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# Effect of caffeine on metabolism of L-arginine in the brain

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## Abstract

Methylxanthines are widely consumed because of their stimulating effect primarily on the central nervous system. Their diuretic and respiratory stimulant action is used in clinical medicine. L-Arginine metabolism in the brain is very important for normal brain function. In addition to brain protein synthesis, arginine is a substrate for the production of urea, creatine, nitric oxide, agmatine, glutamic acid, ornithine, proline and polyamines. As known, many of these compounds are very important in brain function. There is no information relating to effects of caffeine on arginine metabolism in the brain, however, there is a lot of new information about arginine metabolism and caffeine action on the central nervous system. So, we have hypothesized the existence of a relationship that may be of interest in understanding mechanisms of caffeine effects on the central nervous system that may have utility in the clinical applications.

In our experiment protocol we used male Wistar rats weighing about 200 g. Caffeine was added to the drinking water in gradually increasing amounts, from 2 g/l over the first 3 days, to 4 g/l over the last 7 days. A control group was given drinking water without caffeine. The level of lipid peroxidation, arginase and diamine oxidase (DAO) activity in the brain was measured. The results of our study show that arginase and diamine oxidase were decreased in animals treated with caffeine. The level of lipid peroxidation (MDA) was decreased also.

The inhibitory effect of caffeine on arginase activity indicates that caffeine provides more arginine for consumption in other metabolic pathways. Considering the central stimulant effects of caffeine and the decreased lipid peroxidation level, it can be assumed that moderate short-term consumption of caffeine may be beneficial for brain function. (Mol Cell Biochem **244**: 125–128, 2003)

*Key words*: caffeine, arginase, brain, lipid peroxidation, polyamines, diamine oxidase

## Introduction

Methylxanthines: caffeine, theophylline and theobromine are present in several food products such as coffee, tea, cocoa, chocolate, etc. They are widely consumed primarily because of their stimulating effect on the central nervous system. Xanthine drugs are used in clinical medicine as diuretics, analgetics, in the treatment in brain disorders such as vascular headaches, Parkinson's disease [1–3].

L-Arginine metabolism in the brain is very important for normal brain function. The physiological significance of arginine includes protein synthesis, production of urea, agmatine, nitric oxide (NO), proline, glutamate and polyamines [4]. As it is well known, all of these components have very important contributions to brain function. Metabolism of arginine depends on the activity of arginase, argininosuccinate synthetase, arginine decarboxylase and nitric oxide synthetase. Changes in activities of these enzymes will have effects on the metabolic fate of L-arginine in the cell.

We have studied the influence of caffeine on metabolism of L-arginine by measuring arginase activity. As it is known, arginase is an enzyme that leads to the degradation of L-arginine to urea and ornithine, which is the main function of this liver enzyme. In extrahepatic tissues arginase is more important in the synthesis of other products such as NO, proline, polyamines, creatine-P, glutamic acid [5, 6]. The effects of caffeine on the central nervous system is very much dependent on dose. The cortical stimulation produced by small

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amounts of caffeine results in mental alertness, decreased fatigue and decreased drowsiness. Large doses of caffeine may produce irritability, insomnia, tremor and headache. Rats ingested high doses of caffeine reproduce the self-destructive behavior that have obtained in Lesch- Nyhan syndrome [7, 8].

The aim of this study was to investigate the possible effect of moderate doses of caffeine during short-term consumption on arginine metabolism, considering the importance of its metabolic products on brain functions.

## Materials and methods

Male Wistar rats weighing about 200 g were used in each experiment. Caffeine was added to the drinking water in gradually increasing amounts: 2 g/l for 3 days and then 4 g/l for the next 7 days. A control group was given drinking water without caffeine. Rats were killed by decapitation and the brains were quickly removed and frozen.

Brain arginase activity was measured in whole brain homogenate on the basis of formed ornithine, according to the method of Porembska and Kedra [9]. Lipid peroxidation levels (MDA) were determined utilizing thiobarbituric acid [10]. Polyamine oxidase activity (PAO) was measured according to the method of Bashrach and Reches, using spermidine as substrate [11]. Proteins in tissue homogenates were estimated according to Lowry *et al.* [12]. Blood urea, creatinine and uric acid levels were measured by standard biochemical analyses.

Statistical significance between groups was determined using Student's *t*-test.

## Results

Blood levels of urea, creatinine and uric acid were increased in the group of animals treated with caffeine with respect to the control group (Table 1). Urea and creatinine were elevated but not significantly. Elevation of uric acid in caffeine treated group of animals was significant, $p < 0.001$.

The influence of caffeine on brain arginase is illustrated in Fig. 1. Arginase was significantly reduced ($p < 0.01$) compared to control. Diamine oxidase activity was depressed also in caffeine treated rats ($p < 0.01$, Fig. 2).

Malon dialdehyde, a measure of the level of lipid peroxidation, was decreased with respect to the control group ($p < 0.01$, Fig. 3).

*Table 1.* Caffeine effects on blood levels of urea (mmol/l), creatinine (μmol/l) and uric acid (μmol/l)

| | Urea | Creatinine | Uric acid |
|---|---|---|---|
| Control | 7.35 ± 0.07 | 44.70 ± 5.90 | 67.82 ± 10.7 |
| Caffeine | 9.05 ± 0.08 | 54.51 ± 9.22 | 115.6 ± 21.2*** |

Urea, creatinine and uric acid were determinated in plasma using heparin as a anticoagulant. Results are mean ± S.D. ***$p < 0.001$.

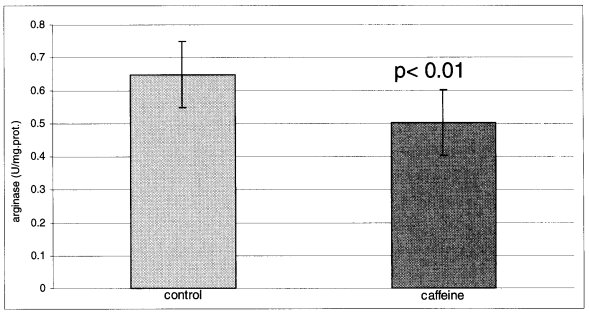


*Fig. 1.* Arginase activity in the brain of caffeine treated rats. Results are expressed as mean ± S.D.

## Discussion

The results of our study show that short-term treatment of animals with small doses of caffeine decreases brain arginase activity. There are two distinct isoenzymes of arginase. Type I arginase is highly expressed in liver as an enzyme of the urea cycle. Type II arginase is expressed in brain, kidney, mammary gland, small intestine and macrophages. The existing differences in regulation of arginase isoenzymes in response to diet, hormones and cytokines show that arginase may be a regulator of the metabolic fate of arginine [4, 13].

Methylxanthines (i.e. caffeine, theophylline and theobromine) belong to a chemical group of purine bases that include important endogenous substances such as adenine, guanine, hypoxanthine and uric acid. This chemical similarity of

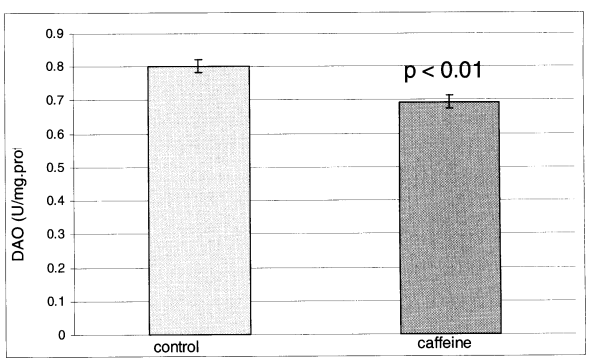


*Fig. 2.* DAO activity in caffeine treated rats. Enzymes activities are expressed as mean ± S.D.

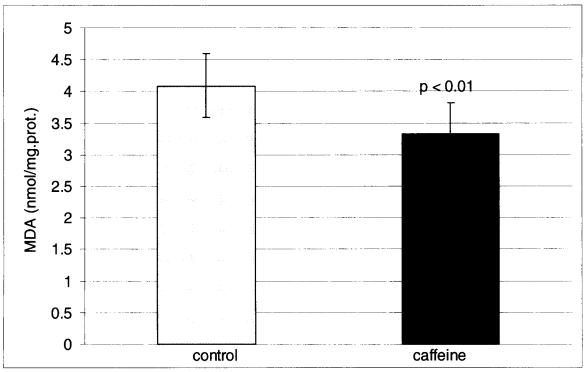


*Fig. 3.* MDA levels in the brain of caffeine treated rats. Results are expressed as mean ± S.D.

xanthines to purine molecules is important with respect to their interaction with the important biochemical regulator, cAMP. Depressed arginase activity in the brain after consumption of caffeine may be a result of caffeine's effect on the level of 3′5′-cyclic adenosine monophosphate (cAMP). A number of metabolic reactions are controlled via cAMP levels. The cellular level of cyclic AMP depends of the activity adenylate cyclase and phosphodiesterase. Xanthines inhibit phosphodiesterase and breakdown cAMP. Cyclic AMP was found to be increased after caffeine treatment [14, 15].

Caffeine stimulates adrenocortical and adrenomedular hormone secretion [16]. Cyclic AMP is a secondary messenger molecule of many hormones, such as ACTH, catecholamines, glucagon, thyroxine and insulin. Glucocorticoids, catecholamines and glucagon increase adenylate cyclase activity. Increased levels of cAMP may affect tissue metabolism in different ways.

Adenosine may be involved in modulation of brain arginase activity. The central stimulant effect of caffeine is linked to the blockade of adenosine receptors [17]. Caffeine removes adenosine from its receptors and increases free adenosine level. Adenosine, adenine, inosine and uric acid are competitive inhibitors of arginase [18]. Changes in the aminoacid pool in the brain may also affect arginase activity. Valine, leucine, isoleucine and ornithine all have inhibitory effects on arginase activity [19].

The important metabolic pathway for arginine is the synthesis of polyamines. Ornithine, a product of arginase activity, is a precursor for synthesis of polyamines. Ornithine decarboxylase catalyses the first step in the biosynthesis of polyamines and its activity is controlled by cAMP [20]. Decarboxylation of ornithine leads to synthesis of putrescine, which is the precursor of spermidine and spermine. Ornithine decarboxylase, the limiting enzyme in polyamine synthesis is not increased in the brain after caffeine treatment [21]. Results of our study show that caffeine leads to a decrease in polyamine catabolism by depression of diamine oxidase activity.

Considering that the activity of arginase and NO synthetase have different regulation it is likely that depressed arginase activity is followed by an increase in arginine levels that can be utilized in the production of NO. Nitric oxide has vasodilatatory and antioxidative effects, and acts as important modulator of brain function.

The results of our study show that caffeine decreases the level of lipid peroxidation in the brain. The lipid peroxidation levels may be influenced by the caffeine itself, although arginine, adenosine and nitro oxide are also antioxidants [22–24]. The relationship between caffeine and L-arginine metabolism indicates a new aspect of caffeine action. It is of interest because both caffeine and arginine have important functions in different physiological and pathological conditions such as vascular tone, neurotransmission, immune response, tumor biology, intoxication, inflammation, etc. [25–28].

## Conclusions

The results show that caffeine changes the metabolism of L-arginine in the brain. Arginase, an enzyme that hydrolyses L-arginine to ornithine and urea, is decreased after caffeine treatment.

Catabolism of polyamines is depressed as a result of decreased activity diamine oxidase. Caffeine consumption decreases the lipid peroxidation level in the brain.

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