Distribution of Argininosuccinate Synthetase-like Immunoreactive Neurons in the Rat Myenteric Plexus: A Whole Mount Study

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Summary: The distribution of argininosuccinate synthetase (ASS)-like immunoreactive neurons in the myenteric plexus of the rat alimentary tract were immunocytochemically studied using whole-mount tissues. The present study revealed ASS-like immunoreactive meshworks of ganglia and interconnecting nerve strands in the myenteric plexus of almost all parts of the alimentary tract. ASS-like immunoreactive neurons constituted about 11% of the myenteric cell.

Argininosuccinate synthetase (ASS, EC.6.3.4.5.) is a well-known and important enzyme in the arginine-synthesis pathway; this enzyme catalyzes the synthesis of L-argininosuccinate from L-aspartate and L-citrulline. Although ASS is believed to play a role in urea synthesis in the liver and in arginine synthesis in the kidney (Ratner, 1976; Funahashi et al., 1981), our recent work (Nakamura et al., 1990) suggested a further role for this enzyme in the synthesis of neuromodulators or substances functionally related to neurotransmitters. This conclusion was reached on the basis of the anatomical distribution of the immunoreactivity to ASS antibody observed in the rat brain. Another series of studies showed the direct product of this enzyme, L-argininosuccinate, to modify the neuronal response induced by glutamate (Nakamura et al., 1991) or smooth muscle contraction produced by acetylcholine (Gold et al., 1989).

In a previous study (Nakagawa et al., 1991), we demonstrated the presence of ASS of the peripheral nervous system of the Japanese monkey, including the myenteric plexus of the small intestine. However, several questions remained concerning whether the homology of the ASS distribution existed across mammalian species or whether the same results would be obtained throughout the length of alimentary tract.

To address these issues, we examined the distribution of ASS labeling in the myenteric plexus over the overall length of the rat alimentary tract.

Materials and Methods

The production, specificity, and characterization of the primary antibody to rat liver ASS have been previously described (Saheki et al., 1977; Ichiki et al., 1987).

Five male Wistar rats were used in this study. They were divided into two groups and used for whole-mount preparations. The first group (two rats) was used for the observation of ASS-like immunoreactive structures in the myenteric plexus by the indirect immunofluorescence method. The second group (3 rats) was used to count the number of immunoreactive nerve cells in this plexus by the peroxidase anti-peroxidase (PAP) method.

All animals were sacrificed by the sodium pentobarbital overdosing (60 mg/kg, i.p.). Samples of the esophagus, stomach, and small and large intestines were immediately removed and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, containing 0.2% picric acid for 2 days at 4°C. After fixation, samples were subjected to the whole-mount preparation technique of Costa et al. (1980).

For immunocytochemistry, whole-mounts were preincubated in 1% goat serum and 0.3% bovine albumin in PBS, and then incubated for 7 days at 4°C with rabbit antiserum to ASS (IgG concentration:

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2 mg/ml), diluted 1:200 in PBS containing 1% goat serum, 0.3% bovine albumin and 0.3% Triton X-100. Subsequently, either the FITC-conjugated secondary antiserum or the indirect method involving PAP was used.

**Results**

ASS-like immunoreactivity was found in the myenteric plexus of the esophagus, stomach, small intestine, cecum and colon. The patterns of immunoreactivity were basically similar throughout the alimentary tract, although the density slightly differed among regions.

As shown in Fig. 1, ASS-like immunoreactive varicose nerve fiber meshworks were observed in the circular muscle layer, ganglia and longitudinal layer. The many ASS-like immunoreactive varicose nerve fibers seen in the myenteric plexus were located in the ganglia and internodal strands (Fig. 1). The ASS-like immunostained varicose fibers in the muscle layers ran along the muscle fibers (Fig. 3). Much denser networks composed of these immunostained nerve fibers were seen in the stomach and the small intestine, whereas there was a much sparser meshwork in the esophageal region.

Most of the myenteric ASS-like immunostained ganglion cells were preferentially located in the periphery of the plexus (Fig. 2). As shown in Table 1, about 11% of the myenteric plexus nerve cells showed ASS-like immunoreactivity: the greatest number of immunostained cells were found in the esophageal region, whereas the least number were observed in the stomach.

In addition, ASS-like immunoreactive structures were detected in other areas of the alimentary tract, such as the submucosal plexus and the nerve fibers distributed over the mucosa.

<table>
<thead>
<tr>
<th>Region</th>
<th>Total neurons</th>
<th>ASS neurons</th>
<th>% Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td>408</td>
<td>116</td>
<td>28</td>
</tr>
<tr>
<td>Stomach</td>
<td>928</td>
<td>78</td>
<td>8</td>
</tr>
<tr>
<td>Duodenum</td>
<td>2093</td>
<td>308</td>
<td>15</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1491</td>
<td>163</td>
<td>11</td>
</tr>
<tr>
<td>Ileum</td>
<td>1991</td>
<td>235</td>
<td>12</td>
</tr>
<tr>
<td>Cecum</td>
<td>1378</td>
<td>163</td>
<td>12</td>
</tr>
<tr>
<td>Colon</td>
<td>879</td>
<td>96</td>
<td>11</td>
</tr>
</tbody>
</table>

**Discussion**

The present results confirm and extend our previous findings on the topochemistry of ASS in the myenteric plexus of the Japanese monkey (Nakagawa et al., 1991). In the present study, ASS-like immunostained nerve cell bodies and fibers were observed in the myenteric plexus of almost all parts of the rat alimentary tract in a pattern similar to the distribution reported in the Japanese monkey. However, the density of these immunoreactive myenteric cells appears to be less than that reported for the monkey, although the significance of this species difference is not apparent at the present time. As expected from results in the Japanese monkey, most ASS-like immunoreactive fibers appear to arise from the intrinsic neurons, evidenced by the lack of ASS-like immunostained fibers in the vagus or sympathetic nerves.

The exact functional role of the ASS-containing neurons remains to be elucidated. However, ASS-like immunoreactive fibers were found with varicosities as both bundles and single fibers. The present of these varicosities suggests that they may exert an effect on nearby tissues such as smooth muscles. In fact, the direct product of this enzyme, L-argininosuccinate, has been reported to modify smooth muscle contraction produced by acetylcholine (Gold et al., 1989). In addition, considering the evidence that nitric oxide synthase, which catalyzes the synthesis of nitric oxide from L-arginine, is concentrated in the myenteric plexus of neurons in both cell bodies and fibers of the small intestine (Snyder and Bredt, 1991), it appears a subject deserving closer investigation in relation to the pursuit of simultaneous localization of ASS and this enzyme in the myenteric neurons.

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**References**


Explanation of Figures

Plate I

Fig. 1. Fluorescent photomicrograph of a whole-mount preparation showing the distribution of ASS-like immunoreactivity in the myenteric plexus of the rat jejunum. x 180.

Fig. 2. High-power photograph of the myenteric ganglion represented in the rat jejunum. Note that the ASS-like immunostained nerve cell bodies tend to be located near the peripheral region. x 210.

Fig. 3. ASS-like immunostained varicose fibers running along the smooth muscle fibers of the rat jejunum. Dark-field photomicrograph. x 440.
Additions and Corrections
A Golgi Study on the Red Nucleus in the Mouse.
Because of the error of bookbinding, this report lacks the following page 80.

reported in the mouse by Ramón y Cajal (1911). He noted that these fibers were derived from the medial lemniscus. However, after damaging the dorsal column nuclei in the cat (Matzke 1951) and monkey (Bowsher 1958), no degenerated fibers were seen in the RN. Therefore, the lemniscorubral fibers should originate from other areas than the dorsal column nuclei. In the present study, some tips of the main axons and collaterals of the cerebellar afferent fibers could be followed into the medial lemniscus. Thus, there exist possibilities that at least some of the lemniscal fibers projecting to the RN may originate in the deep cerebellar nuclei. Furthermore, the RN has been known to receive fibers from the cerebral cortex (Rinvik and Walberg 1963; Kuypers and Lawrence 1967; Brown 1974). The corticorubral fibers were reported to reach the RN from the ventrolateral aspect. A few fibers from the ventrolateral aspect could be traced retrogradely up to a region near the crus cerebri and may represent in part the corticorubral fibers.

References