

MORPHOLOGIC AND QUANTITATIVE STUDY OF THE MYENTERIC NEURONS OF THE JEJUNUM OF MALNOURISHED RATS (*RATTUS NORVEGICUS*)

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ABSTRACT - We studied the effects of maternal proteic desnutrition on the neurons of the myenteric plexus of the jejunum of rats from *Rattus norvegicus* species. It was used litters of female rats which received diet with normal proteic level during gestation and lactation (group NN), normal diet during gestation and hypoproteic diet during lactation (group ND); hypoproteic diet during gestation and normal diet during lactation (group DN); hypoproteic diet during both gestation and lactation (group DD). After weaning all the animals received diet of normal proteic level until the 60th day of age, when they were killed. The jejunum of the animals was subjected to whole-mount preparations stained by the method of Giemsa and used for the morphologic and quantitative analyses of the neurons of the myenteric plexus. We verified that maternal proteic malnutrition does not cause decrease on the number of myenteric neurons per unit area of jejunum in rats, but elicits mechanisms which assure that, when the animal again receives normal proteic level diet (22%) there occurs storage of proteic material on the cytoplasm of the neurons, thus rendering them larger and strongly basophilic.

KEY WORDS: jejunum, myenteric neuron, maternal malnutrition, proteic desnutrition.

Estudo morfológico e quantitativo dos neurônios mientéricos do jejuno de ratos desnutridos (*rattus norvegicus*)

RESUMO - Estudamos os efeitos da desnutrição protéica materna sobre os neurônios do plexo mientérico do jejuno de ratos da espécie *norvegicus*. Utilizamos filhotes de ratas que receberam dieta com teor protéico normal durante a gestação e a lactação (grupo NN), dieta normoprotéica durante a gestação e hipoprotéica durante a lactação (grupo ND); dieta hipoprotéica durante a gestação e normoprotéica durante a lactação (grupo DN); dieta hipoprotéica durante a gestação e lactação (grupo DD). Após o desmame todos os animais receberam dieta com teor protéico normal até os 60 dias de vida ocasião em que foram sacrificados. Preparados de membrana do jejuno foram corados pelo método de Giemsa e foram utilizados para análises morfológicas e quantitativas dos neurônios do plexo mientérico. Verificamos que a desnutrição protéica materna não provoca redução no número de neurônios mientéricos por área do jejuno de ratos, porém desencadeia mecanismos que fazem com que quando o animal volta a receber dieta com teor protéico normal (22%) ocorra acúmulo de material protéico no citoplasma dos neurônios, o que os torna maiores e fortemente basofílicos.

PALAVRAS-CHAVE: jejuno, neurônios mientéricos, desnutrição materna, desnutrição protéica.

The effect of nutritional deficiency on the structure and functions of the central and peripheral nervous systems, as well as on the other systems of the organism, has been the subject of several studies. It is known that the development and growth of the nervous system occurs essentially during the embryonic life, with myelinization beginning on the second half of fetal life and continuing

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during the first post-natal year¹. On the pre- and post-natal periods, chemicals from nutrients are incorporated on the cells of the nervous system to promote its development, differentiation, energy production and neural activities as a whole. Nutritional deficiencies, impinging upon these fast stages of growth, cause morphologic and physiologic changes on the central nervous system (CNS) which, as a function of their intensity, can be irreversible, with decreases of 30 to 50% on the numbers of neurons². The decrease on a given cellular population may have important consequences. If one considers a neuronal population, this acquires a special meaning both because of its functional significance and because of the non-renewable feature of neurons³.

The wall of the digestive tract has extensive plexuses of nerve fibers which are partially derived from pre- and post-ganglionic sympathetic and parasympathetic fibers and also from neurons which are intrinsic to the intestinal wall. These plexuses are related to the maintenance of the rhythmic peristaltic activity of the alimentary canal, although the intrinsic neuronal system *per se* is incapable of keeping this activity when the sympathetic and parasympathetic nerves are destroyed. The two major plexuses of the intestinal wall are the myenteric plexus (or Auerbach's), located between the circular and longitudinal muscle layers and the submucous plexus (or Meissner's), embedded on the submucosa. The myenteric plexus has morphologic and quantitative features which vary along the length of the digestive tract, both in man and in animals³⁻⁷.

The effect of maternal malnutrition on the enteric nervous system and the sympathetic system was investigated through histoenzymology and histofluorescence, in the jejunum of adult rats whose mothers were subjected to diet deficient in proteins during gestation⁸. There was a decrease of 27% on the number of enteric neurons and a decrease on the fluorescence level on the malnourished animals as compared to the controls. The reduction on the number of enteric neurons indicates that the circuitry of the enteric nervous system and thus the control of the intestinal motility can be unfavourably affected by malnutrition.

These data elicited us to carry out an experimental study to contribute to the knowledge concerning the effects caused by an adverse nutritional condition on the myenteric plexus.

METHOD

1. Obtention of experimental groups.

We carried out the mating of nulliparous females of the *Rattus norvegicus* species, *albinus* variety, *wistar* strain, for a period of seven to 10 days. After that, rats considered to be pregnant were isolated in individual cages, and their litters named according with the type of chow offered to the mothers:

- Group NN, normal proteic diet during gestation and lactation;
- Group ND, normal proteic diet during gestation and hypoproteic diet during lactation;
- Group DN, hypoproteic diet during gestation and normal diet during lactation;
- Group DD, hypoproteic diet during gestation and lactation.

Within 24 hours of birth, neonates exceeding six in each litter, as well as litters under 6, were discarded.

After weaning, at the 21 days of age, all the animals received control diet (22% protein) until the 60th day, when they were killed. We used NUVILAB rat chow (recommended by the National Research Council & National Institute of Health, USA) containing 22% protein as control diet and chow containing 8% protein as hypoproteic diet, prepared from the original chow through the addition of corn starch and vitamin and mineral salt supplementation. Chows were prepared as pellets and proteic levels tested through nitrogen dosage (according to semimicro Kjeldahl method). Chow and water were offered *ad libitum* to all the animals.

For the collection of material, the rats were initially anesthetized through inhalation of sulfuric ether for about three minutes so that they did not react to painful stimuli. Next the laparotomy was carried out, one minute being necessary to remove the intestine and immerse it in Giemsa for the whole-mounts. The animals were then killed.

2. Morphologic and quantitative study of the myenteric plexus.

For the morphologic and quantitative analyses of the neurons of the myenteric plexus, we used the jejunum of five rats from each group, which were used for whole-mount preparations by the method of Giemsa, according to Barbosa⁹.

The method used on the quantification of neurons was the counting by sampling. Each whole-mount was divided in four quadrants, in each ten microscopic fields were randomly selected and all their neurons were counted. At the end, considering the whole-mount of each animal, the neurons of 40 fields had been counted. The

area of each microscopic field obtained through diameter measure with Zeiss micrometer ruler was 0.166 mm², yielding a total area in each animal of 6.64 mm².

The morphology of 100 neurons of each group was analyzed from the whole-mounts under Olympus light microscope, 10X lens coupled to micrometer disc and 40X objective. The major longitudinal and transverse axes of the cellular body of the myenteric neurons were measured for further comparison and classification^{10,11}. In addition to the measurements, it was observed in each neuron: cytoplasmic basophilia, cell shape, number of nucleoli and nucleus position.

Whole-mounts were photographed in photomicroscope Wild M20 and photographic equipment Wild MPS-55.

3. Statistical Analysis.

Quantitative data obtained from the four nutritional groups were statistically compared through variance analysis with significance level of 5%.

RESULTS

1. Incidence of myenteric neurons on the jejunum.

Table 1 displays the number of myenteric neurons in 6.64 mm² of jejunum (40 fields of 0.166 mm² each) of the animals studied. Data analysis concerning the mean of neurons did not reveal statistically significant differences ($F=0.72$; critical value=3.24).

2. Morphology of the cellular body.

Through the sum of the major longitudinal and transverse axes of 100 neurons from animals of group NN, neurons were categorized as small, medium and large. Small neurons were considered those whose values varied from 10.52 to 21.52 μm ; medium neurons were those varying from 21.3 to 33.28 μm and large neurons had values ranging from 33.29 to 47.7 μm . This classification of the neurons on the different groups is found in Table 2. The cellular bodies of the medium and large neurons displayed varied shapes, determined by the cytoplasmic content. There were oval, pyramidal and elongated neurons, the last ones differing from the first by possessing tapering ends. The cellular bodies of the small neurons were predominantly round (Fig 1).

Table 3 displays the incidence of small, medium and large neurons with strong, moderate and weak cytoplasmic basophilia on the nutritional groups. As for the stain affinity, considering observations carried out on the four groups altogether, it was verified neurons with strongly stained cytoplasm in 5/74 (6.76%) of the small neurons, 51/241 (21.58%) of the medium neurons and 43/85 (50.59%) of the large neurons.

It was also found neurons whose cytoplasm stained weakly, differing little from the nucleoplasm staining. This feature was observed in 41/74 (55.4%) of the small neurons, 65/241 (26.97%) of the medium neurons and 10/85 (11.76%) of the large neurons.

Table 1. Incidence of neurons in 6.64 mm² of jejunum of rats subjected to different nutritional conditions.

Animals	NN	ND	DN	DD
1	3295	2811	3432	2928
2	2853	3449	2275	2451
3	3471	2716	1307	2647
4	1445	1960	1292	1436
5	2260	1774	1780	1975
Mean	2664.8 (ns)	2542 (ns)	2017.2 (ns)	2287.4 (ns)
SD	826.8	680.2	589.5	888.1

ns, not reveal statistically significant differences; NN, normal proteic diet during gestation and lactation; ND, normal proteic diet during gestation and hypoproteic diet during lactation, DN; hypoproteic diet during gestation and normal diet during lactation, DD; hypoproteic diet during gestation and lactation

Table 2. Relative incidence of small, medium and large myenteric neurons on the jejunum of rats subjected to different nutritional conditions.

Groups	Small	Medium	Large	Total
NN	21	63	16	100
ND	37	51	12	100
DN	11	69	20	100
DD	5	58	37	100
Total	74	241	85	400

NN, normal proteic diet during gestation and lactation; ND, normal proteic diet during gestation and hypoproteic diet during lactation; DN, hypoproteic diet during gestation and normal diet during lactation; DD, hypoproteic diet during gestation and lactation.

It was considered an intermediate neuron that with irregular cytoplasmic staining, of granular aspect. This type was found in 28/74 (37.84%) of the small neurons, 124/241 (51.45%) of the medium neurons and 32/85 (37.65%) of the large neurons.

In all the neurons analyzed the nucleus was weakly stained, the nucleolus was easily identified and often central, and the nucleus periphery exhibits a reticulum of chromatin. As for the position, nucleus can be polar, central or peripheral (Fig 1). Most of the neurons had a single nucleolus (98.75%).

DISCUSSION

According to literature data, malnutrition impinging upon the pre- and post-natal periods causes decreases of 30 to 50% on the number of neurons of the CNS², also is reported a 27% decrease on the number of enteric neurons in rats malnourished during the first two weeks of gestation⁸, while on this study the statistical analyses did not reveal significant differences concerning the incidence of neurons on the myenteric plexus. These results demonstrate, therefore, that malnutrition on the studied periods does not cause decrease on the number of myenteric neurons per area of jejunum in rats. On the other hand, in a study carried out on the duodenum of rats⁶, it was found a greater number of neurons per area on the animals malnourished during gestation and lactation. The authors of this research discuss that the values are due to the smaller growth of the intestine on the malnourished rats, which would cause smaller neuronal spreading and not being therefore a real increase on the total number of nerve cells.

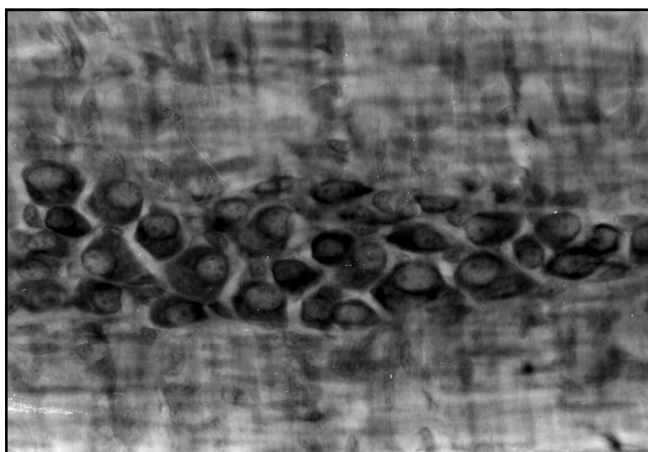


Fig 1. Whole-mount preparation of the jejunum of rat, stained by the method of Giemsa, depicting cellular bodies of neurons in ganglia of the myenteric plexus. 368 X.

Table 3. Absolute incidence of small, medium and large neurons with cytoplasm of strong (S), moderate (M) and weak (W) basophilia in each group.

Group	Small			Medium			Large			Total
	W	M	S	W	M	S	W	M	S	
NN	14	06	01	22	29	12	06	04	06	100
DN	19	16	02	13	23	15	00	05	07	100
ND	06	05	00	19	39	11	03	10	07	100
DD	02	01	02	11	33	14	01	13	23	100
Total	41	28	05	65	124	52	10	32	43	400

ns, not reveal statistically significant differences; NN, normal proteic diet during gestation and lactation; ND, normal proteic diet during gestation and hypoproteic diet during lactation; DN, hypoproteic diet during gestation and normal diet during lactation; DD, hypoproteic diet during gestation and lactation

As for the cytoplasmic basophilia, we verified that among the small neurons there occurs a predominance of weak basophilia (55.4%), while among the medium and large neurons this feature is present in 26.97% and 11.76%, respectively. On the other hand, neurons with strong cytoplasmic basophilia were found on the following proportions: small (6.76%), medium (21.25%) and large (50.59%). The relationship between cytoplasmic basophilia and greater proteic synthesis is classically described on the literature^{13, 14}.

Among the animals which had undergone proteic deprivation, we verified that the incidence of neurons with moderate and strong basophilia is superior to that found on the animals fed with normal diet (NN), weakly basophilic neurons predominating in them. It was also noted that on groups ND and DD there is a greater incidence of large neurons, both features being more evident in group DD. Similar data were verified on the myenteric plexus of the duodenum of rats⁶. We believe that the animals which underwent periods of malnutrition, when returned to normal diet, develop compensatory mechanisms that result on the storage of proteic material on the cytoplasm of the myenteric neurons.

We conclude that maternal malnutrition does not cause decrease on the number of myenteric neurons per area of jejunum in rats, but elicits mechanisms which promote storage of proteic material when normal diet is restored, thus making neurons larger and strongly basophilic.

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