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Effects of Adenine on the Pituitary-Gonad Axis in Newborns Rats

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Abstract: The present study was undertaken to investigate effects of the adenine on the hypothalamus-pituitary-gonad axis and changes in blood hormone concentration such as FSH, LH, progesterone and estrogen in newborn female rats. Adenine is a common organic base and its concentration variations caused by foods, has various effects on the body metabolic systems. In present study, fifty newborns rats were used divided into five groups, of 10s, including control I, control II which received solvent (normal saline) only and three experimental groups which received 50, 100 and 200 mg body weight adenine respectively. All the animals were kept under same condition with plenty food and water and treated Intra Peritoneally (IP) during days 2-16 after birth. At the end of experiment, all the animals were weighed, their ovaries were removed and blood samples were taken for hormone analysis. The results showed that dose dependent adenine solution significantly reduced the body and ovarian weight on 30 and 70 days after birth. In addition adenine led into no significant difference in concentration of FSH and LH in the experimental groups relative to the control on 30th day of life. But on the 70th day, the levels of these hormones raised significantly in the experimental groups. Furthermore, the adenine solution significantly increased the levels of progesterone and estrogen hormones in the experimental groups relative to the control on the 30th day, while decreased their concentration significantly on the 70th day. This situation has close similarities to metabolic disorders present in human caused by excessive use of adenine. High amounts consumption of adenine in can lead into hormone abnormality, weight loss and metabolic anomalies.

Key words: Adenine, estrogen, progesterone, FSH, LH

INTRODUCTION

Adenine is an organic base belonging to purine family. The structural formula of this crucial molecule is $C_5H_5N_5$. It is a fundamental unit of DNA and RNA that plays a role in energy transfer and the processes of energy storage as well as in cell metabolism (Houghton, 2006).

Adenine is a nucleic acid coenzyme and plays a vital role in regulation of most intracellular processes of renal cells and in pathogenicity of cell damage, nephritic toxicity and renal ischemia and nephritis. in addition, it is being used for improvement of white blood cell disorders (Zhang and Lindup, 1997). Adenine has two sources: external source (compounds present in foods) and an internal source (being synthesized in cells) (Danialzadeh and Zareian, 2001).

During the process of adenine catabolism in the AMP molecule (adenosine monophosphate), first the amino group is cleaved through activities of adenilate

desaminase and Inosine 5-monophosphate (IMP) is produced. The resulting IMP is then converts into hypoxanthine via activities of: nucleotides and nucleotide phosphorylase, respectively. In the human body, the final product of adenine breakdown is uric acid, (75%) which excreted by urine and the rest(25%) excreted by feces (Shahbazy and Maleknia, 2002).

Adenosine is a ubiquitous, biologically important molecule, that is a precursor of other biologically active molecules. It is a component of some co-factors and has distinct actions. The daily turnover of adenosine is very high and can act as a hormone by binding to its specific receptors. Four adenosine receptor subtypes have been identified and as an intracellular modulator, after transport into the cell by membrane transporter proteins (Scaramuzzi and Baker, 2003).

Extra cellular nucleotides containing adenine, trigger a vast range of cellular and metabolic responses in most cells through their interactions with specific ion

receptors. A group of their effectors includes immune neural and cardiovascular systems (Levy and Brene, 1999). Furthermore, adenine derivatives have important functions in the cell metabolism; some of these derivatives are AMP (Adenosine Mono Phosphate), NAD (Nicotinamide Adenine Dinucleotide), NADP (Nicotinamide Adenine Dinucleotide Phosphate), ATP (Adenosine Tri Phosphate), ADP (Adenosine Di Phosphate) and types of RNA among others (Shahbazy and Maleknia, 2002). The non-cyclic nucleoside phosphate 9-(2-phosphonilethoxy ethyl) adenine (PMEA) is regarded as a powerful anti-HBV agent and HIV infected patients can take it orally. PMEA has a specific anti-tumor influence (Hatse *et al.*, 1998). Among the plant compounds containing purine one can mention caffeine and theobromine; A small amount of these compounds are present in coffee and tea and these can intensify or lengthen the epinephrine effect (Danialzadeh and Zareian, 2001).

Adenosine has effect on the central nervous system, heart and vascular system, skeletal muscle, the immune system, the gonads and the other organs (Scaramuzzi and Baker, 2003).

The process of homeostasis and thrombus formation can be also affected by adenine nucleotides and adenosine (Pochmann *et al.*, 2004). On the other hands Xu *et al.* (2001) reported that estrogen as a target adenosine molecule influences the mechanisms responsible for ADP-induced vasodilation. Present study investigates the effect of the adenine on the Pituitary-Gonad axis in newborn rats.

MATERIALS AND METHODS

Animals: Twenty five pregnant rats were obtained from Shiraz University, in 2005, Shiraz, Iran each rat had the weighing about 300 ± 50 g were prepared. They were transferred to cages of poly carbonate material, with $50 \times 40 \times 25$ cm dimensions, topped with perforated ceilings of steel material. Cage floors were covered by sawdust, which were changed twice a week. These cages were kept in animal house which was cleaned and disinfected each week. During the study, temperature was set at $23-25^\circ\text{C}$ with 12 h light and 12 h darkness and plenty food and water.

Since the experiment was being conducted on newborn female rats, at the evening of the 21st gestational day, the newborn female rats were separated and were kept in separate cages in groups of 12 rats plus a mother rat. The five extra rats in each group were kept for the purpose of replacing those dying during the experiment. Newborns were weighed using a digital scale and their average weight was about 4 g.

Preparing the adenine solution: The white odorless crystalline adenine was obtained from Merck Company. This compound dissolves well in dilute alkaline hydroxides. Adenine solution was prepared as a full transparent solution, using a pH meter, distilled water, sodium hydroxide and adenine powder.

Experimental procedure: From the second to the 16th day of their life (i.e., for 15 days), all newborn female rats in control and experimental groups were subjected to IP injection of adenine solution or normal saline. There were five experimental groups of 10 rats including: Group I or control, which received nothing; group II or Sham, which received the same amount of normal saline as injected adenine solution; Group III-V or experimental groups were injected 50, 100 and 200 mg adenine solutions, respectively.

Blood sample collection: On days 30th and 70th, 5 rats of each group were randomly selected for blood sample collection and hormone assessment. Blood samples were centrifuged at 10 min on 5000 rpm and the resulting serums were frozen at -20°C to be analyzed later at a convenient time. Hormones assessment of FSH, LH, estrogen and progesterone was done by RIA (Radio Immune Assay) method (Berson and Yallow, 1973).

At the end of each experimental period and before blood collection, body and ovary weight of each group were determined for the relevant analyses.

Statistical analysis: The results were analyzed by SPSS (Statistical Package for Social Scientists) programs. To study the differences between the groups, the experimental data for different groups were compared using one-side ANOVA, Tokay-test and significance value was set at ($p \leq 0.05$).

RESULTS

The results obtained from the influences of injection of various doses of the adenine solution on the body and ovarian weight of rats on their 30th and 70th day of life. As shown, on days 30 and 70 after birth, the mean body weight and the mean ovarian weight in experimental groups declined significantly relative to the control ($p \leq 0.5$, Table 1).

The serum concentrations of LH in experimental groups receiving adenine solution showed no significant difference on the 30th day after birth, though a small raise was seen ($p \leq 0.05$, Table 2).

Table 1: Effects of different doses of adenine on the means of body and ovarian weight in rats on the 30th and the 70th days after birth.

Groups	Means of body weight (g)		Means of ovarian weight (g)	
	30th day	70th day	30th day	70th day
I	74.2±2.77	163.0±3.39	68.9±0.96	151.60±7.47
II	70.0±4.12	159.6±7.30	66.2±1.52	142.20±5.11
III	66.6±2.40*	135.0±3.84*	64.2±1.35*	119.80±8.22*
IV	55.0±1.58*	136.6±2.40*	62.2±1.68*	115.72±2.46*
V	55.0±1.58*	136.0±2.60*	61.4±0.96*	114.16±1.50*

Results are presented as Mean±SEM N = 5, *p≤0.05

Table 2: Effects of difference doses of adenine on the means of serum concentration LH and FSH in rats on the 30th and the 70th days after birth.

Groups	Means of concentration LH (mu mL ⁻¹)		Means of concentration FSH (mu mL ⁻¹)	
	30th day	70th day	30th day	70th day
I	0.54±0.08	0.29±0.12	0.36±0.07	0.43±0.01
II	0.45±0.01	0.39±0.15	0.12±0.01	0.47±0.01
III	0.72±0.06	0.72±0.20*	0.35±0.13	0.93±0.02*
IV	0.68±0.15	0.53±0.15*	0.30±0.01	0.83±0.01*
V	0.63±0.06	0.63±0.06*	0.30±0.01	0.80±0.01*

Results are presented as Mean±SEM N = 5, *p≤0.05

Table 3: Effects of difference doses of adenine on the means of serum concentration Progesterone and Estrogen in rats on the 30th and the 70th days after birth

Groups	Means of concentration progesterone (ng mL ⁻¹)		Means of concentration estrogen (ng mL ⁻¹)	
	30th day	70th day	30th day	70th day
I	13.88±1.01	64.26±0.99	6.71±0.95	6.06±0.65
II	13.70±1.58	52.54±2.04	5.54±1.18	0.38±0.27
III	28.20±8.94*	44.06±1.69*	10.52±1.68*	3.78±0.78*
IV	21.32±3.62*	45.00±1.62*	7.04±0.96	4.05±0.91*
V	40.96±2.42*	45.26±1.05*	7.68±4.62	3.28±0.36*

Results are presented as Mean±SEM N = 5, *p≤0.05

However, on the 70th day after birth, the serum content of LH hormone in experimental groups showed a significant increase relative to the control group (p≤0.05, Table 2). In addition, The FSH level in experimental groups showed no significant difference to the control group on the 30th day after birth, even though there was a small reduction in experimental groups (p≤0.05, Table 2), while on the 70th day after birth, the serum concentrations of FSH in experimental groups was significantly higher than that of the control group (p≤0.05, Table 2).

The serum concentrations of progesterone in experimental groups were significantly higher than that of the control group on the 30th day after birth (p≤0.05, Table 3), while on the 70th day after birth, concentrations of progesterone were significantly lower than that of the control group (p≤0.05, Table 3). In addition, the serum estrogen content significantly improved only in group III on the 30th day Table 3, while on the 70th day, its concentration decreased significantly in all experimental groups (Table 3).

DISCUSSION

According to the results the injection of dose dependent adenine solution led to the loss of body and ovarian weight in experimental rats ones on days 30 and 70 after birth (Table 1). Based on this weight loss, it is

probable that the inhibition of ornithine decarboxylase enzyme leads to specific losses of the total tissue weight, especially the ovaries and a tangible reduction in the protein level of organs. Numerous other studies support this weight loss and suppression of this enzyme (Pegg *et al.*, 1981; Bethell *et al.*, 1982; Pegg, 1986).

During polyamine biosynthesis, the enzyme ornithine decarboxylase is needed for the step of ornithine → putrescine conversion. This enzyme plays a fundamental role in the sexual maturation of mammals, so that ornithine decarboxylase antagonists such as αDFMO cause specific organ (including ovaries) defects and their weight loss. Moreover, a tangible reduction in the level of nucleic acids and proteins is seen in these organs (Abraham, 1981; Mamont *et al.*, 1978; Slotkin *et al.*, 1984). As an intracellular regulator, the ornithine decarboxylase enzyme, functions mainly in the biosynthesis of macromolecules, in differentiated cells (Muller *et al.*, 1986; Gray and Kavlock, 1991).

The pathway of cAMP signal in hormonal actions on sertoli cells, spermatogenesis and spinal cord injury is associated with changes in FSH and/or testosterone regulation (Huang *et al.*, 2003). Furthermore, Yazawa *et al.* (2003) also reported that the synergistic action of cAMP response element (CRE) binding protein, steroidogenic factor 1 (SF-1) and the rapid down-regulation of Dax-1 are responsible for induction of

gonadotrophin inducible ovarian transcription factor 1 (GIOT1) gene by gonadotrophin.

Based on Table 2, administration of adenine solution had no significant influence on the serum LH level on the 30th day, but on the 70th day, this hormone showed a significant increase in experimental groups. The reason for this raise may be related to adenine effects on the kidneys which results in uremia. According to the reports made by Damassa *et al.* (1977), De Kretser *et al.* (1973) and Holdsworth *et al.* (1977), the LH level increases in organisms with uremia, which results from a reduction in metabolic clearance. Moreover, the serum level of FSH in experimental groups on the 30th day showed no significant change while on the 70th day, it increased significantly relative to the control (Table 2); The reason may be a progressive renal insufficiency. This result is in accord with the findings provided by Chen and Liu (1981), Holdsworth *et al.* (1977) and Nazian and Dietz *et al.* (1987), who regard the raise in FSH level to be the result of a reduction in its clearance and an increase in its release.

The high level of intracellular cAMP, observed by treatment with cholera toxin and isobutyl methyl xanthine or by addition of 8-bromo-Camp, result in 6-to 7-fold increases in the intracellular content of progesterone receptor. In addition estrogen and insulin-like growth factor z (IGF-Z) are important regulators of the level of progesterone receptor in uterine cells (Aronica and Katzenellenbogen, 1991). On the other hand, the estrogen has a major effect on the levels of gonadotrophin receptors expressed in response to FSH and other cAMP -inducing ligands (Knecht *et al.*, 1984).

As seen in Table 3, the injection of adenine solution, caused a significant raise in the serum progesterone concentration in experimental rats on the 30th day, while on the 70th day, it showed a significant reduction (Table 3). In addition, on the 30th day, a significant increase in estrogen level was seen only in group III, while on the 70th day, its serum concentration showed a significant reduction in all experimental groups (Table 3). The reason for this initial increase and then a decline in the levels of the both hormones may be due to the role played by adenine; this means that, on the 30th day, adenine regulates the activity of the enzyme 17-beta hydroxyl steroid oxidoreductase and, consequently, raises the level of the two precursors of estrogen and progesterone hormones, i.e., 17-alpha hydroxy progesterone and androstenedione; while on the 70th day, their levels decrease due to renal insufficiency. This is in accord with findings provided by Nazian and Dietz (1987). They state that the hypothalamus pituitary axis is damaged in organisms with chronic renal insufficiency.

In general the findings of this research show that administration of adenine, in addition to affecting the hypothalamus-pituitary-gonad axis, has other effects on organs such as ovaries and kidneys as well, which result in hormone disorders and weight loss.

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