Cerebellar Purkinje Cell-Specific Protein-Like Immunoreactivity in Noradrenalin-Chromaffin Cells and Ganglion Cells but Not in Adrenaline-Chromaffin Cells in the Rat Adrenal Medulla

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Received June 20, 1985

Summary. By means of immunohistochemistry combined with fluorescence microscopy, a cerebellar Purkinje cell-specific protein, also named spot 35 protein, was found to occur in all noradrenaline-chromaffin cells and some ganglion cells of the rat adrenal medulla. The immunoreactivity was localized in the chromaffin granules as well as the cytoplasmic matrix. The nucleus as well as the interior of the membranous organelles were free of immunoreaction. No immunoreactivity was found in most of the intra-adrenal nerve fibers except for a few nerve fibers derived from the immunoreactive ganglion cells. Neither Schwann, satellite cells nor cortical cells were immunoreactive. It is suggested that some intracellular phenomenon possibly relating to the regulation of intracellular Ca-ion concentration is common to noradrenaline-chromaffin cells, medullary ganglion cells and cerebellar Purkinje cells.

A cerebellar Purkinje cell-specific protein, termed spot 35 protein has recently been isolated from the soluble fraction of the bovine and rat cerebella (Yoshida and Takahashi, 1980). This protein has a molecular weight of 27 K dalton and a pI of 5.3, and is characterized by a high content of glutamic acid, aspartic acid and leucine. Immunohistochemistry reveals that Purkinje cells with their complicated dendritic arborizations and their single axons in the cerebellum are intensely and selectively stained with the antiserum against spot 35 (Yamakuni et al., 1984).

Further analysis in our laboratory with immunohistochemistry has revealed the occurrence of spot 35-like immunoreactivity in several receptor cells including olfactory epithelial cells, taste cells of the tongue, hair cells of the inner ear and horizontal cells in the retina (Iwanaga et al., 1985). Moreover, some endocrine paraneurons such as chromaffin cells of the adrenal medulla, islet cells of the pancreas, gut endocrine cells, and thyroid parafollicular cells have been shown to possess the spot 35-like immunoreactivity (Iwanaga et al., in preparation). It is to be noted, however, that the distribution of the immunoreactivity is generally heterogenous within each sensory or endocrine organ. Thus a proportion of the adrenal chromaffine cells has been demonstrated to be immunopositive for spot 35.

The present study was undertaken to examine which types of adrenal chromaffin
cells, noradrenaline- or adrenaline-cell, exhibit the spot 35-like immunoreactivity. The precise localization of the immunoreactivity was also examined and the functional significance of the existence of the protein common to the Purkinje cells and chromaffin cells is discussed.

MATERIALS AND METHODS

Young male rats weighing about 150 g, under a Nembutal anesthesia (35 mg/kg), were fixed by transcardiac perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 10 min. The adrenal glands were extirpated and immersed in the same fixative for a further 2 hr. The glands, after rinsing overnight with the phosphate buffer containing 30% sucrose, were frozen in liquid nitrogen and 20 μm thick sections were made on a cryostat. The sections were mounted on glass slides and first observed under the fluorescence microscope using a filter D for catecholamines. After taking photographs of the fluorescent cells, the sections were incubated for 20 hr at 4°C with rabbit anti-spot 35 antiserum at a dilution of 1:1,000. The sites of antigen-antibody reaction were visualized with the peroxidase-antiperoxidase (PAP) method according to Sternberger. As the control serum, the anti-spot 35 antiserum was immunoabsorbed

![Fig. 1. and 2. Micrograph showing spot 35 immunoreactive chromaffin cells (Fig. 1) and fluorescence micrograph showing fluorescent noradrenalin cells (Fig. 2) in the same section of rat adrenal medulla. Note that all spot 35-immunoreactive cells are noradrenaline-fluorescent (arrows). ×115](image-url)
with spot 35 protein (10 μg/ml, 1:1,000 dilution). For electron microscopy, the sections after the completion of the PAP procedure were postfixed with 1% OsO₄ in 0.1 M cacodylate buffer, pH 7.4, for 30 min, and embedded in Epon according to conventional procedures. Ultrathin sections were examined with brief staining by uranyl acetate.

RESULTS

Chromaffin cells with the spot 35-like immunoreactivity appeared either in small clusters or singly and were distributed throughout the adrenal medulla with no special topographical relation (Fig. 1). The immunoreactive cells occupied approximately one fifth of the total population of the chromaffin cells. They were polygonal in shape, 15–20 μm in diameter, and had no distinct cytoplasmic processes.

In fluorescence microscopy, approximately one fifth of the total chromaffin cells emitted intense blue-white fluorescence. The fluorescent cells occurred in small groups or singly throughout the adrenal medulla (Fig. 2). After processing the same sections for immunohistochemistry, all spot 35-immunoreactive chromaffin cells were intensely fluorescent (Fig. 1, 2).

In immuno-electron microscopy, the immunoreactive chromaffin cells were characterized by abundant chromaffin granules, whose cores were mostly rounded, though there were pleomorphic cores intermingled among them (Fig. 3). The electron density of the granule core was remarkably high due to the immunoreaction. The immunoreactive material was also located diffusely in the cytoplasm, resulting in increased electron density and a granular appearance to the cytoplasm. However, the interior of the mitochondria and other membranous organelles as well as the nuclei were free of the immunoreaction. Non-immunoreactive nerve fibers were seen in synaptic contact with the immunoreactive chromaffin cells. Non-immunoreactive chromaffin cells contained abundant chromaffin granules whose cores exhibited considerably low electron density as compared to those of the immunoreactive cells.

In addition to chromaffin cells, the spot 35-like immunoreactivity was found to occur in some medullary ganglion cells. They were 30–40 μm in diameter and had a large nucleus with prominent nucleoli. They extended multipolar processes among adjacent chromaffin cells as thin nerve fibers. The immunoreactivity appeared diffusely in the perikarya but their nuclei were free of the immunoreaction. In electron microscopy, the immunoreactive ganglion cells were characterized by a well developed rough endoplasmic reticulum and Golgi apparatus, and by abundant small mitochondria; they contained no chromaffin granules (Fig. 4). The immunoreactive material was located diffusely throughout the cytoplasm except for the interior of the membranous organelles, including the mitochondria and endoplasmic reticulum. Non-immunoreactive nerve fibers containing abundant small clear vesicles were seen to make synaptic contact with the immunoreactive ganglion cells. Most of the intra-adrenal nerve fibers were immunonegative except for a few nerve fibers derived from the immunoreactive ganglion cells. Neither glial elements nor cortical cells were immunoreactive for spot 35.

After incubation with the antiserum preabsorbed with the antigen (spot 35 protein 20 μg/ml, 1:1,000 dilution), no immunoreactivity was seen in any portions of the adrenal medulla.
Fig. 3 and 4. Legends on the opposite page.
DISCUSSION

Fixation with 4% paraformaldehyde used in the present study is known to be sufficient for the detection of fluorescence specific for noradrenaline (Falck and Torp, 1961; Eränkö, 1967). Through a combination of immunohistochemistry with fluorescence microscopy, it is now clear that noradrenaline-containing chromaffin-cells are immunoreactive for spot 35. This finding of differences between noradrenaline-cells and adrenaline-cells in the presence of spot 35-like immunoreactivity is intriguing. It is possible that spot 35 is present in both types of chromaffin cells but at such a low concentration in adrenaline-cells that it is not detected in immunohistochemistry. Alternatively, it likely reflects some major differences between the two cell types in their cell metabolism and function.

The occurrence of the spot 35-like immunoreactivity in the chromaffin granules in addition to the cytoplasmic matrix is rather unexpected, since the original study of this protein stated that it is found in the cytosol (Yoshida and Takahashi, 1980; Yamakuni et al., 1984). This suggests the possibility that spot 35 is partially released from the chromaffin cells during exocytosis of the granule contents, including noradrenaline. Another study from our laboratory (Iwanaga et al., in preparation) indicated that the localization of the spot 35-like immunoreactivity outside the cerebellum is largely similar to that of the vitamin D dependent Ca-binding protein already reported by other authors (Wasserman and Taylor, 1966; Wasserman et al., 1968; Morisse et al., 1975; Roth et al., 1982; Taylor et al., 1984). In agreement with this coincidence in localization, spot 35 protein has recently been revealed to also be a Ca-binding protein (Yamakuni et al., 1985), although several differences in chemical characterizations have been pointed out between both proteins: the molecular weight 27 K dalton of the spot 35 versus 25 K dalton of the vitamin D dependent Ca-binding protein, and pI 5.3 of the spot 35 versus 4.1 of the Ca-binding protein (Yamakuni et al., 1984). Since the spot 35 possesses a Ca-binding ability, and because Ca-ions are known to play important roles in the process of secretion (Douglas, 1974), the occurrence of the spot 35-like immunoreactivity in the chromaffin cells, especially in intimate relation to chromaffin granules, may indicate the significant involvement of this protein in the process of noradrenaline secretion. In this regard, it should be noted that differential releases of the two catecholamines have been known to occur under various stimuli (Edwards et al., 1980).

The present study is the first to report on the occurrence of the Purkinje cell-specific spot 35 protein in nerve cells in the peripheral nervous system. This, together with its occurrence in noradrenaline-cells, may underline some intracellular phenomena in those cells common to the Purkinje cells and to other neurons and paraneurons (see Introduction) which share this specific protein. Further chemical characterization of the spot 35 protein is crucial to more clearly understand this point.

Fig. 3. Electron micrograph showing a spot 35-immunoreactive chromaffin cell (*) adjacent to immunonegative chromaffin cells (n). Note the increased electron density due to the immunoreaction in the chromaffin granules and in the cytoplasmic matrix, and the negative immunoreaction in the nucleus (N). Note also pleomorphic chromaffin granules (arrows) in the immunoreactive cells. ×24,000

Fig. 4. Electron micrograph of a spot 35-immunoreactive ganglion cell and an adjacent immunonegative chromaffin cell (n). Note the diffuse localization of the immunoreactive material throughout the axoplasm except for the interior of various membranous organelles. ×12,000
Acknowledgements. The authors wish to thank Prof. Y. Takahashi of the Brain Research Institute, Niigata University, for kindly providing us with the anti-spot 35 antiserum.

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