values are expressed in nmol . h^{-1} . mg $protein^{-1}$ and CPS I and OTCase in μmol . h^{-1} . mg $protein^{-1}$; numbers in parentheses indicate size of sample

4. Discussion

Considering that 40 mg caffeine, the average ingested per rat per day, could be transformed into a maximum of 400 μmol urea, and that the daily protein intake ($1.8\ g\ .\ rat^{-1}\ .\ day^{-1}$) can produce 10.8 mmol urea, it is apparent that the increased levels of urea in blood cannot be explained simply as the result of urea produced from caffeine metabolism but rather by increased synthesis, since the activities of the enzymes CPS I and OTCase are increased.

Addition of methyl-xanthines to particulate fractions of tissue homogenates increases cyclic 3′,5′-AMP levels by inhibiting the cyclic 3′,5′-nucleotide phosphodiesterase [23]. The increased urea synthesis could be due to the stimulation of mitochondrial function as a consequence of the high cAMP levels produced by the administration of caffeine.

Many attempts have been made to produce uremia in animals as a model for renal failure [24–26]. These results indicate that caffeine is an effective agent for increasing the urea levels in rats, so it may prove useful for such studies.

Although 4 of the 6 enzymes of pyrimidine biosynthesis have been found elevated in HGPRTase-deficient erythrocytes, the mechanism has not yet been established [17,18].

The decrease in activity of HGPRTase in animals treated with caffeine and showing self-destructive behavior resembles those first shown in Lesch–Nyhan syndrome [27]. However, HGPRTase activity in patients was decreased to <1% of controls, while in rats showing neurological symptoms HGPRTase activity was usually undetectable but was occasionally decreased only 50% that of the controls. From these results it appears that caffeine may be helpful in clarifying this disease, particularly since there are many variants of the syndrome. That symptoms develop in rats with apparently less depletion of the enzyme than in humans may reflect their lower normal levels of enzyme activity. A cup of coffee or tea may contain up to 150 mg caffeine, which would be equivalent to some 750 mg/l. This is approximately half the concentration needed as drinking fluid to increase blood urea levels 30–40% or 1/8th of that needed to develop autodestructive behavior in rats.

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