Enkephalin-like immunoreactivity in the SIF cells of sympathetic ganglia of frogs

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ABSTRACT

SIF cells exhibiting positive immunoreactivity to met-enkephalin were found underneath the ganglionic sheath in the second, third and fourth sympathetic ganglia of bullfrogs. They were located in close to small blood vessels and isolated from principal ganglion cells by thin layers of the connective tissue. Immunoreactive materials appeared granular suggesting that they are located in the granular vesicles characteristic of SIF cells. No long cytoplasmic processes of the SIF cells were seen and no nerve fibers with this immunoreactivity were found in the ganglia.

KEY WORDS SIF cells / enkephalin / frog sympathetic ganglia

Recent immunohistochemical studies have revealed positive immunoreactivity with antisera to several biologically active peptides in autonomic ganglia and suggested the functional significance of the peptides in the ganglionic transmission (2, 3, 4, 7). SIF (small intensely fluorescent) cells, which are characterized by the presence of abundant granular vesicles and the high concentration of catecholamines in the cytoplasm (1, 6, 12, 13), exhibit enkephalin-like immunoreactivity in mammalian sympathetic ganglia. However, no immunohistochemical analyses have been made on SIF cells of amphibian sympathetic ganglia. In the course of immunohistochemical studies on various peripheral ganglia, we encountered frog ganglionic SIF cells exhibiting enkephalin-like immunoreactivity, which will be reported in this paper.

Adult bullfrogs (*Rana catesbeiana*) of both sexes were used in the present study. Under ether anesthesia bullfrogs were perfused with Bouin's fixative via the ventricle for 15 min. All paravertebral sympathetic ganglia of both sides (from the first to 10th ganglia) were then dissected out and processed for embedding in paraffin. Deparaffinized sections, 6 μ m thick, were incubated with the rabbit anti-synthetic methionine- (met-) enkephalin antiserum (S.

Ito, Niigata) at the dilution of 1:300 for 12 hr at 4°C. The sites of antigen-antibody reaction were detected with the peroxidase-antiperoxidase (PAP) method according to Sternberger (9). The control serum was obtained by addition of 10 μ g of the synthetic met-enkephalin to 1 ml of the antiserum diluted at 1:300. After incubation with the antiserum to met-enkephalin, positive immunoreactivity was found in SIF cells in the second, third and fourth sympathetic ganglia. Immunoreactive SIF cells occurred sparsely and were found in small clusters of 5-9 cells lying underneath the connective tissue sheath of the ganglion (Fig. 1). They were isolated from adjacent principal ganglion cells by thin layers of connective tissue, and surrounded small blood vessels. The SIF cells were approximately 13 μ m in diameter and angular in shape and had a round nucleus. No long cytoplasmic processes were observed. Immunoreactive materials appeared granular and were widely distributed throughout the whole cytoplasm. After incubation with the control serum, the SIF cells exhibited no immunoreactivity. No SIF cells with immunoreactivity to met-enkephalin were found in lower sympathetic ganglia and no nerve fibers with this immunoreactivity were found within any ganglia.

Fig. 1 A group of SIF cells exhibiting the immunoreactivity to met-enkephalin is seen around a small blood vessel (V) close to the connective tissue sheath of the third sympathetic ganglion. G: principal ganglion cells. $\times 920$

There has been increasing evidence suggesting the coexistence of biologically active peptides and classical transmitters such as catecholamines in the same cells. Enkephalin-like immunoreactivity was exhibited in some noradrenergic sympathetic ganglion cells and SIF cells as well as adrenomedullary cells in mammals (7, 8). Somatostatin-like immunoreactivity was observed in certain noradrenergic ganglion cells (2). The present finding gives another evidence suggesting such coexistence.

The granular appearance of immunoreactivity suggests that the immunoreactive material is localized in the granular vesicles which are characteristic of SIF cells. Although the absolute number of SIF cells in frog sympathetic ganglia is known to be small as compared with that in mammalian ganglia (10, 11), the sparse occurrence of enkephalin-like immunoreactive SIF cells only in the upper three ganglia of the sympathetic chain infers that the enkephalin-like immunoreactive substance is present only in a small population of SIF cells.

Although it has been proposed that SIF cells are adrenergic inhibitory interneurons in mammalian ganglia, there has been much controversy on their synaptic relation to principal ganglion cells and preganglionic nerve terminals (5, 12, 13). Since electron microscopic studies have failed to detect any efferent synapses

from SIF cells to other neuronal elements in amphibian ganglia (10, 11), it is suggested that SIF cells in this vertebrate class may function as neurosecretory cells rather than interneurons. If they function as neurosecretory cells, the intraganglionic effect of their secretory substances on adjacent principal ganglion cells by diffusion as well as the intra or extra ganglionic remote effect of them by vascular transport should be considered. Physiological studies on the response of ganglion cells to enkephalin in these upper ganglia of sympathetic chain of frogs may lead us to better understanding the functional significance of SIF cells.

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