Asian Journal of Chemistry Vol. 19, No. 6 (2007), 4135-4142

Biochemical and Histochemical Study on the Hypothalamus at Some Periods of Starvation and Refeeding After Starvation

A. TEMUR*, M. ATLI, H. KARADAG†, H.H. DONMEZ† and F. ASKEROV‡
Faculty of Education, Yüründü Yil University, 65080 Van, Turkey
E-mail: temurat@yahoo.com

This study was carried out to determine biochemical and histochemical alterations in the lateral (feeding centre) and ventro-medial (satiety centre) nuclei of hypothalamus in rats starved and refed after some periods of starvation. 180 Wistar albino rats were used in the investigation. The rats were starved for a periods of 1, 3, 5 and 7 d. The rats starved 5 d were refed for a periods of 5, 10, 15 and 30 d. The activity of the neutral peptide hydrolase was biochemically determined in the hypothalamus. In addition, histochemical investigation was made in the feeding and satiety centres. It was observed that the activity of the neutral peptide hydrolase became closer to the levels of the control group at 30th d of refeeding after 5 d starvation. It was concluded that the feeding centre was more sensitive to starvation than the satiety centre and the satiety centre was restored earlier than the feeding centre.

Key Words: Hypothalamus, Biochemical study, Histochemical study, Starvation.

INTRODUCTION

It has been reported that more than 50 neuropeptides were synthesized in the hypothalamus[1]. The transport proteins in the hypothalamo-neurohypophyseal system are effective on the formation of a lot of polypeptides such as neurophysin I and II, the neuro-hormones dilated the vessels of heart[2]. In addition, it was noted that β-lypothropins and endopeptidases help to formation of the endorphins and enkephalins in the hypothalamus[3,4].

Marks *et al.*[5] and Walter and Glickson[6] brought forward that the neutral peptides and the neutral peptide hydrolases are synthesised in the hypothalamus. These peptides are charged in a rather complex interaction mechanism and regulates the peripheral hormonal system by the central system[7,8].

†Faculty of Veterinary, Yüründü Yil University, 65080 Van, Turkey.
‡Institute of Physiology n.a. A.I. Karayev, Azerbaijan Academy of Sciences, Baku, Azerbaijan.

While some neuropeptides, especially endorphins and vasointestinal peptides, affect transmitters in the neurones, some of them also affect animal behaviour. For example, glucagon and holohistokinin regulate the capacity of appetite, thus, they regulate feed intake and feeding time in animals[9]. Anochin[10] suggested that holohistokinin provide the satiety sensation. Kangava and Matsuo[11] reported that holohistokinin enhance the inhibitor effects of somatostatin by effecting the growth hormone.

Whereas Oomura *et al.*[12] stated that the feeding centre (Lateral nucleus of the hypothalamus) responded earlier and faster than the satiety centre (Ventro-medial nucleus of the hypothalamus) to starvation, Zakyskin *et al.*[13] suggested that there is a close relation between the neurone functions and the morphology of nucleoproteides.

The restoration in the feeding centre could be observed in 60-70th day of the refeeding after starvation[14,15].

All of these peptides are classified in the category of the proteins based on their origin and synthesized by the neutral peptide hydrolases. Therefore, the determination of the neutral peptide hydrolase levels are important in the some periods of the starvation and the refeeding after starvation. The aim of this study was to determine neutral peptide hydrolase levels and histochemical changes in the feeding and satiety centres of hypothalamus of rats starved and refed after starvation.

EXPERIMENTAL

A total of 180 adult, healthy male Wistar albino rats were used in this study. 20 Rats were randomly allotted into each treatment group. 10 Rats from each treatment group were utilised for histochemical and biochemical studies.

Control animals: In this group, 20 rats were used. These animals were fed with commercial diet (Van-Yem A.[a]., TURKEY) and watered *ad libitum*.

Starved animals: In this group 80 rats were used. They were divided into 4 groups. These groups were starved for a periods of 1, 3, 5 and 7 d, respectively. They were watered *ad libitum*.

Restored animals (refed animals group after starvation): In this group 80 rats starved 5 d were used. The rats were divided into 4 groups and fed with commercial diet and watered *ad libitum* for a periods of 5, 10, 15 and 30 d, respectively.

Biochemical methods: The rats were killed by cervical dislocation at the end of the treatment. The activity of the neutral peptide hydrolyzes was detected by establishing the arginin levels in the homogenate of the hypothalamus and the fractions of the hypothalamic cells[16]. The findings in the

periods of the starvation and the restoration were compared with those of the control group.

Histochemical methods: To determine the nucleoproteins in the neurones, histological sections of the Carnoy's solution-fixed, paraffin embedded the feeding and satiety centre were prepared and stained with cresyl violet[17].

Statistical analysis of data: All data were subjected to analysis of variance using General Linear Model Procedure of SAS[18]. Mean Treatment differences were determined by Duncan's t-test with a level of statistical significance of 5 %[19].

RESULTS AND DISCUSSION

Biochemical results: The arginin levels in homogenate of the hypothalamus and fractions of the hypothalamic cells in the control group and different periods of the starvation groups are shown in Table-1.

TABLE-1
ALTERATIONS OF THE ARGININ LEVELS IN HOMOGENATE OF THE HYPOTHALAMUS AND FRACTIONS OF THE HYPOTHALAMIC CELLS AT THE PERIODS OF STARVATION

Groups	Homogenate (µg)	Fractions		
		Nucleus (µg)	Mitochondria (µg)	Cytosol (µg)
Control	1.74 ± 0.11	0.78 ± 0.06	0.49 ± 0.03	1.80 ± 0.06
1 d starvation	2.26 ± 0.67*	1.20 ± 0.08*	1.49 ± 0.07‡	1.52 ± 0.07‡
3 d starvation	2.75 ± 0.19†	2.87 ± 0.17‡	4.23 ± 0.21‡	1.68 ± 0.09
5 d starvation	2.93 ± 0.21†	10.10 ± 0.23‡	6.30 ± 0.20‡	2.90 ± 0.07‡
7 d starvation	3.52 ± 0.18‡	3.01 ± 0.12‡	7.71 ± 0.13‡	8.68 ± 0.14‡

The difference in important compared with the control *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.

While the neutral peptide hydrolase activity increased 1.3, 1.5 and 3; and 1.5, 1.5 and 8.6 folds in homogenate, nucleus, and mitochondria, respectively, it decreased 16 and 7 % in cytosol at first and third day of starvation, respectively, compared with that of control.

The increases in the neutral peptide hydrolase activity were 1.7, 1.6, 13 and 13; 2, 4.8, 3.8 and 16 folds in homogenate, cytosol, nucleus and mitochondria, respectively, at 5th and 7th day of starvation, respectively, compared with that of control.

This findings indicated that the levels of the neutral peptide hydrolase continuously increased in homogenate from the 1st to 7th day of starvation. Their levels in mitochondria increased from the 1st to 5th day of starvation, but at 7th day of starvation, their levels rapidly decreased to the levels of the 3rd day of starvation.

It was noted that the NPH activities in cytosol were low at 1-3 day of starvation, but started to increase from the 5th day of starvation and continued to increase.

The levels of the arginin in the hypothalamus of the rats refed after 5 days starvation are shown in Table-2. It was noted that the levels of the neutral peptide hydrolase in homogenate and fractions started to become close to the levels of control group at 30th day of refeeding after 5 days starvation.

TABLE-2
ALTERATIONS OF ARGININ LEVELS IN HOMOGENATE OF HYPOTHALAMUS AND FRACTIONS OF HYPOTHALAMIC CELLS AT THE PERIODS OF REFEEDING AFTER 5 DAYS STARVATION

Groups	Homogenate (μg)	Fractions		
		Nucleus (μg)	Mitochondria (μg)	Cytosol (μg)
Control	1.74 ± 0.11	0.78 ± 0.06	0.49 ± 0.03	1.80 ± 0.06
5 d starvation	3.09 ± 0.22†	2.40 ± 0.08‡	0.40 ± 0.03*	1.70 ± 0.07
10 d starvation	3.19 ± 0.25†	1.58 ± 0.10‡	6.10 ± 0.22‡	4.37 ± 0.32‡
15 d starvation	2.52 ± 0.14†	8.58 ± 0.31‡	5.27 ± 0.27‡	0.76 ± 0.05‡
30 d starvation	2.03 ± 0.13*	0.92 ± 0.05*	0.84 ± 0.04‡	1.52 ± 0.10*

The difference in important compared with the control *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.

Histochemical results of starvation periods

The neurones of the feeding centre in the control group formed the neurone groups. These groups were composed of 2-3 large, 5-6 middle and a few small neurones and glia cells. The apical dendrites of neurones were fully observed. In the neurones of the satiety centre in the control group, the diffuse granular nucleoproteides settled next to the plasmatic membrane and at the same level. The nuclei of the cells in this centre was seen to be euchromatic, while the nucleoli was small and dark.

It was seen that the nucleoproteides in both the feeding and satiety centres decreased and the basophilic substance in some glial cells destructed at 1st day of starvation. It was found that nucleoproteides in the large pyramidal neurones of the feeding centre increased and the neurone-glial cell complexes occurred.

The mobility of the rats and search for food at 3rd day of starvation gradually increased. It was seen that there were central chromatolysis in the neurones of the feeding centre and nucleoproteides were dragged to the periphery of the cytoplasma. The morphological changes in the satiety centre started to appear obviously. The nuclei and nucleoli in most of neurones

had an eccentric localization. On the other hand, there was a decrease of nucleoproteides in some neurones.

At 5th day of starvation, it was observed that movement of the rats reduced and some of them inactivated. In addition, the drinking action in rats decreased.

The typical morphological alterations related to starvation in the neurones of the feeding and satiety centres appeared. Hydropia and total chromatolysis were seen in the neurones of the feeding centre. In addition, it was found that there were a phagocytosis of the remnant neurones by the glial cells. However, the phagocytosis by the glial cells in the satiety centre was less than in that of the feeding centre, the morphological changes in large neurones were also seen in the satiety centre. Hydropia and chromatolysis appeared in the neurones. The neurones formed the neurone groups.

At the 7th day of starvation, 70 % of the animals died because of starvation. The histopathological changes were occurred in the feeding and satiety centres. Ectopia, severe hydropia and chromatolysis were occurred in the neurones of the feeding and satiety centres.

Essential histochemical alterations were seen in the feeding centre during starvation. In addition, histochemical changes were also observed to increase in the satiety centre as starvation period increased. The alterations occurred faster, more remarkable and severe in the feeding centre than in the satiety centre.

Histochemical results of refeeding periods after 5 days starvation

There was no evidence of a remarkable change in satiety and feeding centres at 5th day of refeeding after 5 days starvation.

It was observed that the protein concentrations increased in the glial cells, the nucleoproteides in the cytoplasma began to increase, the glial cells were activated in the feeding centre. However, neurophagia was continuing in the centres at 10th and 15th day of refeeding after 5 days starvation.

Although the histochemical structure of the satiety centre resembled to that of control group, it was seen that the nucleoproteides were continuing to increase in some neurones of the feeding centre at 30th day of refeeding after 5 days starvation.

It was observed that the satiety centre restored earlier than the feeding centre during the refeeding after 5 days starvation.

Palladin *et al.*[20] stated that the neutral proteins abundantly was present in the mitochondria. The neutral proteins in the mitochondria entered into the cytosol and were used for neural function, when the neurones need them[21]. It was established that the protein concentrations decreased in the cytosol, but increased in the nucleus and mitochondria at 1st and 3rd day of starvation. On the other hand, the activity of arginin increased 8-12 times

in the nucleus and mitochondria in comparison with the control group. This can be explained by the fact that the neurones receive the proteins required from the cytosol and the structural proteins in the nucleus and mitochondria were transformed into the free proteins at the beginning of starvation. The reason of the transformation proteins from the structural proteins into the free proteins is that the neurones are stimulated by the feeding and satiety centres owing to starvation. Likewise, Graft *et al.*[3] and Kenessay and Graft[4] reported that the neutral proteins in the nucleus, especially in the mitochondria involve in forming of the synthesis mechanism of new hormones originating from the peptides at the beginning of starvation and these hormones had a role in a new catabolising system that mobilize the stored food.

At the 7th day of starvation, the reason of increased the neutral peptide hydrolase activity in all of the fractions, especially in mitochondria (16 times in comparison with the control group) is the increased action of the transformation of protein from the structural proteins into the free proteins for the vegetative functions of the neurones during the endogen feeding. The activity of the neutral peptide hydrolase is more excessive in the mitochondria than in the other fractions of the neurones. Because, as Askerov[21] stated, the intracellular protein requirement is obtained much more from the mitochondria than the other fractions of neurones.

The enzyme activity in the mitochondria had increased 10-12 times in comparison with the control group at 10th-15th day of refeeding after 5 days starvation. This increase may be considered that the mitochondria and nucleus have an important role in the cell adaptation during the periods of the starvation and refeeding periods. It was suggested that the neutral proteins in the outer membrane of the mitochondria had an important role in the cell adaptation after starvation[15,20,22,23].

It was established that the enzyme activity became close to the levels of the control group at the 30th day of the refeeding after 5 days starvation in the present study.

It can be considered that the feeding centre is more sensitive to starvation than the satiety centre, because of the fact that the histochemical alterations in the feeding centre is more evident than that of the satiety centre at 1st day of starvation. Likewise, Juravlov[24] suggested that the feeding centre is more sensitive to starvation than the satiety centre at first period of the starvation in rabbits. In addition, the cytoplasmic transparency and the rare chromatolysis in some large cells noted at 1st day of starvation pointed out that the sensitivity of the satiety centre is less than that of the feeding centre. This result is supported by Oomura *et al.*[12] findings.

It was found that the bodies and dendrites of the neurones in the feeding centre enlarged, the central chromatolysis occurred and the nuclei settled eccentrically at the 3rd day of starvation in this study. Thereby, it can be

considered that these neurones have a high functional capacity. This is also supported by the biochemical findings of this study. Similarly, Zakyskin *et al.*[13] stated that there were a relation between neuronal function and morphology of nucleoproteides.

Leontoviç[25] reported that the neurones of the satiety centre were small, reticular and serrated gluco-reseptoric and had a strong functional communication. The decreasing of the nucleoproteides and the eccentric placement of nuclei and nucleoli in the neurones of the satiety centre at the 3rd day of starvation prove that the satiety centre essentially begins to react to starvation at this period.

The typical histochemical alterations in the satiety and feeding centres were observed at the 5th day of starvation. The changes such as hydropia, total chromatolysis, ectopia of nucleus and phagocytosis by glial cells are supported by the electron microscopic study of Abushov and Verbitskaya[26].

The histochemical structure in the satiety and feeding centres at the 5th day of refeeding after 5 days starvation was not significantly different from that of 5 days starvation.

It was considered that the increase of the proteins in the glial cells and the nucleoproteides in the neurones at the 10-15th day of refeeding after 5 days starvation was a signal of the beginning of the restoration in the satiety and feeding centres. On the other hand, the continuation of the neurophagia by the glial cells in the feeding centre proves that the destruction of starvation in the feeding centre is stronger than in the satiety centre. The neurophagia in this centre creates a favourable microenvironment for restoration. The first remarks of the starvation were seen in both centres at the 10-15th day of refeeding after 5 days starvation. The results of Abushov and Verbitskaya[26] support the results of the current study.

As Askerov[21] stated, it was observed that the nucleoproteide levels increased in some neurones of the feeding centre at 30th day refeeding after starvation. However, the restoration of the satiety centre was faster than that of the feeding centre and the histochemical structure in the satiety centre resembled to that of control group at this time. Abusov[14] and Askerov[21] suggested that the restoration of the feeding centre could be completed at 60 to 70 days of refeeding after starvation.

Finally, the neurones obtain their protein requirement from the cytosol at 1st day of starvation and from the neutral proteins in the outer membrane of the mitochondria and the nucleus at the latter periods of starvation. In addition, the activity of the neutral proteins become close to the normal levels at the 30th day refeeding after 5 days starvation. Therefore, it can be considered that time required for the restoration of the intracellular proteins after starvation requires minimum of 30 day.

Starvation have caused faster and more intensive destruction in the feeding centre than in the satiety centre based on the histochemical findings. During the refeeding after starvation, the satiety centre have began to restore, and the restoration have been completed at the 30th day of refeeding after 5 days starvation. Although the significant remarks of the restoration have occurred, the restoration in the feeding centre at 30th day of feeding regime could not been completed.

REFERENCES

1. S.K. Sudakov, *Uspechi Sowremyonnoy Biologii*, **105**, 100 (1988).
2. B.A. Tkacuk, Vvedeniye k molekularnoy endokrinologii, p. 207, MGU, Moskva (1983).
3. L. Graft, A. Ronal and L. Bajurz, *FEBS Lett.*, **64**, 181 (1976).
4. A. Kenessay and L. Graft, *Brain Res.*, **2**, 247 (1978).
5. N. Marks, A. Galoyan, A. Grinbaum and A. Lojitha, *J. Neurochem.*, **22**, 73 (1979).
6. R. Walter and J.D. Glickson, *Proc. Natl. Acad. Sci.*, **70**, 1199 (1983).
7. S. Joseph and H.D. Brahmachari, *Indian J. Exp. Biol.*, **10**, 272 (1973).
8. N.A. Yudayev and Z.F. Uteseva, Gurmoný gipotalamusa v kn: Biochimiya Gormonov Gipotalamieskoy Regulyasii. Nauka, Moskva, pp. 10-14 (1976).
9. M.T. Lin and C.V. Su, *J. Physiol.*, **295**, 179 (1979).
10 P. K. Anochin, Cholosistokinin u sistemných mechanismach pisevogo nasýþeniya. Tes. Docl. Mejdunarodnogo soveta-amer. Pavlovsk. Conf. Pamyati, Emosiya i Pavedeniya Sistemny Podchool. Jyn., Moskva, pp. 19-20 (1984).
11. K. Kangava and H. Matsuo, *Biochem. Biophys. Res. Commun.*, **86**, 153 (1979).
12. J. Oomura, T. Oko, H. Oomura and M. Wayner, *Nature*, **222**, 282 (1969).
13. S.L. Zakyskin, L.Y. Nemirovsky, A.B. Jukovisky and I. Drugiye, *Sitologiya*, **22**, 982 (1980).
14. B.M. Abushov, *Biull. Eksp. Biol. Med.*, **2**, 205 (1986) (Russian).
15. F. Askerov, Morphokemiçeskiye zakanamernosti adaptatsyonno-kompensatorných reaksiy yader gipotalamusa pri izmenenii piþevoy i pityevoy motivasii. Avtoreferat doktorskoy dissertasii. Doktora Biologiçeskih Nauk., Kiev. (1991).
16. J.V. Belik, A.Q. Grinenko and L.S. Smercinskiy, *Ukr. Biochem. J.*, **40**, 532 (1968).
17. C.F.A. Culling, R.T. Allison and W.T. Barr, Cellular Pathology Technique, Butterworth and Co. Ltd., London (1985).
18. SAS User's Guide, Statistics, SAS inst., Inc. Cary, NC, Version 5 ed. (1985).
19. R.G. Steel and J.H. Torie, Principle and Procedures of Statistics, McDonald Book Co., Inc. New York, edn. 2 (1980).
20. A.V. Palladin, J.V. Belik and N.M. Polyakova, Belki golovnogo mozga i ých obmen. Nauka Dumka, Kiev, p. 315 (1972).
21. F. Askerov, Ucastiye belkov ZNS kak osnova funkzionalnoy spezificnosti struktur mozga. II Resp. Bioch. Conf., Baku, pp: 32-35 (1993).
22. A. Palladin and V.I. Trudi, Nauka Dumka, Kiev, p. 415 (1975).
23. M.T. Genkin, A.G. Grinenko and I.I. Zaitsva, Peptid gidrolaznaya aktivnost submitochondrialnýh fraksiy serogo veþestva bolþih poluþariy mozga koþek. Metabolism Belkov CNS: Tez. Dok. Sessayuz Simposyum., Dnepropetrovsk, 12-14 Sept. p. 54 (1978).
24. B.V. Juravlov, *Physiol. J.*, **62**, 1578 (1979).
25. T.A. Leontoviç, Nöronnaya organizatsiya i nekotoriye svyazi gipotalamusa. 2. Sessayuz Konf. Po Vopurosan Fizyologii Vegetativnoy Nervnoy Sistemý. Tez. Dokl. Erevan., pp. 104-106, (1966).
26. B.M. Abushov and L.B. Verbitskaya, *Arch. Anat. Histol. Embryol.*, **84**, 40 (1984).

(*Received*: 31 May 2005; *Accepted*: 28 March 2007) AJC-5533