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ULTRASTRUCTURAL ASPECTS CONCERNING THE HYPOTHALAMUS-PITUITARY COMPLEX REACTIVITY FOLLOWING CHRONIC ADMINISTRATION OF ASPARTAME IN JUVENILE RABBITS

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Abstract. Experimental groups: C group - control group consists of 5 juvenile male rabbits. Our results showed that administration of 2 mg/kg b.w. of ASP induced some neurodegenerative injuries of ventraly-medialy hypothalamic neurons. Groupping of neurons with strong alteration of all neuronal ultrastructure in lesioned regions hippocampus and hypothalamus were observed in A group of rabbits. Degenerative aspects of all cellular constituents which are typical features for apoptosis were observed manifested by irregular outline of nuclei; picnotic and intense electrondense nuclei; mitochondrial and rough endoplasmic reticulum swellings; demyelination of the myelin sheath; intense vacuolisation both of the cytoplasm and axoplasma. The GH cells in A group are polymorphic in regards to the shape and structure of both the nuclei and cellular organelles. The nuclei have uneven shapes; many of them are hyperchromic with condensed chromatin; presenting dilated intermembranary spaces. The pituitary LH-FSH cells seemed to have an altered structure; and they have few secretion granules; both in rats and rabbits. The results of the electron microscopy study attest the neurotoxic effects of Aspartame on the hippocampus; hypothalamus and the anterior hypophysis cellular structures in juvenile rabbits.

INTRODUCTION

Aspartame (Equal; Nutrasweet); is one of the most videly low-calorie used artificial sweetener which is approximately 200 times sweeter than sucrose and it is added to over 5.000 food and drink products. Aspartame is hydrolysed in the body to three chemicals; aspartic acid (40%); phenylalanine (50%) and methanol (10%).

Aspartic acid is an amino acid; and it is an aspartate precursor. Aspartate and glutamate act as neurotransmitters in the brain; carrying information from neuron to neuron. When there is an excess of neurotransmitter; certain neurons are killed by allowing too much calcium into the cells. The neural cell damage that is caused by excessive aspartate and glutamate is the reason they are referred to as ‘excitotoxins’. The excitotoxins are substances; usually acidic amino acids; that react with specialized receptors in the brain in such a way as to lead to destruction of certain types of neurons. Glutamate and Aspartame are two of the more commonly known excitotoxins; (Ranney 1976; Roberts ; 1991; Stegink and Filer; 1984).

Phenylalanine is one of the "essential" amino acids; meaning that humans must get it from their diet. It is a precursor for the synthesis of tyrosine and several neurotransmitters. A high level of phenyalanine in the brain is extremely harmful and sometimes fatal. For this reason; products containing Aspartame carry an information label for phenylketonurics. *Methano*l is commonly encountered in the diet. Methanol is highly toxic; it is gradually

released in the small intestine when the methyl group of the aspartame encounters the enzyme chymotrypsin; (Troncho and Rardo; 1998; Walters; 2001).. The aim of this study was to determine the effects of Aspartame (ASP) chronic ingestion for a period of 30 days on ventraly-medialy hypothalamic area and CA1 hippocampic area neurons ultrastructure and on anterior pituitary GH; LH-FSH cells ultrastructure in juvenile rabbits.

MATERIAL AND METHODS

Three months-old healthy prepuberous; juvenile males Supercuni rabbits (*Oryctolagus cuniculus* L.); weighing initially 1200 ± 50 g.; were used. The rabbits were housed in cages of one animal each; in a controlled environment (21-22'C; 70-75% relative humidity; lights on from 08:00 to 20:00); and were fed a standard chow rabbit pellet and tap water ad libitum. Experimental groups: *C group* - 5 juvenile male rabbits and *A group* of 5 male juvenile rabbits treated 30 days by intragastrical gavage with ASP in a dose of 2 mg/kg b.w. Each rabbit received 4 mL of the diet slurry. At the end of the experiment animals were sacrificed by decapitation; the brains and the pituitaries were immediately removed after sacrifice; and prepared for electron microscopy investigation; according to Ploaie and Petra (1979); and Weakley (1981) methods. The neuronal ultrastructure of hypothalamic ventraly-medialy (VMH); area and of anterior pituitary GH; LH-FSH cells have been investigated.

RESULTS

Neuronal ultrastructure of VMH neurons

Neuronal ultrastructure of medial-basal hypothalamus nuclei has shown that in the case of the **C group** the morphological aspect of the neurons is normal; with large nuclei; euchromatic; round or oval; with an even outline; that contain one or two reticular nucleoli; with a clear outline. The neuronal perikaryon contains cellular organelles with a normal constitution: numerous polisomes and mitochondria; which have distinct cristae; as well as a rough endoplasmic reticulum; which is placed in narrow profiles; distributed throughout the cytoplasm. The axons and the dendritic extensions are covered in a myelin sheath; with normal constitution; but the myelin sheath is much thiny comparative to axonal and dendrtitic fibers in rats; (figure 1).

In the case of the **A group**; chronically treated with ASP the overall image of the VMH structures shows a reduced number of normal neurons. The general aspect of the surrouding basal hypothalamus in the case of A group of rabbits revealed the moderate to intense alteration of the neuronal cells ultrastructure. Large groups of the neurons have nuclei with an uneven outline; heterochromatic; the chromatin presenting condensation centers and condensations. Numerous neurons are being in strong stages of ultrastructural alteration. They contain hypercromic and picnotic nuclei with uneven outlines; with numerous invaginations and with a low caryoplasmatic content. The nucleols are enlarged. The cytoplasmic organelles have alterative degenerative modifications characterized by swelling; vacuolisation and loss of cristae of mitochondria; disintegrated mitochondria; and dilatation of the endoplasmic reticulum and Golgi system; comparative to normal aspects of the neurons in C group of rabbits. ASP-treatment induced degeneration of axons (demyelination and interruption of the myelin sheath; vacuolisation at the level of axoplasma; accompanied by losses of axonal substance); in lesioned VMH regions of hypothalamus; (figure 2).

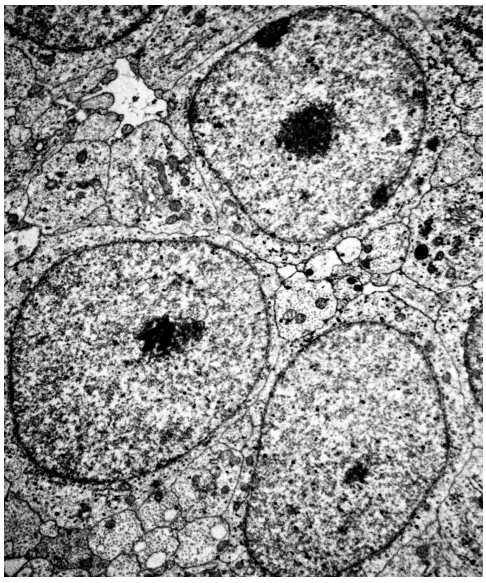

Figure 1 – Groupping of VMH neurons with normal ultrastructural aspect in C2 group, (magnification 10.000 x).

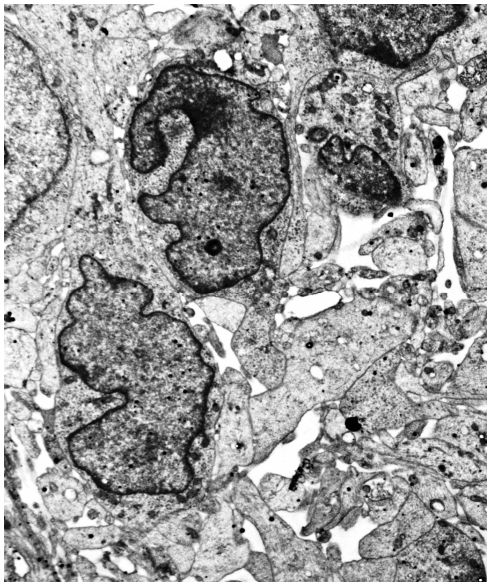

Figure 2 – Groupping of VMH neurons in A2 group. Strong degenerative changes of neuronal ultrastructure: picnotic nuclei, swelling of mitochondria, demyelinisation of the dendritic surfaces, (magnification 10.000 x).

Ultrastructure of the somatotrophs GH cells

Electron-microscopy study in **C group** have shown that there are numerous classical type of somatotroph GH cells; of normal aspect. Their cytoplasm contains a high number of large secretory granules (diameter 250-350 nm) and small secretory granules (diameter 100-150 nm). GH cells are polygonal or oval; sometimes irregular in shape; and medium in cell size. The rough endoplasmic reticulum and the Golgi apparatus are well developed in this stage of onthogeny; (figure 3). A difference in the number and in form of GH cells was found after ASP-treatment in **A group** of rabbits. Our study showed an accentuated polymorphism of shape and nucleus and cellular organelles structure of GH cells. The majority of GH cells have altered nuclei which become oval; reduced in volume; with close to normal organized chromatine. The majority of mitochondria are slightly inflated; but with clear visible cristae. The completely degenerated GH cells have picnotic and reduced in size of the nuclei. There are various degrees of vacuolization of cytoplasme; few secretion granules; altered shapes and sizes of cellular organelles; both of the endoplasmic reticulum; with dilated profiles. Mitochondria are highly inflated; and intense vacuolised; without the cristae; (figure 4).

Ultrastructural study of the gonadotrophs LH-FSH cells

LH-FSH gonadotrophs cells have a distinctive morphology on electron microscope examination The gonadotrophs cells that secrete glycopeptide hormones in **C group** are less represented; probably because of the sexual immaturity of the animals. These cells are scattered as single cells or small groups throughout the gland. Ultrastructurally the immature and round granules are between 150-400 nm in diametter. The cells have globular; euchromatic cells; with one or two nucleoli. The cellular organelles involved in the process of hormonal secretion have a low representation; and they are situated on the periphery of the cytoplasme; especially. The ribosomes are numerous disposed on all the surface of the cytoplasme; suggesting a less to intense synthesis activity. Mitochondria are numerous;

globular; with clear visible cristae; and ribosomes are numerous; distributed throughout the cytoplasm. In the cytoplasme the majority of the secretion granules are immature; disposed in certain areas; especially at the periphery of the cell; (figure 5).

LH-FSH cells in **A group** are highly polymorphic in their general aspect; with uneven nuclei; with numerous invaginations and dilated intermembranary spaces. Some gonadotrophic cells have different degrees of alteration of their shape and structure of cellular organelles. Nuclei are globular or oval; euchromatic; or heterochromatic. Mitochondria are moderately inflated; the Golgi system and the ribosomes (grouped in synthesis centers) are less represented; there is a relative low number of secretion granules. The majority number of LH-FSH releasing cells showed significant alterations of nuclei; which are of an uneven shape; heterochromatic; with tendencies of condensation; or picnotisation. The gonadotrophs developped extensive cytoplasmic vacuolation; the mitochondria are inflated; the endoplasmic reticulum has dilated profiles; and the Golgi apparatus is weakly represented. The number of hormonal secretion granules is highly decreased in the majority of cells. The ribosomes are few in number; (figure 6).

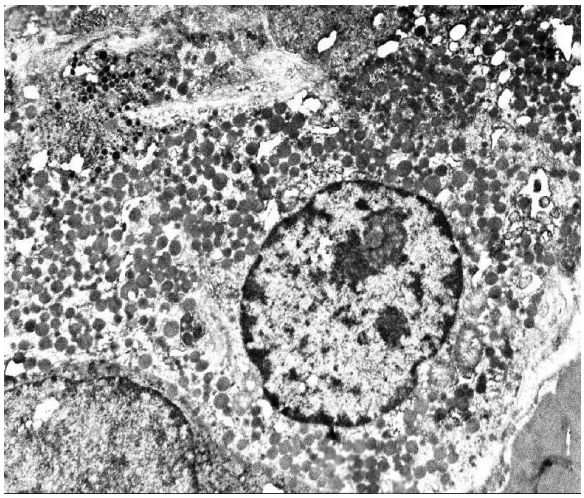

Figure 3 - Round GH cell with high density of the secretory granules in C group, (magnification 10.000 x).

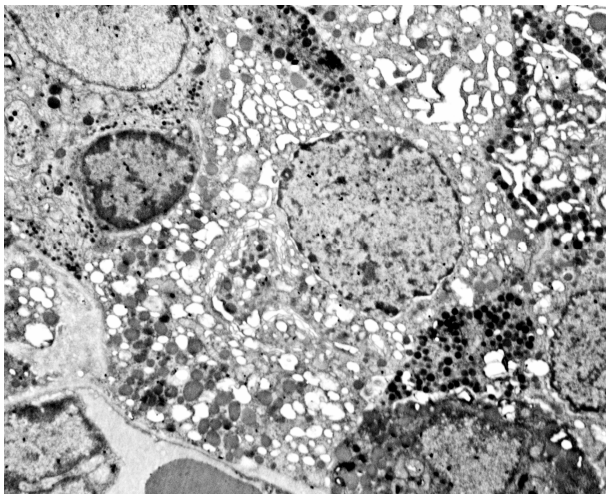

Figure 4 - Grouping of round and polygonal GH cells in A group which are devoid of secretory granules,. (magnification 8000 x).

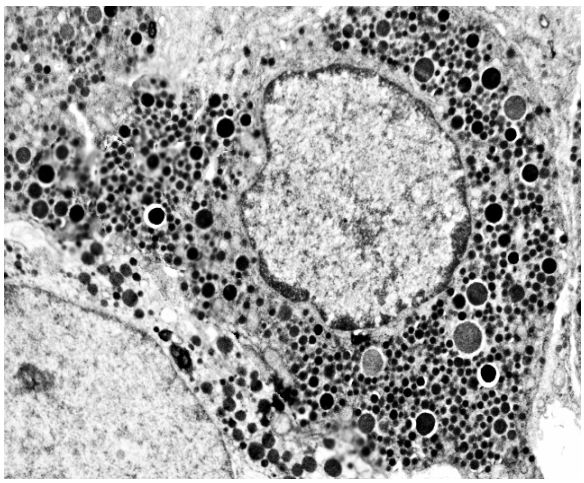

Figure 5 - LH-FSH secretory cells in C2 group. There are numerous immature and few mature secretory granules disposed throught in the cytoplasme, (magnification 10000 x).

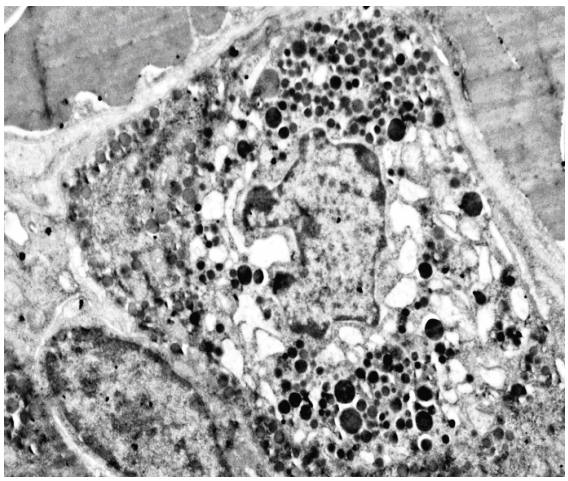

Figure 6 - Gonadotroph cells in the A2 group The cytoplasme contains balloonised mitochondria. The secretory granules have a relative few number, (magnification 12000 x).

DISCUSIONS

The electron-microscopy analysis revealed that chronic administration of 2 mg/g b.w. of ASP for 30 days induced selective degeneration of all subcellular neurons ultrastructures both in CA1 neurons of hyppocampus and in VMH area of hypothalamus in juvenile rabbits.

The results of electron microscopy have pointed out their concordance with the data from literature; that show that different ways of administration (parenteral or direct intrahypothalamic injections) of some excitotoxic amino acids (glutamate; aspartic acid; cysteine); and their homologues (ibotenic; kainic acids) to lab rodents; caused aspects of neuronal degeneration; associated with axonal-dendritic lesions; (Blaylock; 2002; Puică et al.; 2006; Stegink and Filer; 1984).Ultrastructural studies (Olney; 1980); localized the apparent site of toxic action to postsynaptic dendrosomal membranes where glutamate excitatory synaptic receptors are believed to be localized. By electron microscopy it has been shown that the toxic action of monosodium glutamate and aspartate impinges selectivelly on dendritic and somal surface of the neuron that posses excitatory receptors trough which the depolarizing effects of glutamate and aspartate putativelly are mediated. The precise mechanisms by which excessive EAA receptor activation leads to acute neuronal death are not well understood; although increased membrane permeability and abnormal Na^{+}; Cl^{-} and Ca^{2+} influx into cells and mitochondria are assumed to play important roles; (Meldrum; 2000).

Myelination can also be affected by neurotoxins. In general; excitotoxic substances affect dendrites and neurons more than axons but axon demyelination has been demonstrated. During the myelination process; each fiber tract has its own spatiotemporal pattern of development; accompanied by significant biochemical changes; especially in lipid metabolism. In general; the excitotoxic lesions (neuronal lesions and those of the axonal and dendrite extensions) caused by the high levels of excitotoxins in the sanguine flow; are extremely harmful during the intrauterine development of the fetal brain; these manifesting themselves starting with the third trimester of pregnancy; continuing in the first years after birth too; during to prepuberty stage of development; under conditions of incompletely developed brain; (Blaylock; 2002; Erselius and Wree; 1991; Hull; 2000; Puică et al.; 2008).

In this way; Blaylock (1997; 2002); Bowen (2007); Coyle; (1981); Olney et al.; (1980); Pardridge; l986; Puică et al.; (1997; 2004a; b; 2006; 2008) described that exposure to excitotoxins (such as glutamate; aspartate and Aspartame) during fetal and prepubertal life in rodents may cause alteration in brain development. As it is well known; the periadolescence is classically defined in rodents as the ontogenetic period including the week preceeding the onset of puberty and few days thereafter. During this period; brain areas show proliferation and maturation of axon terminals and synapses. (Harder 2002; Butchko et al.; 2002; and Yin et al.; 2006) mentioned that Aspartame attacks and destroys the nervous system.

In conclusion; our researches pointed that the very young prepuberal rabbits appear to be most susceptible to the deleterious effects of Aspartame.

Our animal experimental data put the problem of extrapolation into the human **to predict human risks concerning the Aspartame consume**. It was indicated that the highly reliable extrapolation into the human; based on the animal experimental data; could be done by the reason that the animal finding predicted effects of Aspartame in humans. Consumption of Aspartame containing products by childrens during this critical juvenile period of life of brain formation is of special concern and **should be discouraged**.

CONCLUSIONS

- The chronic administration of Aspartame at prepubertal stage on juvenile rabbits induced neurodegenerative effects especially in the circumventricular organs (CVO) of hypothalamus; and severe structural and functional alterations in hypothalamic-pituitary axis.
- The experimental results show that there is an increased sensibility of the immature brain in prepubertal stage of onthogenetic development following chronic exposure. to Aspartame
- The degenerative aspects of brain and pituitary observed in Aspartame-treated rabbits; suggests that it is reasonable to assume that the same infant-to-adult relationship would be true for the Aspartame consumption in humans to children in the prepubertal period of development.

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