Peptidergic innervation irregularities in Hirschsprung’s disease

Immunohistochemistry – Radioimmunoassay

Tomoaki Taguchi¹, Kenzo Tanaka¹, Keiichi Ikeda², Sunao Matsubayashi³, and Noboru Yanaihara³

Department of Pathology¹, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812, Japan
Department of Pediatric Surgery², Faculty of Medicine, Kyushu University, Fukuoka, Japan
Laboratory of Bioorganic Chemistry³, Shizuoka, College of Pharmacy, Shizuoka, Japan

Summary. The distribution of vasoactive intestinal polypeptide (VIP)-containing nerves and the contents of both VIP and substance P (S-P) in the intestines from 12 children with Hirschsprung’s disease were examined using immunohistochemical methods and radioimmunoassay. VIP-containing nerve fibers were markedly decreased in number in the true muscle coats of aganglionic segments, while extrinsic hypertrophic nerve bundles in these segments showed positive VIP-immunoreactivities. This finding suggests the existence of extrinsic origins of VIP-containing nerves in the human gut. The contents of VIP were 44.5 ± 8.2 in aganglionic segments and 130 ± 17.1 pg/mg wet tissue weight in normoganglionic segments. The contents of S-P were 0.42 ± 0.18 in aganglionic segments and 6.38 ± 2.3 pg/mg wet tissue weight in normoganglionic segments. Both VIP and S-P contents in aganglionic segments were significantly reduced as assessed by the use of radioimmunoassay (p < 0.001 and p < 0.05).

These abnormal peptidergic patterns of innervation might relate to the non-peristaltic state in Hirschsprung’s disease.

Key words: Hirschsprung’s disease – Vasoactive intestinal polypeptide – Substance P – Immunohistochemistry – Radioimmunoassay

The pathophysiology of the occurrence of the narrow segment in Hirschsprung’s disease has not been fully defined. The abnormal autonomic nerve supply in the diseased bowel is, however, considered to be a causative factor (Whitehouse and Kernohan 1948; Kamijo et al. 1953; Ehrenpreis 1966; Meier-Ruge 1968).

Deficiencies in the non-adrenergic inhibitory system (Burnstock et al. 1963; Crema et al. 1968) have been demonstrated physiologically in the aganglionic segments (Frigo et al. 1973). The finding that impairment of

Offprint requests to: K. Tanaka at the above address
the non-adrenergic pathway blocks peristaltic activity (Crema et al. 1970) suggests the significant role of this system in the pathogenesis of Hirschsprung’s disease. The failure of the non-adrenergic-related relaxation may be one of the main factors related to the non-peristaltic state (Frigo et al. 1973).

Vasoactive intestinal polypeptide (VIP) is a putative neurotransmitter in the non-adrenergic inhibitory nerve system (Bryant et al. 1976; Fahrenkrug 1979; Goyal et al. 1980). This system is also referred to as the peptidergic nerve system (Sundler et al. 1980). Recently the content of VIP has been revealed to decrease in the aganglionic bowels by using radioimmunoassay (Freund et al. 1979; Dupont et al. 1980; Bishop et al. 1981). These authors estimated the content of VIP in aganglionic segments, compared with that in normoganglionic segments.

The distribution of VIP-containing nerves in the bowel of patients with Hirschsprung’s disease should be determined morphologically, in order to elucidate the pathophysiology of the non-peristaltic state of aganglionic bowel. Two differently innervated forms of bowel are present in the aganglionic segments. One is the aganglionic segment with extrinsic hypertrophic nerve bundles and the other is that part without these bundles. These two forms of aganglionic segments are thought to have different peptidergic innervations.

Substance P (S-P), a regulatory peptide in both central and peripheral nerve system (Euler and Gaddum 1931), is reported to act as an excitatory neurotransmitter in peristaltic movement (Leander et al. 1981). Ehrenpreis and Pernow (1953) described bioassay evidence for a decrease of S-P activity in the aganglionic segments. The depletion of S-P innervation is also related to the non-propulsive effect of the aganglionic segments, therefore the content of S-P has to be estimated in detail with radioimmunoassay.

We examined immunohistochemically the detailed distribution of VIP-containing nerves. We measured the contents of both VIP and S-P, using radioimmunoassay, in resected intestines from 12 children with Hirschsprung’s disease. We investigated the contents of VIP and S-P in 3 different parts of the resected bowel, the oligoganglionic as well as the aganglionic and normoganglionic segments.

Materials and methods

Twelve Japanese children ranging in age from 2 months to 17 years were clinically diagnosed as Hirschsprung’s disease, following barium enema, manometric study and histochemical study using acetylcholine-esterase staining in suction biopsy material (Karnovsky and Roots 1964; Meier-Ruge et al. 1972). The intestine was resected using the Z-shaped anastomosis method (Ikeda 1967).

As a control study, transverse and sigmoid colons, and anorectal portions obtained at the time of autopsy on patients without gastrointestinal disease were studied. Here, the ages ranged from newborn to 16 years. All materials were obtained within 5 h after death.

Histology and immunohistochemistry

The materials were fixed with Zamboni’s solution (1967) for 1 h at 4° C, then were cut 2 mm thick and fixed in the same solution for an additional 24 h at 4° C. Fixation was followed