EFFECT OF SHORT DURATION CAFFEINE TREATMENT ON THE JEJUNUM OF ADULT ALBINO RATS

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ABSTRACT: The effect of the short duration (21 days) of caffeine at two different doses (12 mg & 30 mg/kg body weight/day) on the activities of adenosine triphosphatases in jejunum was studied. The activity of Na+, K+-dependent ATP-ase was reduced significantly in the upper jejunal mucosal and serosal layers of low and high dose groups. Such effect was observed only in the lower jejunal serosal layer while a marked reduction in the activity of Mg2+ATP–ase was seen in mucosal and serosal layers of the jejunum at both doses. There was a significant increase in the activities of Ca2+-dependent and HCO3-dependent ATP–ases in these regions of the lower jejunum.

INTRODUCTION

Food toxicology is an emerging area of nutritional sciences. It is important to note that food toxicants counter the beneficial effects of biological effects would be further aggravated and under the conditions of malnutrition. It is very essential to examine the food toxicants effects in animals and assessment of risk to humans.

Caffeine is a xanthine compound used widely as a stimulant all over the world. It has toxic effect on bone mineralization, 2 and cholesterogenesis. It is known to stimulate the Ca+ Mg+ excretion. A sex linked Ca2+ interaction was apparent tin Ca2+ concentration after administration as only females exhibited an increasing concentration of Ca2+ with surcorose. The main pharmacological actions of caffeine is exerted on the central nervous system (CNS) and the cardiovascular system. In addition, the drug is a diuretic and stimulated gastric secretions.

Very little work has been done with regard to caffeine’s effect in the gut, hence the present investigation is an attempt to study the probable effect of caffeine on some transport enzymes like the adenosine triphosphatases in the jejunum of adult male albino rats. As these enzymes are very essential for transport of glucose amino acids etc., any impairment in their activity will surely affect the gut activity as well as its motility.

MATERIALS AND METHODS

Caffeine citrate was procured from bakul products, Bombay (India). Male adult albino rats of wistar strain weighing 150-200 g weight were maintained in a well ventilated animal house with constant 10 hrs. of darkness and 14 hrs. of light schedule. The rats were given standard pellet diet Hindustan Lever Ltd., India) with free access to water. The animals were divided into 3 groups with 5 animals in each groups.
Group I: Controls, treated intraperitoneally (ip) with 1% saline for 21 days.

Group – II: Low dose group, treated with 12 mg caffeine/kg body wt/rat per day for days.

Group – III: High dose group, treated (ip) with 30 mg caffeine/kg body wt/rat/day for 21 days.

The body weight of each animal was recorded before and after the treatment schedule. After the last injection, animals were fasted for about 20 hrs with access to water only, before sacrifice. The animals were scarified by cervical dislocation 24 hrs, after the drug treatment as per the schedule. The jejunum was dissected from the connective tissues and blood vessels were removed. The intestinal lumen form each not was flushed with cold saline and the jejunum was divided into upper and lower regions. Mucosal and serosal layers were separated. From each segment of the jejunum the whole amount of the mucosal and serosal tissues were immediately blotted and weighed accurately to nearest milligram. The tissues were homogenized in ice cold 0.1 ml Tris Hcl buffer pH 7.5 in a Teflon homogenizer 7-10 min. the whole homogenates were centrifuged for 15 min at 10,000 g at 4oc. The supernatant was used for assaying the specific activities of Ca2, Mg2+, Na+, K+ and HCO3 – dependent ATP –ases. The inorganic phosphorous present in the supernatant was also determined. The results were analysed statistically 10 using student’s ‘t’ test.

RESULTS AND DISCUSSION

Table 1 and 2 indicate the effect of Caffeine treatment on the activities of Na+, K+ and Mg++ -ATP –ases. A marked reduction (P<0.001) in the activities of these enzymes was observed at both doses.

On the other hand the activity of Ca ++dependent ATP –ase in the jejunum of adult albino rats was significantly increased (P<0.001) in these regions by caffeine treatment (Table 3)

Table 4 represents the effect of caffeine treatment on the activity of HCO3 – dependent ATP –ase in the jejunum. A marked increase (P<0.001) in the enzyme activity was observed in the upper jejunal mucosal layer of high dose group, while it was significantly reduced (P<0.01) in the serosal layer of low dose group. In the lower jejuna mucosal layer, the low dose of caffeine caused a significant increase (P<0.05) in the enzyme activity. However a decreased activity (P<0.01, P<0.05) was seen in high dose drug treated a groups in the enzyme activity. However a decreased activity (P<0.01, P<0.05) was seen in high dose drug treated groups in both serosal and mucosal layers respectively.

In the present investigation caffeine administration appeared to inhibit the sodium pump and activate Na+ + K+ exchange in the upper jejunum only, compared to the lower jejunum. This may be due to the interactions between Caffeine and adenosine at the adenosine receptor sites and activation of this enzyme system with in the jejuna cells as suggested by Batting. Since the Na+, K+ ATP -ases system is dependent on the Mg2+ ions for its activity, the moderate decrease of the Mg2+ dependent ATP –ases system after caffeine treatment indicates a supporting efflux of the Mg2+ ions.

Das and chatterjee12 have shown the HCO3-ATP ases to exhibit decreased affinity towards substrate molecules.
Therefore, the inhibition of jejunal serosal membrane bound HCO3-ATP – ases activity under caffeine treatment appears to be mediated through changes in membrane fluidity. Further, the stimulated Ca2+ dependent ATP ase activity in the serosal layer under caffeine influence might lower the HCO3-ATP ase activity to an extent as suggested by Ray et al.13 Ca2+ ions, which act as second messenger and are critically essential for gastric secretion in the stomach also inhibit the HCO3-ATP ase reaction in the digestive system.13 This point may suggest a precise and delicate regulatory mechanism for maintenance of the Ca2+ in different intracellular compartments of the jejunal tissue. It also emphasizes the role of Ca2+ as a member of a signal transducing cascade system in the process of jejuna H+ transport. Thus, caffeine administration seems to alter the electrochemical gradient and plasma membrane permeability by permitting Ca2+ influx into the serosal cells of the jejunum.

Thus, the data obtained in this study suggest an adverse effect of caffeine on jejunal mucosal and serosal layers. Further, it suggests that the altered ionic transport, indicated by changed activities of the ATP ase systems was a signal for altered cell permeability probably due to cell injury.

This substantiates the work of Hartiala14,13 That caffeine causes damage to the gut epithelium and thereby its function . since ATP ase are membrane bound enzymes, the role of membrane lipids and its microenvironmental changes at the physical and chemical level maybe responsible for the differential response as observed in the serosal and mucosal layers at the level of the ATP ase activities under both low and high dose caffeine treatment.

Table -1 Effect of caffeine treatment on the activity of Na+, K+ -dependent ATP –ase in the jejunum of adult albino rats @

| S. NO | GROUPS SHORT DURATION | MUCOSAL LAYER | SEROSAL LAYER |
|-------|------------------------|---------------|---------------|
|       |                        | UPPER JEJUNUM | LOWER JEJUNUM | UPPER JEJUNUM | LOWER JEJUNUM |
| 1. 2. | Control Low dose       | 23.40 ± 0.46 6.09 | 15.25 ± 0.54 5.6 ± | 14.84 ± 0.43 6.33 ± | 13.44 ± 0.70 9.04 ± |
|       | High dose              | 27.08 ± 0.85 7.63 | 20.52± 0.22 15.24 ± | 21.96± 0.45 7.46 ± | 15.61 ± 0.72 9.91 ± |
|       |                        |               |               |               |               |

Each value is mean ± SEM of 5 animals *p<0.05; **p<0.01; ***p<0.001 Control Vs other groups Group –I, Group-II Group-III as given in the text @ The values are expressed as µg of Pi formed hr/gm tissue.
Table -2 Effect of caffeine treatment on the activity of Mg++ -dependent ATP –ase in the jejunum of adult albino rats @

| S. NO | GROUPS SHORT DURATION | MUCOSAL LAYER | SEROSAL LAYER |
|-------|------------------------|---------------|---------------|
|       |                        | UPPER JEJUNUM | LOWER JEJUNUM | UPPER JEJUNUM | LOWER JEJUNUM |
| 1. 2. | Control Low dose High dose | 23.40 ± 0.46 6.09 | 15.25 ± 0.54 5.6 | 14.84 ± 0.43 6.33 | 13.44 ± 0.70 9.04 |
| 3.    |                        | ± 0.33*** 17.55 | ± 0.33*** 13.75 | ± 0.34*** 9.23 | ± 0.38*** 8.59 |
|       |                        | ± 0.50***      | ± 0.96               | ± 0.51***          | ± 0.63***          |

Each value is mean ± SEM of 5 animals *p<0.05; **p<0.01; ***p<0.001 Control Vs other groups Group –I, Group-II Group-III as given in the text @ The values are expressed as µg of Pi formed hr/gm tissue

Table -3 Effect of caffeine treatment on the activity of Ca++ -dependent ATP –ase in the jejunum of adult albino rats @

| S. NO | GROUPS SHORT DURATION | MUCOSAL LAYER | SEROSAL LAYER |
|-------|------------------------|---------------|---------------|
|       |                        | UPPER JEJUNUM | LOWER JEJUNUM | UPPER JEJUNUM | LOWER JEJUNUM |
| 1. 2. | Control Low dose High dose | 27.08 ± 0.85 7.63 | 20.52± 0.22 15.24 | 21.96± 0.45 7.46 | 15.61 ± 0.72 9.91 |
| 3.    |                        | ± 0.53*** 22.85 | ± 0.46*** 12.32 | ± 0.54*** 14.33 | ± 0.68*** 10.42 |
|       |                        | ± 0.50**      | ± 0.17***              | ± 0.55***          | ± 0.57***          |

Each value is mean ± SEM of 5 animals *p<0.05; **p<0.01; ***p<0.001 Control Vs other groups Group –I, Group-II Group-III as given in the text @ The values are expressed as µg of Pi formed hr/gm tissue
Table 4 Effect of caffeine treatment on the activity of HCO3-dependent ATP–ase in the jejunum of adult albino rats

| S. NO | GROUPS SHORT DURATION | MUCOSAL LAYER | SEROSAL LAYER |
|-------|----------------------|---------------|---------------|
|       |                      | UPPER JEJUNUM | LOWER JEJUNUM | UPPER JEJUNUM | LOWER JEJUNUM |
| 1. 2. 3. | Control Low dose High dose | 23.40 ± 0.46 6.09 ± 0.33 *** 17.55 ± 0.50*** | 15.25 ± 0.54 5.6 ± 0.33 *** 13.75 ± 0.96 | 14.84 ± 0.43 6.33 ± 0.34 *** 9.23 ± 0.51*** | 13.44 ± 0.70 9.04 ± 0.38 *** 8.59 ± 0.63*** |
|       | GROUPS SHORT DURATION | MUCOSAL LAYER | LOWER JEJUNUM | UPPER JEJUNUM | LOWER JEJUNUM |
| 1. 2. 3. | Control Low dose High dose | 27.08 ± 0.85 7.63 ± 0.53 *** 22.85 ± 0.50** | 20.52 ± 0.22 15.24 ± 0.46 *** 12.32 ± 0.17*** | 21.96 ± 0.45 7.46 ± 0.54 *** 14.33 ± 0.55*** | 15.61 ± 0.72 9.91 ± 0.68 *** 10.42 ± 0.57*** |

Each value is mean ± SEM of 5 animals *p<0.05; **p<0.01; ***p<0.001 Control Vs other groups Group – I, Group – II Group – III as given in the text @ The values are expressed as µg of Pi formed hr/gm tissue

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