STRAIN DIFFERENCES IN THE MORPHOLOGY OF MYENTERIC GANGLIA IN THREE STRAINS OF RABBIT

M.Kemal IRMAK , M.D., Tufan KARAOSMANOĞLU*, M.D., Osman ÖZCAN, M.D.

**Gülhane Military Medical Academy, Departments of Histology and Embryology,
Hacettepe University, Faculty of Medicine, Departments of Histology and Embryology*,
Ankara, Turkey**
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SUMMARY : *Craniofacial features, pigmentation, stature and body proportions are certain racial characteristics. It was suggested that a racial difference might also be present in the morphology of peripheral nervous system. The purpose of the present study was to investigate the morphology of myenteric plexus in three strains of rabbit as a model of peripheral nervous system. Our study has revealed significant inter strain differences in the myenteric plexus morphology. In the light of these results, we suggest that human races might also have these differences.*

Key Words : *Enteric neurons, Strain Differences, Rabbit.*

INTRODUCTION

Enteric nervous system is the system of neurons and their supporting cells that is found within the walls of the gastrointestinal tract, including the neurons within the pancreas and gall bladder (17). It has connections with autonomic ganglia outside the walls of the gastrointestinal tract and with the central nervous system and contains entire reflex pathways through which the contractions of the muscular coat of the alimentary tract, the secretion of gastric acid, intestinal transport of water and electrolytes, mucosal blood flow, and other functions are controlled (3).

It is capable of mediating intestinal reflexes in the absence of connections to the central nervous system (4).

The enteric nervous system consists of a number of interconnected plexuses. Within some parts of these plexuses are small groups of nerve cell bodies, the enteric ganglia. The plexuses lie very close to the structures they control. The myenteric (Auerbach's) plexus is a network of nerve strands and small ganglia that lie in the plane between the external longitudinal and circular muscle coats of the intestine (11).

The enteric plexuses are ideally suited for quantitative studies on size and spatial density of neurons, as these can be established with a fair degree of accuracy in whole-mount preparations. Counts have been carried out on many parts of the gut, in different animal species and with different staining methods. Some consistent differences were found, for example a higher neuronal density in the duodenum than in the jejunum, or higher in the large than in the small intestine, or beneath the taeniae than between taeniae. In general the spatial density of neurons is higher in small animal species than in larger species (7).

It was suggested that a racial difference might be present in the morphology of peripheral nervous

system (10). Since we could not find any literature data about the strain differences in peripheral nervous system morphology, we have planned a study to show the differences if any, in the structure of myenteric plexus in three strains of rabbit. We have chosen the myenteric plexus as a model of peripheral nervous system because it would be easy to count the neurons on such a whole-mount preparation and the results would be comparable between the groups.We have concentrated on the quantitative aspects of the myenteric plexus and measured the number of neurons per ganglion and the the number of ganglia per unit area.

MATERIALS AND METHODS

Tissue preparation

36 adult male rabbits from three different strains (12 rabbits from each strain : New Zealand, Chinchilla and California) were used in the experiments. All the animals were purchased from the Research Institute of Ministry of Agriculture and they were maintained in similar conditions. After decapitation, 5 cm long intestinal segments were washed with Krebs solution then opened along the mesenteric border and fully stretched up to 2 cm x 6 cm rectangles. All were pinned flat under Krebs solution in a dish that was lined with silicone elastomer (Sylsaard, Dow Corning, Midland, MI). The segments were then fixed with formalin for 8 hours.

Preparation of whole mounts

After fixation, intestines were dissected as described previously to reveal the myenteric ganglia (14). First, the mucosal and attached submucosal layers of the intestinal wall were separated from the underlying circular muscle. The circular muscle fibers were then stripped away from the remaining intestine to expose the myenteric plexus and the longitudinal layer of smooth muscle. The whole mounts with the longitudinal muscle having the adharent myenteric plexus (Longitudinal muscle-myenteric plexus = LMMP) were thus obtained.

Staining and counts

After washing in distilled water, the whole-mount preparations were stained with 0.5 % methylene blue for three minutes (16). The preparations were washed in distilled water again and dehydrated through graded alcohol, exposed to graded glycerine solutions and mounted in 97 % glycerine. After mounting; neurons and ganglia were counted and pictures were taken under a light microscope (Olympus BHS/BHT). In all segments methylene blue was supposed to stain selectively all the ganglionic nerve cells in the whole mount preparations. In each preparation neurons and ganglia were counted by two independent observers. Care was taken to ensure that all neurons in a ganglionic area were counted. The average number of ganglia per unit area and the average number of neurons per ganglion and per unit area were then calculated. The data were expressed as mean ± SD. The significance of differences between the groups was analzed by the Student's - t test. In all instances, p values less than 0.05 were considered to be statistically significant.

RESULTS

At low magnifications, the outlines of the ganglia were clearly recognizable. At higher magnifications, neuronal cells were easily recognizable with negative nuclear image within the blue cytoplasm and prominent dark stained nucleoli. Nissl substances and nucleoli stained blue. Staining appeared to be limited to the perikaryon, as Nissl substances do not extend into the axons. In California strain, large ganglia were closely packed and connected by internodal strands (Fig 1). In contrast to the California, New Zealand strain had finer ganglia with no visible internodal strands (Fig 2). The average number of neurons per ganglion which was 20 for New Zealand, 33 for Chinchilla (Fig 3) and 27 for California showed inter - strain variations ($P<0.05$). The number of ganglia per unit area was also significantly different between the groups ($P<0.05$).

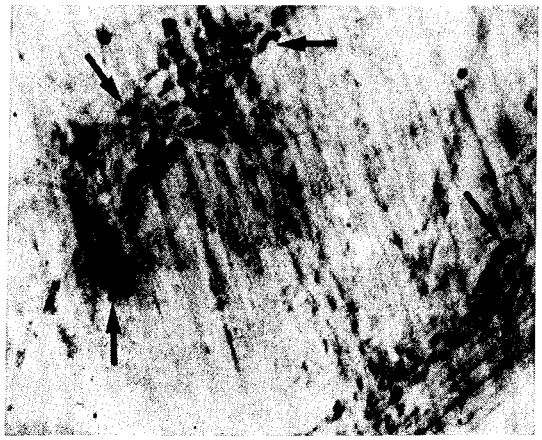

Figure - 1 : A photomicrograph from a preparation of California strain; large ganglia were closely packed and connected by internodal strands. Neuronal cells (arrows) are easily recognizable with negative nuclear image within the dark cytoplasm (Methylene blue, x460).

Figure - 2 : New Zealand has finer ganglia without prominent internodal strands. Arrows : Neuronal cell bodies (Methylene blue, x460).

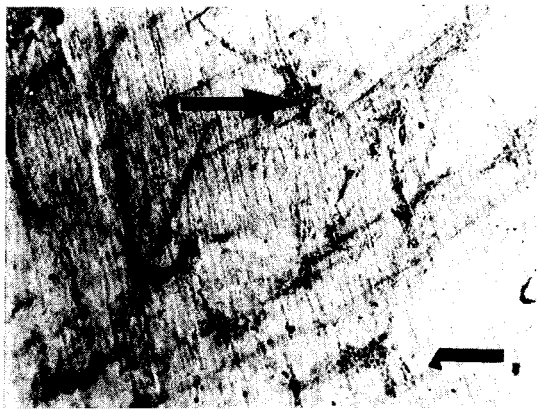

Figure - 3 : A photomicrograph from a preparation of Chinchilla strain; the average number of neurons per ganglion was highest in this group. Arrows : Myenteric ganglia (Methylene blue, x55).

These values were lowest in Chinchilla while they were highest in the California type (Table 1). Total number of neurons per unit area is also different between the groups ($P<0.05$).

	New Zealand	Chinchilla	California
Neuron/Ganglion	20 ± 1.5	33 ± 2.1	27 ± 1.8
Ganglion/cm^2	238 ± 28	207 ± 10	383 ± 29
Neuron/cm^2	4554 ± 169	6573 ± 148	9621 ± 449

Table 1 : The results of the counts in the myenteric ganglia.

DISCUSSION

The myenteric ganglia vary in size, shape and orientation among various species and from one part of the intestine to another; but in any one area from a particular species the shape of the meshwork is quite characteristic and readily identified (2, 6).

In a study, neuronal loss was reported in aging guinea pigs (5). To avoid the age based variations we used animals of the same age group.

Early morphological and quantitative studies were performed on the serial sections of the intestine (3). Neuronal counts of the myenteric ganglia on the serial sections are less informative than other methods. A proportion of the ganglia may be missed in sections of the intestine and it is considerably time consuming to count the cells in serial sections (23). Thus, in the present study, whole - mount preparations were used to get information about the number of ganglia and neuronal cells concurrently.

A number of methods have been used to stain enteric neurons in intestinal whole mounts to estimate the total number of nerve cell bodies. Methylene blue and toluidine blue are the cationic dyes (13) generally used to stain neuronal cell bodies. Cuprolinic blue is an alternative method developed by Heinicke for staining the myenteric neurons in whole mount preparations of rat small intestine (9). In our study, because of the precision in the counts, we have preferred methylene blue to stain neuronal cells in whole mount preparations of the intestinal tract (24).

It was shown that the colon had a higher neural density than the small intestine, and the duodenum had a higher density than jejunum (4). Young et al. used the number of neurons per ganglionic area counts for quantitative studies in rats (24).

In our study, we have used the number of neurons per ganglion and per unit area and the number of ganglions per unit area to compare the groups.

The average number of neurons per centimeter in rabbit jejunum was 3500 in a report (16). It is about 6900 in our study. The difference might result from the serial sections or different stretching levels of the intestine segments during fixation or from the different number of stained neuronal cell bodies.

Three rabbit strains are recognized easily with their physical characteristics. For example, New

Zealand type is white, Chinchilla is gray and California type is black and white (18). The cranium is larger in Chinchilla; while the ear is the longest in the New Zealand type (15). In this study, we have demonstrated an additional strain characteristics for rabbits. The difference we found in the myenteric plexus morphology might be the first report of the peripheral nervous system morphology. The myenteric ganglia vary in size, shape and orientation among the rabbit strains.

All racial and strain characteristics were suggested to be the results of adaptation to environment over long periods of time (21). Different morphology of the myenteric ganglia might also show the adaptive changes in the rabbit intestines.

In humans, racial differences are present in certain functions related to peripheral nervous system such as symphathetic nervous system-mediated energy expenditure (19). In addition, adults in most populations have low levels of lactase activity and may suffer from intestinal symptoms of intolerance including bloating, flatulence, and diarrhea upon the ingestion of milk products high in lactose (20). Population differences in lactose intolerance might be attributable to the permanent functional differences in enteric nervous system, mucosal nerve fibers which control the transport across the intestinal lining and the epithelial enzyme activity.

Furthermore, additional inter racial differences are also present in certain intestinal diseases of human. For instance, constipation and colorectal adenocarcinomas in the adult show a higher incidence in blacks than in whites (8, 12). The prevalance of small intestinal atresia and necrotizing enterocolitis were found to be significantly higher in black children, than in white children (1, 22).

In conclusion, differences in the myenteric plexus morphology might also be present in human races and it needs further investigation.

Correspondence to : Dr.M.Kemal IRMAK
GATA
Histoloji - Embriyoloji Bilim Dalı
Etlik
06018 ANKARA - TÜRKİYE
Phone : 312 - 325 12 11 / 2396

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