Effect of Protein Malnutrition on Efferent Projections of Amygdala to the Hippocampus

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ABSTRACT

Introduction: Previous investigations have shown that protein malnutrition can alters the structure and function of some areas of hippocampal formation. We investigated the effect of protein malnutrition on amygdaloid projections to the CA1 hippocampal area. In this study we investigated level and pattern of distribution of efferent projections from amygdala to hippocampus in the rat by Horseradish Peroxidas (HRP) neural tract tracing in 2 groups; Control group fed with regular diet (% 18 proteins)and case group fed with low protein diet (%8). We used SPSS 11.0 (T test & mann-withney) Software for data analysis.

Methods: Following injection of HRP to CA1 region of hippocampus in the control group Rats, Labelled neurons showed more density in the Basolateral, Cortical and Medial nuclear Groups. Having done the analysis and examining the relations between the case data and those of the control groups, we found that number of labelled neurons in the Basolateral, Cortical & medial nuclei were decreased in the case group($p<0.05$). Our findings showed that different nuclei of amygdala (Basolateral, Cortical and Medial) send projections to CA1 region of hippocampus; Among, them basolateral nuclei group send the most projections .

Discussion: This results may be caused by decrease of activity of neural cells after protein malnutrition, that can results in impairment in growth and development of nervous system. Also it is possible that axoplasmic transfer rate maybe decreased in this condition.

1. Introduction

Malnutrition is associated with severe health and socioeconomic problems worldwide(Morgane, Mokler, & Galler, 2002).Malnutrition during development results in long-lasting structural and biochemical abnormalities in different brain regions(Bedi, 2003), especially in the neocortex and hippocampal formation(Cordero, Valenzuela, Rodriguez, & Aboitiz, 2003). Moreover, pre- and early postnatal malnutrition produces behavioral impairments and memory deficits(Valadares & de Sousa Almeida, 2005) .Protein malnutrition produces a number of effects in the hippocampal formation, such as increased genesis of cells in the anterior dentate granule cell layer. Histological changes also have been demonstrated, include decreased dendritic spines in the cingulate cortex and

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hippocampus of malnourished rats (Garcia-Ruiz, Diaz-Cintra, Cintra, & Corkidi, 1993; Schonheit & Haensel, 1984) .

In the glutamatergic granule cells, malnutrition reduces the mossy fibre axonal area(Granados-Rojas, Aguilar, & Diaz-Cintra, 2004) and the number of dendrites and spines (Diaz-Cintra, Garcia-Ruiz, Corkidi, & Cintra, 1994). Similar morphological effects have been found in CA3 pyramidal cells(Garcia-Ruiz, et al., 1993). In addition, it has been reported that prenatal malnutrition increases the frequency of miniature inhibitory postsynaptic currents in CA1 and CA3 interneurons (Chang, Galler, & Luebke, 2003). Malnutrition` differently affected glutamic acid decarboxylase 67 (GAD-67)-positive hippocampal interneurons in young and adult rats. These alterations might partially explain the changes in inhibitory activity recorded in the hippocampus, as well as the deficits in attention, learning, and memory processes observed in malnourished animals(Diaz-Cintra et al., 2007).

Early nutritional deficiencies, even when transient, may affect long-term disease risk, possibly by modifying cellular differentiation and/or function at critical stages of development(Caballero, 2002). A study showed that pre- and postnatal malnutrition in rats reduced proliferation of neuronal and glial cells (Giuffrida, Hamberger, Serra, & Geremia, 1980). Hippocampal formation system consists of hippocampus, subiculum and adjacent parahippocampal region is known to play an important role in learning and memory processes.

It has been showed that amygdala (accessory basal, medial basal, cortical nuclei and cortical amygdaloid transition area) send projections to the hippocampal formation. Projections from magnocellular part of the accessory basal nucleus and cortical nuclei terminate in the molecular layers of CA3, CA2, and CA1 subfields. These connections may be important in the limbic memory system(Saunders, Rosene, & Van Hoesen, 1988) . Electrical stimulation of amygdaloid body in conscious humans evokes feelings of fear and sometimes of general irritability of even anger. Effects of destructive lesions in the temporal lobes consists of docility, loss of ability to learn, abnormal sexual activity and loss of short time memory(Amaral & Witter, 1989). Another study showed that presubiculum, basolateral nucleus of amygdala and contralateral presubiculum innervated by the CA1 area of hippocampus(van Groen & Wyss, 1990).

A recently revealed important function of the amygdala is that it acts as the brain's lighthouse, constantly monitoring the environment for stimuli which signal a threat to the organism (Liddell et al., 2005). The posterior nucleus of amygdala send projections to adjacent area of CA1 of hippocampus , subiculum and Entorhinal cortex (Canteras, Simerly, & Swanson, 1992). It was demonstrated that vasopressin cells in the medial amygdaloid nucleus project to: Lateral septum by the ventral amygdalofugal pathway and ventral hippocampus via the Amygdalohippocampal transition zone(Caffe, van Leeuwen, & Luiten, 1987). Injection of Horseradish Peroxidas (HRP) as a tracer, in CA1 hippocampal area showed that pyramidal cells of this area receive afferents from anterior part of amygdala(Wyss, Swanson, & Cowan, 1979). Our study focused on effect of protein malnutrition on amygdaloid projections to CA1 hippocampal area.

2. Methods

Male wistar Rats were used (2+103 gr ; n: 20); they were divided into two groups (case & control) in a simple randomized manner, prior to use. During 7 months control group was being fed with normal diet (% 18 protein) and case group with low protein diet (%8 protein). They were kept in a 12 hours light /dark cycle.

All of the experimental procedures were approved by the animal ethics committee of the medical university of Qazvin.

Rats were anaesthetized by interaperitoneal injection of ketamine (40mg/kg) and xylasine (5mg/kg)(Anesthesia and analgesia in laboratory animals, 2008). HRP enzyme was microinjected by stereotaxic surgery in the CA1 region of dorsal hippocampus in both groups (Bregma -3.30 , Lateral 1.5 , Deep 2.8 mm). 48 hours after surgery, the rats died because of the lethal dose of ketamine and xylasine, and then perfused transcardially with saline followed by

Karnovsky's fixative (0.1 M phosphate buffered mixture of 2.5% glutaraldehyde and 2% paraformaldehyde, pH 7.6). After removal, the brains were cut in 40 micrometer thick coronal sections by a freezing microtome (Cryocut 1800), and then stored in 0.1 molar phosphate buffer. Then sections were reacted with Tetra Methyl Benzidin (TMB) according to the procedure of Mesulam (Mesulam, Van Hoesen, Pandya, & Geschwind, 1977). Sections were lightly counterstained with neutral red. After assessment of injection Site, we studied the slides with light microscope and took photographs

from all amygdaloid areas. Topographical studies on distribution of labeled cells with HRP were performed. Image toll 2 and SPSS11.0 software (T-test and Mann-withney) for analysis of data were used.

3. Results

Two groups were fed by specific diet for 7 months. After injection and perfusion, brains were removed. Injection site in hippocampus were defined by dark blue color after histochemical reaction. After injection of HRP this substance absorbs by axonal endings and transfers retrogradly to perikaryons that send projection to injection site and accumulate in several vesicles, these cells were determined as HRP labeled cells.

In this study, after injection of HRP in CA1 hippocampal area, the number and pattern of distribution of labeled cells in amygdaloid nuclei in case and control groups were counted and compared. Because the injection of enzyme was performed unilaterally, other amygdala was considered as contralateral.

Amygdaloid nuclei are divided in 4 groups: basolateral, cortical, centromedial and other amygdaloid nuclei groups.

After injection of tracer in CA1 hippocampal area, most of the labeled cells in amygdala were in basolateral(BL) nuclei group.

Basolateral(BL) group includes 3 nuclei: basolateral(BL) , accessory basal (AB) and lateral (LA) nuclei. Most labelled cells were seen in anterior part of the basolateral nucleus (BLA), posterior part of accessory basal nucleus and anterior region of dorsolateral nucleus(LaDL). Among Lateral nuclei, Basal and Accessory Basal nuclei have more connections than other nuclei. The number of labelled cells in basolateral group of contralateral side was less than ipsilateral and showed the same pattern of distribution (Fig. 2).
Comparing the case and control groups, we found that in case group, the number of labelled neurons decreased significantly (t test, $p<0.05$, Mean ± SEM of control group is 62.17 ± 1.424 N=6 and Mean ± SEM of case group is 31.17 ± 0.8724 N=6)

Cortical-like nuclei group: The number of labelled cells in cortical group showed that the posterior part of cortical nuclei (PMCO) sends more projections than anterior (ACO) and posterolateral nuclei (PLCO) to CA1 region. Number of labelled cells in the contralateral side was less than ipsilateral and showed the same pattern of distribution . Comparing the case and control groups, we found that in case group the number of labelled neurons in ACO , PMCO, and PLCO nuclei decreased significantly (t test, $p<0.05$, Mean ± SEM of control group is 31.17± 0.8724 N=6, Mean ± SEM of case group is 12.00 ± 0.6831 N=6).

Centromedial nuclei group includes 3 nuclei groups: central nuclei, medial nuclei and bed nucleus of stria terminalis. In bed nucleus of stria terminalis area no labelled cells were seen in both groups. In central nuclei the most projections to the hippocampus were originated from the central and medial parts of central nuclei (Cec,Cem). In medial nuclei the most projections to the hippocampus were originated from the centrum of postrolateral part of this group.

The labelled cells of contralateral centromedial group were less than ipsilateral and showed the same pattern of distribution (Fig 1). Comparing the case and control groups (Fig. 2), we saw a significantly decreasing of labelled neurons in the former group (t test, $p<0.05$, Mean ± SEM of control group is 26.00 ± 0.6831 N=6 and Mean ± SEM of case group is 8.000 ± 0.5774 N=6).

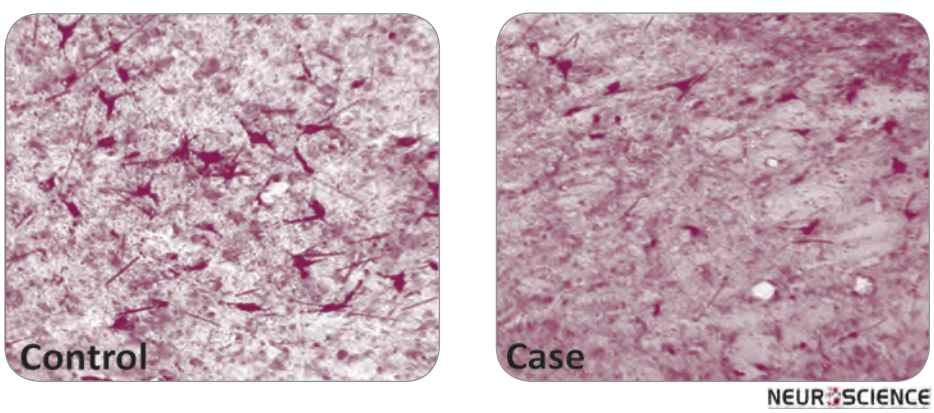


Fig. 1. labeled cells in ipsilateral Centromedial nuclei in control and case

Fig. 2. Topographic drawing of injection site and dispersions of labeled cells in Amygdala of control (left) and case (right) groups

Other nuclei of amygdala includes: anterior part of amygdala (AAD, AAV), Amygdalo-hippocampal area (AHiPM, HiAL) and Intercalated nucleus .

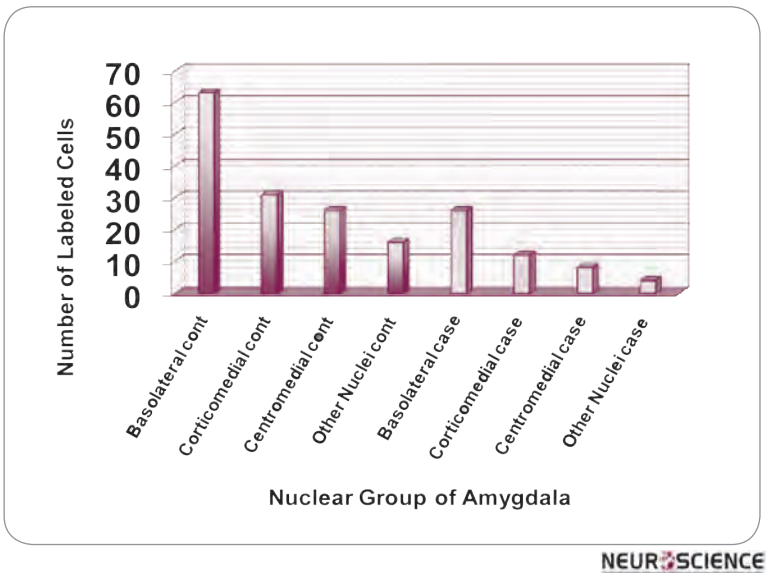
Labeled cells were seen in posterolateral division of the anterior part of amygdala group and anterior part of Amygdalo-hippocampal area . No labeled cell was seen in Intercalated nucleus of both groups.

The number of labeled cells of this group in the contra lateral side was less than ipsi lateral, and showed the same pattern of distribution. Comparing the case and control groups, the number of labelled neurons of the area in case group was found to be reduced considerably (t test, $p<0.05$, Mean ± SEM of control group is 15.67 ± 0.6667 N=6 and Mean ± SEM of case group is 4.000 ± 0.3651 N=6) but no changes were seen in pattern of distribution of connection (Table 1) (Fig. 2).

4. Discussion

Previous studies have shown that basolateral nuclear group has extensive connections with different parts of hippocampus. In the study of pikkarainen et al, Phaseolus Vulgaris-leucoagglutinin as antrograde tracer was injected in various divisions of the lateral, basal and accessory basal nuclei of the rat amygdale; their results showed that the most projection to hippocampus originates from the basal nuclei (Pikkarainen, Ronkko, Savander, Insausti, & Pitkanen, 1999); The most projections are to CA1 and CA3 areas of hippocampus (Pikkarainen & Pitkänen, 2001). Results of this study also indicated that most of the projections to hippocampus originate from basal nuclei group. Other studies have shown that efferents from the magnocellular part of accessory basal nucleus terminate in the molecular layer of CA3, CA2 and CA1 areas of hippocampus(Saunders, et al., 1988). The organization of axonal projections from the Basolateral nucleus of amygdala was examined with Phaseolus Vulgaris-leucoagglutinin (PHAL) method in another study. The results indicated the anterior part of BMA mainly to innervate Olfactory and Insular areas, whereas the posterior part of BMA also innervates inferior temporal (Perirhinal, Entorhinal) cortex and medial prefrontal (Infralimbic, Prelimbic) areas of the hippocampal formation(Petrovich, Risold, & Swanson, 1996). Also, one study showed that different areas of basolateral nuclei group and accessory basal nucleus project to the Stratum Lacunosum- molecular of CA1(Pikkarainen, et al., 1999). Our results showed that posterior part of accessory basal nucleus sends more projections to the hippocampus .

Table 1. Comparisonal columnar chart of number of labeled cells in ipsilateral Amygdala of case and control groups



Pikkarainen and Pikanen have shown that the largest number of projections from the amygdala to the hippocampal formation and the parahippocampal areas are originated from the lateral, basal and accessory basal nuclei. These projections terminate in the rostral half of the Entorhinal cortex, temporal end of CA3 and CA1 subfields or the subiculum and the parasubiculum(Pikkarainen, et al., 1999). fallout of this study indicated that LaDL nucleus of Lateral nuclei send the most projections to the hippocampus in comparison with other nuclei of Lateral nuclei. Among Lateral nuclei, Basal and Accessory Basal nuclei have more connections than other nuclei. In 2002 kemppainen indicates that hippocampal formation, stratum lacunosum-molecular of temporal CA1 subfield and the adjacent molecular layer of the proximal temporal subiculum received a moderate projections(Kemppainen et al., 2002). Pikanen Also showed that posterior cortical nuclei send projections to the CA1, CA3 and Subiculum areas of Hippocampus(Pitkänen, Pikkarainen, Nurminen, & Ylinen, 2000). Our findings are also the same: nuclei of posterior part of the cortical group (PMCO, PLCO) send more projections to the hippocampus in comparison with the anterior part of the cortical group (ACO). Majak & Pitkanen indicated in their study that periamygdalo-hippocampal area sends projections to the CA1 area of hippocampus (2003). Yet, this current research by HRP signifies that the periamygdalo-hippocampal area sends lesser projections to the CA1 area compared with other parts of amygdale.

As seen in graph 1 all of the 4 nuclear groups of amygdala send projection to the CA1, but projections of Basolateral nuclear group is more than other nuclear groups.

Proteins constitute about 8% of brain's weight. Biosynthesis of Proteins in brain depends on persistent availability of sufficient amounts of amino acids. Regarding the importance of dietary protein, many studies have assessed the effects of protein malnutrition on different parts of brain. Results of one study in 1999 showed that mild prenatal malnutrition (8% Protein) is able to produce deleterious effects on cortical neuronal density(Soto-Moyano et al., 1999).

Andradeh and colleagues examined the part and found out that protein deprivation in adult life can lead to deficits in the number of hippocampal granular cells and CA3-CA1 pyramidal cells, and changes the dendretic domain of granule and CA3 pyramid cells. The volume of the subiculum and the total number of its neurons were reduced in malnourished animals. Their study also revealed marked regressive changes in the apical and basal dendretic trees of the pyramidal subicular neurons. However, the spine density was increased in malnourished rats (Andrade, Madeira, & Paula-Barbosa, 1998).

Prenatal protein malnutrition has deleterious effects on hippocampal structure and function that likely is the result of a decreasing in synapse number. Moreover it can produce a significant decrease in the volume of

the mossy fibbers of CA3 area (Granados-Rojas et al., 2002).

Hippocampal formation undergoes significant morphological and functional changes after prolonged food restriction; dentate granule cells are selectively vulnerable among hippocampal neurons (Andrade, Lukoyanov, & Paula-Barbosa, 2002). Benitez-Bribiesca, De la Rosa-Alvarez, & Mansilla-Olivares pointed out in a research that early protein-caloric malnutrition during the critical brain development period (first 24 months of age), causes severe alteration in the dendretic spine apparatus of neurons of the fifth cortical layer. The Changes comprise: shortening of the apical dendrites, significant decrement of the spines and presence of abnormal forms (1999).

Oxidative damage may be in relation with the brain changes induced by protein malnutrition in hippocampus. Caloric and/or protein prenatal malnutrition has been reported to cause oxidative stress in extracerebral tissues by the changes in antioxidant enzyme activity and/or antioxidant compounds in humans (Gupta, Narang, Banerjee, & Basu, 2004) and rats(Rana et al., 1996). Oxidative damage to lipids and proteins are probably associate with numerous alterations in membrane structure, biochemical parameters, and functional activities such as membrane fluidity and mitochondrial dysfunction(Halliwell, 1992).

In conclusion, we investigated the effect of protein malnutrition on efferent projections of amygdala to hippocampus. Thus it was revealed that the number of labeled cells is decreased in amygdale of protein malnourished rats. This cut was significant in the nuclei of basolateral group, posterior parts of cortical group and some parts of centromedial. Possibly the reduction of neural cell activity following protein malnutrition delete, might cause the mentioned decreasing in amygdale of protein malnourished rats. it is Also possible that the decreasing of axoplasmic transfer rate might be for the protein malnutrition. These changes can alter synaptic connection and/or conduction between neurons. We recommend that the effect of protein malnutrition on neuronal activity, structure and function of amygdala and hippocampal formation and other areas of brain should be studied.

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