Acta Scientiarum 21(2):409-413, 1999.
ISSN 1415-6814.

Quantitative morphological analysis of the myenteric neurons of the ileum in rats under experimental desnutrition

Adriana Fiorini, Sonia Lucy Molinari*, Maria Raquel Marçal Natali e Marcílio Hubner de Miranda Neto

*Departamento de Ciências Morfofisiológicas, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá-Paraná, Brazil. *Author for correspondence.*

ABSTRACT. This paper deals with the effects of proteic desnutrition on the morphology, morphometry and density of the neurons of the myenteric plexus. The ileum of 10 rats was used. For this study five rats aging 90 days were fed during 120 days with hypoproteic ration (experimental group) and five rats (control group) received ration with normal proteic level. Segments of the ileum were obtained for the method of whole-mount preparation, stained with Giemsa (Barbosa, 1978) and for histological routine treatment stained with HE. The neurons were clustered in ganglia located between the circular and longitudinal layers of the muscular tunica. Based on the lengths of the major longitudinal and transverse axes, the neurons were selected in three groups: small, medium and large. The neuronal density on the ileum of rats corresponding to an area of 7.08mm^2 was 1,482 and 2,515 neurons on the control and experimental groups respectively. The data suggested that proteic desnutrition did not cause alteration to the neuronal density.

Key words: enteric neurons, ileum, proteic desnutrition.

RESUMO. Análises morfológica e quantitativa dos neurônios mientéricos do íleo de ratos desnutridos. O objetivo desse trabalho foi verificar os efeitos da desnutrição protéica sobre a morfologia, a morfometria e a densidade dos neurônios do plexo mientérico. Foi utilizado o íleo de 10 ratos. Para este estudo, cinco ratos com 90 dias receberam, durante 120 dias, ração hipoprotéica (grupo desnutrido) e 5 ratos (grupo de controle) receberam ração com teor protéico normal. Segmentos do íleo foram coletados e submetidos à elaboração de preparados de membrana, corados por Giemsa (Barbosa, 1978), e, para tratamento histológico de rotina, corados por HE. Os neurônios agrupavam-se formando gânglios localizados entre os estratos circular e longitudinal da túnica muscular. Com base nos comprimentos dos maiores eixos longitudinal e transversal, os neurônios foram classificados em três grupos: pequenos (14,44 a 22,32μm), médios (22,60 a 39,40) e grandes (40,70 a 63,02). A densidade neuronal no íleo de ratos em uma área de 7,08mm^2 foi em média 1.482 e 2.515 neurônios, respectivamente, nos grupos de controle e desnutrido. Os dados obttidos sugerem que a desnutrição protéica não provocou alteração na densidade neuronal.

Palavras-chave: desnutrição protéica, íleo, neurônios entéricos.

Many of the gastrointestinal functions, such as motility, secretion, water and electrolyte transport and blood flux on the mucosa are controlled or modified by the enteric nervous system. In this way, it is to be expected that pathologies of the enteric nervous system cause a series of gastrointestinal disorders (Furness and Costa, 1987).

The neurons show on their cellular body a large amount of rough endoplasmic reticulum and numerous free polysomes, indicative of high protein synthesis. The amount of rough endoplasmic reticulum varies with the type and functional state of the neurons (Junqueira and Carneiro, 1990; Ham and Cormack, 1991).

The restriction of the supply of aminoacids may affect the processes of normal cellular metabolism, and as the majority of the biomolecular reactions requires the participation of enzymes, it is expected that each cell, as well as the tissues, may be affected when subjected to conditions of proteic desnutrition (Deo, 1978).

Winick and Noble (1966) e Firmansyah *et al.* (1989) highlight that the cellular response to desnutrition depends on the starting period as timing of food restriction period.

The special literature has reported that desnutrition acquired by female rats during the post-parturition period causes decrease in the body weight and in the intestinal length (Hatch *et al.*, 1979; Firmansyah *et al.*, 1989), and decrease in the thickness of the mucosa was described by Firmansyah *et al.* (1989).

In view of these facts, and once the enteric nervous system is composed of tissue of low index of cellular turnover, the purpose of this work was to verify the morphologic, morphometric and quantitative features of the neurons of the myenteric plexus of the ileum in rats under experimental proteic desnutrition for 120 days.

Material and methods

We used the ileum of 10 adult male rats (*Rattus norvegicus* of Wistar strain), from the Central Biotery of the State University of Maringá, with about 90 days of age and weighting an average of 296g and divided into two groups, control (five animals) and undernutrition (five rats).

The rats from the control group (C) were fed during 120 days with "Nuvilab" ration (recommended by the National Research Council & National Institues of Health, USA), with proteic level of 22%, while the rats of the undernutrition group (D) received, for the same period, hypoproteic ration (8%), supplemented with hydrosoluble vitamins of the B complex and mixture of mineral salts (Natali and Miranda-Neto, 1996). After being prepared, the ration was dosed for proteins by the method of Semimicro Kjedahl, which identifies nitrogen atoms (Silva, 1981).

The animals from each group were kept in cages and received ration and water *ad libitum*. After 120 days the animals were weighted, anesthetized with ether inhalation and killed. Segments of the ileum of each experimental group were collected and subjected to techniques of whole-mount preparations stained with Giemsa (Barbosa, 1978), and the histological sections stained with Hematoxilin-Eosin. The segments for the latter were fixed in 10% formol solution and cut transversely and longitudinally at 6mm thickness.

For the quantitative analysis the neurons of five animals of each group were counted using optical microscope Olympus CBB, equiped with WF 10X lens and 40X objective. The method chosen was the counting by sampling. In each whole-mount preparation 40 fields were randomly chosen on the microscope, where all the neurons were counted; half-neurons were counted in alternate fields. The area of the microscopic field, measured with the help of a micrometered ruler coupled to the lens, was of $7.08mm^2$.

With the aim of measuring the major longitudinal and transverse axes of the cell bodies of the myenteric neurons, the morphology of 500 neurons of each group was analyzed through the whole-mounts, with the help of optical microscope equipped with WF 10X lens, with coupled micrometer disc and 40X objective. In addition to measures, the shape of the cell, nucleus position, number of nucleoli and cytoplasmic basophily were also observed.

The mean, standard deviation and variation coefficient of the number of neurons found in each group of animals were calculated. Student's 't' test and the X^2 test were applied and the level of significance adopted for both tests was of 5%.

Results

In both experimental groups, the neurons of the myenteric plexus of the ileum cluster into ganglia, and these are found between the circular and the longitudinal layers of the muscular tunica. The ganglia are generally elongated, disposed in longitudinal rows, parallel with each other along the intestinal wall (Figure 1). Ganglia of semilunar and triangular shape are also observed.

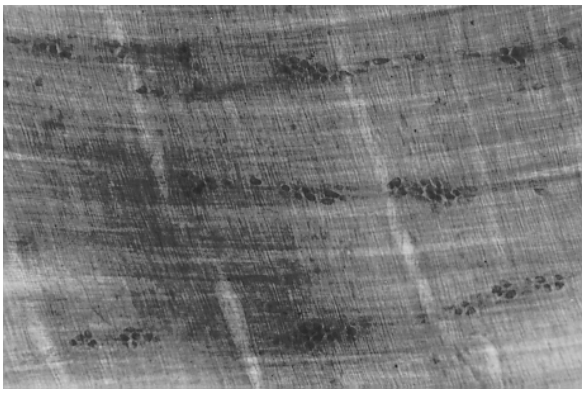

Figure 1. Whole-mount preparation of the ileum of rat showing the organization and disposition of the ganglia of the myenteric plexus. Giemsa, green filter, 151x

The neurons exhibited oval, elongated and round shapes and based on the length of the major longitudinal and transverse axes of the cell body they were grouped as small, medium or large (Figure 2).

Small neurons have size ranging from 14.44 to 22.32μm. Nuclei are mostly central and occupy most

of the cell body, with single nucleoli or only clusters of chromatin on the nucleoplasm.

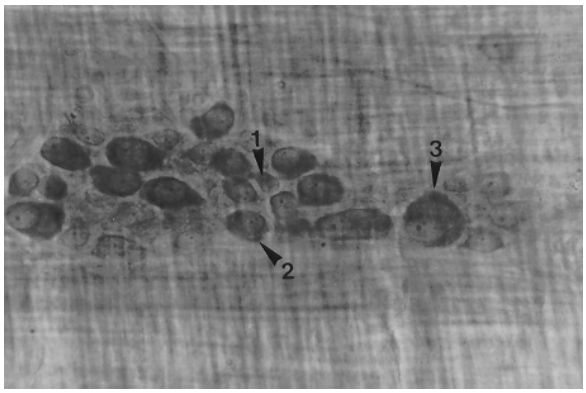

Figure 2. Whole-mount preparation of the ileum of rat evidencing small (arrow 1), medium (arrow 2), and large neurons (arrow 3). Giemsa, green filter, 306x

Medium neurons range between 22.60 and 39.40μm and this nuclei are generally excentric, and some neurons are observed with central nucleus. One or two nucleoli are observed, with few neurons with three nucleoli.

Large neurons have cell body ranging between 40.70 and 63.02μm. The nucleus is mainly excentric, with one or two well-defined nucleoli.

The number of small, medium and large neurons observed in the ileum of adult rats of both groups is presented in Table 1.

Table 1. Number of small, medium and large myenteric neurons present on the ileum of adult rats from the control (c) and undernutrition (d) groups, from a sample of 500 neurons

Size	Number	
	C	**D**
Small (14.44 a 22.32 μm)	62	95
Medium (22.60 a 39.40 μm)	358	380
Large (40.70 a 63.02 μm)	80	25
Sample	500	500

$\chi^2 = 6.42$; c.v.=5.99

The cell body of the neurons from both groups presents weak, strong and intermediate cytoplasmic basophily. The quantification of the neurons with the different basophilies is summarized in Table 2.

After the analysis of the whole-mount preparations, the neuronal density (Table 3) on an area of 7.08mm^2 of the ileum rats from the control group had a mean of 1.482 neurons (20.932,2 neurons/cm^2), and from the undernutrition group of 2.515 neurons (35.522,6 neurons/cm^2).

Table 2. Number os myenteric neurons found on the ileum of adult rats from the control (c) and undernutrition groups (d), related with cytoplasmic basophily, from a sample of 500 neurons

Cytoplasmatic basophily	Number	
	C	D
Weak	116	113
Intermediate	180	175
Strong	204	212
Sample	500	500

$\chi^2 = 0.26$; c.v = 5.99

Table 3. Incidence of neurons found in an area of 7.08mm^2 of ileum of adult rats from the control and undernutrition groups

Animals	Control	Animals	Undernutrition
1	1.308	9	2.866
3	1.302	10	2.335
4	1.857	11	1.860
5	1.711	12	3.426
6	1.232	13	2.089
X	1.482	X	2.515
S	282.06	S	631.66

t = 6.678; c.v. = 2.31

The means of body weight of the rats from groups C and D were 445.20 and 276.46 g respectively. The rats from group D showed a decrease in body weight of about 37.9% relative to the weight of rats from the control group.

Discussion

The myenteric neurons of the ileum of adult rats of both groups studied were placed inside ganglia, arranged in longitudinal rows, similar to those verified by Matsuo (1934), Furness and Costa (1987), Santer and Baker (1988) and Sternini (1988). The arrangement of the neurons in ganglia was also observed in the jejunum-ileum of guinea pigs by Matsuo (1934) and Torrejais *et al.* (1995) in the ileum of undernutrition rats during 60 days after breast-feeding. On the other hand, Gabella (1971) verified in rats that the myenteric neurons, in addition to forming ganglia, may be found isolated within one of the muscular layers.

The ganglia are localized between the circular and the longitudinal layers of the muscular tunica. Similar location was mentioned in humans (Junqueira and Carneiro, 1990; Ham and Cormack, 1991), in animals by Gabella (1979) and in the ileum of undernutrition rats during 39 days after breast-feeding withdrawal by Torrejais *et al.* (1995). The ganglia were abundant and showed predominantly elongated shape. The same was verified by Matsuo (1934) in the guinea pig ileum, by Gabella (1971) in the small intestine of adult rats, and by Torrejais *et al.* (1995) in the ileum of undernutrition rats duraing 39 days after breast-feeding withdrawal.

Through the measuring of the major longitudinal and transversal axes of the cell body, the neurons were grouped in small, medium and large sizes. Small neurons ranged between 14.44 and 22.32μm. An average 62 of them were found in the control group, and 95 on the group under desnutrition. Medium neurons (from 22.60 to 39.40μm) were the most frequent: an average of 358 in the control group and 380 in the experimental group. Large neurons, ranging from 40.70 to 63.02μm, summed an average of 80 in group C and 25 in group D.

The medium size neurons were the most common, representing 71.6% in the sample of group C and 76% in that of group D. The analysis of these values showed by the X^2 test statistically significant differences when both groups were compared (X^2 = 6.42 and critical value = 5.99). We can suppose that the experimental condition led to a decrease in the population of large neurons.

Furthermore, the special literature showed that, Cook and Burnstock (1976), in studies carried out in guinea pigs, described small neurons with 10 to 12μm length, medium neurons from 20 to 25μm and large neurons with 30 to 35μm. Natali and Miranda-Neto (1996), when studying neurons of the duodenum of rats, whose mothers were under proteic desnutrition during gestation, lactation or both, observed small neurons with cell body ranging from 3.92 to 9.16μm, and medium and neurons ranging from 10.47 and 28.80μm.

Torrejais *et al.* (1995) observed in the ileum of undernutrition rats, during 39 days after breast-feeding withdrawal, that 19% of the ganglia were composed of small neurons (10.48 to 19.49μm) and 81% were composed of medium and large neurons (20.95 to 47.14μm), concluding that the size of the neurons does not depend on the nutritional state. The difference of the neuronal size among the authors may be due to the different animal species, ages and experimental conditions, as well as to the different segments of the gastrointestinal tract analyzed.

As for the cytoplasmic basophily, we observed that the cell body of the neurons exhibited staining intensities which varied from weak to strong. According to the statistical treatment used (X^2 = 0.26 and critical value = 5.99), there were no significant differences when the groups were compared.

Strongly stained cell bodies were observed in 40.80% of the neurons from group C and 42.4% of those from group D. According to Junqueira and Carneiro (1990) and the Ham and Cormack (1991), neurons with high synthetic activity present large amounts of rough endoplasmic reticulum and numerous free polysomes. These structures are directly related with the more intense staining verified in the neuronal cytoplasms. In addition, our data suggest that the condition of undernutrition did not alter the staining features, demonstrating that the population of ribosomes was not affected.

Though the small neurons exhibit central nucleus, it was verified that most of the nuclei, independently of the group, were excentric. Similar observations were described by Ham and Cormack (1991) for the neurons of the peripheral nervous system ,by Natali and Miranda-Neto (1996) for the myenteric neurons of the duodenum of desnurtured rats on gestation and lactation, and by Torrejais *et al.* (1995) during the ileum of desnurtured rats during 39 days after breast-feeding withdrawal.

As for the number of nucleoli, small neurons showed single nucleolus or only clusters of chromatin in the nucleoplasm; medium and large neurons, one or two nucleoli. Few large neurons showed three nucleoli, datum similar to that found by Torrejais *et al.* (1995).

The neuronal density observed in an area of 7.08mm^2 of ileum of the group C was an average of 1.482 neurons (20,932.2 neurons/cm^2), while in the ileum of group D there was an average of 2,515 neurons (35,522.6 neurons/cm^2). These data, subjected to Student's 't' test, revealed significant statistical difference. When compared with the number of neurons of both groups. Group D had an increase of 41.07%.

Analyzing the body weight, we observed an average of 445.20g for the rats of group C and of 276.46g for group D at the end of 120 days of treatment, and this difference does attain significance (t = 6.54 and critical value = 2.31). We verified that the rats under hypoproteic diet had a decrease in body weight of 37.9%. The decrease caused by desnutrition is also reported by other authors (Hatch *et al.*, 1979; Firmansyah *et al.*, 1989; Torrejais *et al.*, 1995).

Comparing the data obtained of body weight with those of neuronal density, we verified that the rats of group D had their weights reduced in 37.9% and the neuronal density had an increase of 41%, suggesting that the decrease in weight led to greater concentration of the neurons in the intestinal wall of the undernutrition rats. The data obtained suggest that the adult rats subjected to diet of hypoproteic ration (8%), during 120 days, did not show alteration in the neuronal density, similar to the data found by Torrejais *et al.* (1995), in which the desnutrition

during 39 days after breast-feeding withdrawal did not cause decrease in the neuronal density.

Through the data obtained in this study the medium neurons were the most abundant and represented 71.6% of the sample of the control group and 76% of that of the experimental group.The condition of proteic desnutrition caused by ration with 8% proteic level did not alter the staining conditions of the cell body. Confronting the data of the decrease in the body weight (37.9%) with the increase in the neuronal density (41.07%), we conclude that the proteic desnutrition used did not alter the neuronal population, because the neuronal density is due to the lower physical growth of the animal.

References

Barbosa, A.J.A. Técnica histológica para gânglios nervosos intramurais em preparados espessos. *Rev. Bras. Pesq. Med. Biol. ,11*(2-3): 95-97, 1978.

Cook, D.R.; Burnstock, G. The ultrastructures of Auerbach's plexus in the guinea-pig. 1 - Neuronal elements. *J. Neurocytol., 5*:171-194, 1976.

Deo, M.G. Cell biology of protein-caloric malnutrition. *Wld. Rev. Nutr. Diet., 32*: 49-45, 1978.

Firmansyah, A.; Suwandito, L.; Penn, D.; Lebenthal, E. Biochemical and morphological changes in the digestive tract of rats after prenatal and postnatal malnutrition. *Am. J. Clin. Nutri, 50*:261-268, 1989.

Furness, J.B.; Costa, M. *The enteric nervous system.* New York: Churchill Livingstone, 1987.

Gabella, G. Neuron size and number in the myenteric plexus of the newborn and adult rat. *J. Anat*., *109*:81-95, 1971.

Gabella, G. Innervation of the gastrointestinal tract. *Int. Rev. Cytol., 59*:129-191, 1979.

Ham, A.W.; Cormack, D.H. *Histologia.* 9.ed. Rio de Janeiro: Guanabara Koogan, 1991.

Hatch, T.F.; Lebenthal, E.; Branski, D.; Krasner, J. The effect of early postnatal acquired malnutrition on intestinal growth, disacharidases and enterokinase. *J. Nutrit., 109*:1874-1879, 1979.

Junqueira, L.C.V.; Carneiro, J. *Histologia básica.* 7.ed. Rio de Janeiro:Guanabara Koogan, 1990.

Matsuo, H. A contribution on the anatomy of Auerbach's plexus. *Jpn. J. Med. Sci. Anat*., *4*:417-428, 1934.

Natali, M.R.M.; Miranda-Neto, M.H. Effects of maternal proteic undernutrition on the neurons of the myenteric plexus of duodenum of rats. *Arq. Neuro - Pisiquiatr., 54*(2):273-279, 1996.

Santer, R.M.; Baker, D.M. Enteric neuron numbers and sizes in Auerbach's plexus in the small and large intestine of adult and aged rats. *J. Auton. Ner. Syst., 25*:59-67, 1988.

Silva, D.J. *Análise de alimentos (métodos químicos e biológicos).* Viçosa: Universidade Federal de Viçosa, 1981.

Sternini, C. Structural and chemical organization of the myenteric plexus. *Ann. Rev. Physiol., 50*:81-93, 1988.

Torrejais, M.M.; Natali, M.R.M.; Conegero, C.I.; Miranda-Neto, M.H. Effects of proteic desnutrition after breast-feeding on the morphology of the intestinal wall and enteric neurons of the ileum of rats. *Rev. Unimar, 17*(2):315-327, 1995.

Winick, M.; Noble, A. Cellular response in rats during malnutrition at varion ages. *J. Nutrit., 89*:300-306, 1966.

Received on July 29, 1998.
Accepted on March 01, 1999.