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EVALUATION OF THE AREAS OF NEURONAL CELL BODIES AND NUCLEI IN THE MYENTERIC PLEXUS OF THE DUODENUM OF ADULT RATS

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ABSTRACT - This study compared the areas of cell body and nucleus profiles of the myenteric neurons in the antimesenteric and intermediate regions of the duodenum of adult rats. Five male rats were used. The duodenum was removed and dissected to whole-mount preparations, which were stained by the Giemsa technique. The areas of cell body and nucleus profiles of 100 neurons, 50 from each region, of each animal, were assessed with image analyser. Based on the global mean±SD of the areas of cell body profiles, neurons were labelled as small, medium or large. It was observed that the neurons did not differ significantly in size or incidence between the antimesenteric and intermediate regions. However, the nuclei of the small and medium neurons were significantly smaller in the latter region. It is discussed that the smaller nuclear size could be related to the cell bodies being slightly smaller on this region and to a possible smaller biosynthetic activity which would influence nuclear size.

KEY WORDS: myenteric neurons, neuronal area, duodenum, rats.

Avaliação das áreas dos corpos celulares e dos núcleos neuronais no plexo mientérico do duodeno de ratos adultos

RESUMO - Este estudo comparou as áreas dos perfis dos corpos celulares e dos núcleos neuronais dos neurônios mientéricos nas regiões antimesentérica e intermediária do duodeno de ratos adultos. Cinco ratos machos foram usados. O duodeno foi removido e dissecado a preparados de membrana, os quais foram corados pela técnica de Giemsa. As áreas dos perfis dos corpos celulares e dos núcleos neuronais de 100 neurônios, 50 de cada região, de cada animal, foram avaliadas com analisador de imagens. Com base na média global±SD das áreas dos perfis dos corpos celulares, os neurônios foram classificados como pequenos, médios ou grandes. Foi observado que os neurônios não diferiram significativamente de tamanho ou incidência entre as regiões antimesentérica e intermediária. Contudo, os núcleos dos neurônios pequenos e médios foram significativamente menores na última. Discute-se que o menor tamanho nuclear poderia estar relacionado ao fato dos corpos celulares serem ligeiramente menores nesta região e a uma atividade biossintética possivelmente menor, que influenciaria o tamanho nuclear.

PALAVRAS-CHAVE: neurônios mientéricos, área neuronal, duodeno, ratos.

For many years the enteric nervous system (ENS) has been the goal of studies aiming, first, at unravelling its intricate structure, neuronal cell types, connections and neurochemical coding and, second, at understanding how these aspects are affected by factors such as age and pathophysiological

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conditions. Since the first studies it has been observed that the neurons composing the myenteric plexus - the most prominent plexus of the ENS - show distinct distributions on the various intestinal segments relative to density, morphology and neurochemistry. Quantitative variations along the digestive tract were also reported. In his review on the gastrointestinal tract, Gabella[1] explains with clarity the extensive variability of the enteric plexuses concerning their structural and functional aspects. Similarly, differences were also observed on the sensitivity of the myenteric neurons to the experimental conditions to which the animals are subjected[2,3].

The morphoquantitative studies of the myenteric plexus carried out until the 80s used to consider a given segment of the digestive tube, but recently it was found that different regions of the stomach and of the intestinal circumference also differ in their features and in their sensitivity to experimental procedures[4-6]. This fact deserves attention because phylogenetic, age-related and pathophysiological comparisons can be interpreted erroneously due to the random selection of neurons around the intestinal circumference. Systematic qualitative and quantitative evaluations of the neuronal variations in the myenteric plexus around the digestive tract are necessary to minimize such discrepancies.

For this reason, this study was aimed at comparing the areas of cell body profiles of the neurons of the myenteric plexus in the antimesenteric and intermediate regions of the duodenum of adult rats. The comparison of the areas of nuclear profiles between these circumferential regions was also made.

METHOD

Material processing

For this study five adult male Wistar rats (av. weight 428.8±6.99 gr (mean±SD); av. age seven months) were used, coming from the Central Biotery of the State University of Maringá. The animals were killed under ethylic ether anesthesia and laparotomy was carried out. The material was processed according to the Giemsa technique[7]. Briefly, the duodenum was removed, washed in 0.9% saline solution and kept distended by filling it with acetic formaldehyde fixative and ligating the extremities with cotton threads. After minimal fixation of 48 hours, the segments were opened lengthwise by the mesenteric attachment and the mucosa and submucosa, along with part of the circular muscle layer, were dissected out under the stereomicroscope. The resultant whole-mount preparations were kept in Giemsa staining solution for 16 to 20 hours, and then subjected to the routine histological procedure for mounting between slide and coverslip (dehydration in ascending series of alcohols and diaphanization in xylene).

Morphological analysis

Measurements of the areas of cell body and nucleus profiles of the myenteric neurons were performed on an Olympus BX40 microscope using a 40X objective, coupled to a computerized system of image analysis (Image-Pro Plus 3.0.1). From each animal the cell body and nucleus profiles of 100 myenteric neurons were measured, 50 neurons being from the antimesenteric region (from 120º to 240º, considering the mesenteric attachment as 0º) and 50 from the intermediate region (from 60º to 120º and from 240º to 300º,[5]) of the duodenal circumference.

Statistical analysis

To label the neurons as small, medium and large, a mean and a standard deviation (SD) of the neuronal size were obtained for both regions altogether. Myenteric neurons were considered large when the areas of cell body profiles were above the mean+SD, small when the areas were below the mean-SD and medium when the areas were on the interval of the mean±SD. This procedure of classification was the same as that employed in other morphometric analyses previously carried out in our laboratory[5,6,8].

The proportionality of the areas of cell body and nucleus profiles of the three neuronal categories between the regions was verified. Means±SD of the areas of cell body and nucleus profiles of the neurons were compared between the antimesenteric and intermediate regions using the two-tailed unpaired t test (Prism 2.0) with Welch correction when necessary, and proportions were compared with the test for proportions (Statistica 5.0). Data are presented as mean±SD. The significance level for both tests was $p<0.05$.

Table 1. Size and incidence of small, medium and large myenteric neurons in the antimesenteric and intermediate regions of the duodenum of adult rats.*

Region	Small neurons		Medium neurons		Large neurons	
	μm^2	%	μm^2	%	μm^2	%
Antimesenteric	115.4±3.97	14.61	229.9±3.22	70.38	374.4±6.68	15.00
Intermediate	107.5±3.64	17.05	221.2±3.69	67.05	367.7±9.91	15.89

*global mean=229.7±3.75, SD=85.32 μm^2

Table 2. Mean areas of nuclear profiles of small, medium and large myenteric neurons in the antimesenteric and intermediate regions of the duodenum of adult rats.

Region	Small neurons μm^2	Medium neurons μm^2	Large neurons μm^2
Antimesenteric	58.43±2.91*	90.38±1.39**	127.7±2.83
Intermediate	45.55±1.99*	85.90±1.41**	122.6±4.16

*differ significantly from each other (p=0.0004)
**differ significantly from each other (p=0.0243)

RESULTS

In the duodenum, the areas of neuronal cell body profiles ranged between 52.18 and 482.6 μm^2 in the antimesenteric region (Fig 1) and between 51.15 and 602.4 μm^2 in the intermediate region (Fig 2).

The mean areas of cell body profiles were 234.8±5.08 μm^2 (SD=81.95) in the antimesenteric region and 224.5±5.51 μm^2 (SD=88.44) in the intermediate region. The global mean, obtained from neuronal areas of the two regions, was 229.7±3.75 μm^2 (SD=85.32). Neuronal labelling, based on the global mean, is depicted in Table 1.

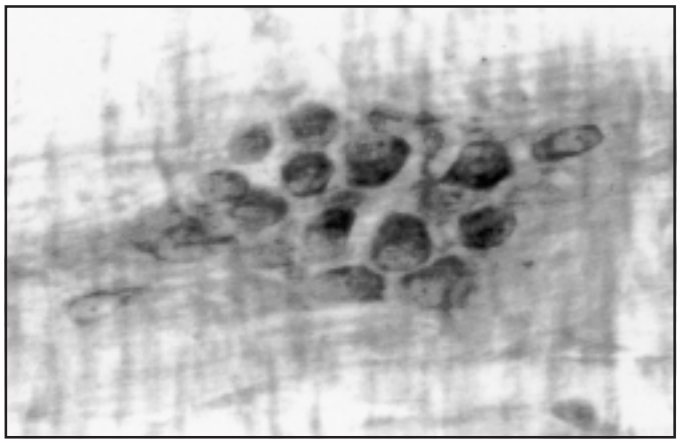

Fig 1. Myenteric neurons in the antimesenteric region of the duodenum of an adult rat. Giemsa, green filter, 367X.

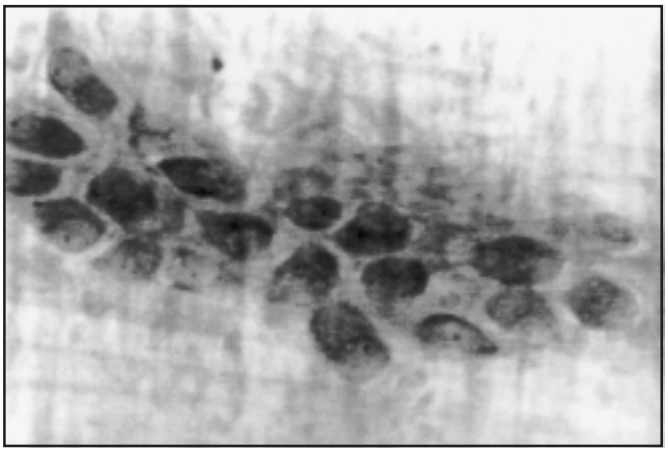

Fig 2. Myenteric neurons in the intermediate region of the duodenum of an adult rat. Giemsa, green filter, 367X.

Significance levels obtained from the comparisons of mean areas of cell body profiles and of incidences of small, medium and large neurons between the two regions had values between p=0.0757 (for neuronal areas of medium neurons) and p=0.5806 (for neuronal areas of large neurons), demonstrating that no significant difference existed on the areas and incidences of the neuronal categories between the antimesenteric and intermediate regions of the duodenal circumference.

The mean areas of nuclear profiles of the small, medium and large neurons in the antimesenteric and intermediate regions are shown in Table 2. Data demonstrated that small and medium neurons of the intermediate region had nuclear areas significantly smaller than those of the antimesenteric region. Large neurons had nuclei essentially equal in size in both regions.

The cell bodies of the small, medium and large neurons from the intermediate region had mean areas equivalent to 93.15%, 96.2% and 98.2% of the corresponding areas in the antimesenteric region, while nuclear areas were 77.9%, 95% and 96%, respectively, of those in the antimesenteric region.

DISCUSSION

The minimum and maximum values of the areas of neuronal cell body profiles found in the antimesenteric and intermediate regions on this study were within the interval described in the small intestine of six-month-old rats[9]. In addition, in the proximal jejunum of age-matched rats, neurons were found with areas equivalent to those described in this work[10]. Nevertheless, in this investigation, cell body areas above 602.4 μm^2 were not observed, while those authors reported the presence of a small proportion (less than 1%) of neurons measuring up to 750 μm^2.

In this study it was investigated whether the areas of neuronal cell body profiles showed variations according to the region of the intestinal circumference, because several authors, when carrying out studies of phylogenetic nature[11-13] or when assessing conditions such as desnutrition[14], aging[9,10,15-17] and diabetes[2,8], label the enteric neurons based on morphometric and quantitative analyses regardless of the region of the intestinal circumference. In some quantitative studies, the authors stress the existence of neuronal density differences according to the circumferential region of the digestive tube. Using methylene blue impregnation, Irwin[18] described qualitative and quantitative variations in the myenteric plexus along and around the intestinal segments. Sant'Ana et al.[4,5] found a significant difference in the neuron counts between the antimesocolic and intermediate regions of

the ascending colon of rats using the NADH-diaphorase technique, and Fregonesi et al.[6] observed a markedly higher number of myenteric neurons on the lesser gastric curvature relative to the greater with this technique. Although these latter observations could be due to a varying expression of NADH-diaphorase-reactive neurons, they indicate that this fact is region-dependent. However, no reports were found about evaluations of neuronal cell body areas between the regions of the intestinal circumference.

In this study, comparisons of mean neuronal sizes and of incidences of small, medium and large neurons between the two duodenal regions were not significant. This demonstrated that, despite there being differences, though not large, on the neuronal density of the myenteric plexus between the regions of the intestinal circumference[4,5,18], there is on the other hand a maintenance of the proportions of small, medium and large neurons, as well as of the mean areas of cell body profiles.

Small and medium neurons of the duodenal antimesenteric region showed mean nuclear areas significantly greater than those in the intermediate region, although the mean areas of cell body profiles of these categories between regions were not significantly different from a statistical point of view, as discussed above. The reasons for this difference on the nuclear area can be two-fold. First, although the cell body size did not differ statistically between the regions, it was verified, on the three categories, smaller neuronal sizes in the intermediate region, keeping a relationship to the nuclear sizes (see Results). Second, the larger nuclear size of the small and medium neurons in the antimesenteric region, specially of the small neurons, indicate that these neuronal categories in the antimesenteric region included neuronal populations with biosynthetic activity inherently greater, possibly due to their neurochemical transmitter system, than in the intermediate region. Increases of nuclear area were already observed on the hypothalamus of rats in certain cases[19,20] and these increases were attributed to a greater nuclear synthesis of mRNA of neuro-hormonal precursors.

The fact that large neurons did not differ significantly between regions either on cell body or nuclear size may indicate that this morphologic population is quite homogeneous around the duodenum, at least as far as neuronal activity (especially biosynthesis) is concerned.

Morphometric analyses such as that described here are only the first step in understanding circumferential differences in a given intestinal segment. Neuron counts made separately for the antimesenteric and intermediate regions[4,5], as well systematic investigations on their neurochemistry must be carried out to give a complete morphophysiological picture of these spatially defined subpopulations of enteric neurons.

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