Fine Structure of the Taste Bud in Guinea Pigs.
II. Localization of Spot 35 Protein, a Cerebellar Purkinje Cell-Specific Protein, as Revealed by Electron-Microscopic Immunocytochemistry

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Received October 25, 1990

Summary. The localization of spot 35 protein, a cerebellar Purkinje cell-specific protein, was studied in guinea pig taste buds by means of electron-microscopic immunocytochemistry. The immunoreactivity was localized in the cytoplasmic matrix of discrete bud cells. The ultrastructural features of the reactive cells indicated that they corresponded to the Type III or gustatory cells making a synaptic contact with the intragemmal nerves. All other cells specified as basal, Type I, and Type II were immunonegative for spot 35 protein. This finding indicates a method for specifically demonstrating the gustatory cells in the guinea pig taste bud and, further, gives new evidence that para-neurons may share neuron-specific substances with neurons.

The gustatory cell in the taste bud comprises a sensory paraneuron together with other chemo- or mechano-receptive cells including the olfactory cell in the nasal mucosa, hair cell in the inner ear, and Merkel cell in the skin and oral mucosa (FUJITA et al., 1988). Notwithstanding their non-neuroepithelial origin, these kinds of cells all exhibit immunoreactivities for certain neuron-specific proteins which originally were believed to be contained only in neurons (for review, see FUJITA et al., 1988; IWANAGA et al., 1989). In the taste buds of several species investigated, a part of the cells have typically been demonstrated as immunoreactive either for neuron-specific enolase (NSE) (FUJITA et al., 1983, 1988; YOSHIE et al., 1988, 1989; HIRATA and KANASEKI, 1989) or for both NSE and spot 35 protein (KURAMOTO, 1988; YOSHIE et al., 1988, 1989). The latter substance, spot 35 protein, is an acidic soluble protein (isoelectric point 5.3, molecular weight 27 kD), which was first isolated from bovine and rat cerebella (YOSHIDA and TAKAHASHI, 1980; YAMAKUNI et al., 1984, 1985) and was immunohistochemically localized in the Purkinje cells (YAMAKUNI et al., 1984).

Our previous immunocytochemical study on the guinea pig taste bud has revealed that certain bud cells display concurrent immunoreactivities for spot 35 protein and NSE (YOSHIE et al., 1988). An ultrastructural examination in the same species has meanwhile disclosed the occurrence of four distinct cell types in the taste bud, among which the Type III cell has been identified as the gustatory cell (YOSHIE et al., 1990).

The present paper aims at light- and electron-microscopic immunocytochemistry of spot 35 protein in the guinea pig taste bud, in order to specify the immunoreactive cell types.

MATERIALS AND METHODS

Five male guinea pigs weighing 350–400 g were used. The antiserum against bovine spot 35 protein raised in rabbits was generously donated by Prof. Y. TAKAHASHI, Department of Neuropharmacology, Brain Research Institute, Niigata University, Niigata (see YAMAKUNI et al., 1984).

Tissue preparation. Anesthetized animals were perfused through the left ventricle with 0.15 M phosphate-buffered saline (pH 7.3) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). The circumvallate papillae were excised from the tongues and fixed in the fixative for 6 h at room temperature.

For light-microscopic immunocytochemistry, the fixed tissue specimens were briefly rinsed in the
buffer, dehydrated through a series of graded ethanol and propylene oxide, and embedded in Araldite. Semithin sections were cut at 0.5 μm on an LKB microtome (Ultrotome V) with a diamond knife and mounted on glass slides by heating (80°C). Resin was removed from the sections with sodium methoxide immediately before the following immunocytochemical procedures (for details, refer to GRUBE and KUSUMOTO, 1986).

For electron-microscopic immunocytochemistry, the fixed specimens were rinsed overnight in the buffer containing 30% sucrose at 4°C and rapidly frozen in liquid nitrogen. Sections were cut at 20 μm on a cryostat, mounted on plastic slides, air-dried, and processed for the following procedures.

**Immunocytochemical procedures.** Immunocytochemistry both at the electron- and light-microscopic levels was performed with the peroxidase-anti-peroxidase (PAP) technique (STERNBERGER, 1986). The sections were incubated with the antiserum against spot 35 protein at various dilutions of 1:2,000–1:8,000 for 24 h at 4°C followed by incubation with porcine anti-rabbit IgG (1:20, 30 min) and rabbit PAP complex (1:50, 30 min). For electron microscopy, the completed sections were postfixed with 1% osmium tetroxide in the phosphate buffer for 30 min, dehydrated through a graded ethanol series, and embedded in Epon 812. Ultrathin sections were stained in uranyl acetate and then examined and photographed with a JEOL 1200EX electron microscope.

The specificity of the immunoreaction has previously been reported (IWANAGA et al., 1985; YOSHIE et al., 1988).

**RESULTS**

The immunoreactivity for spot 35 protein was exclusively recognized in certain bud cells both at the light- and electron-microscopic levels (Figs. 1, 2), confirming the previous findings by our group (IWANAGA et al., 1985; YOSHIE et al., 1988). The immunostaining was localized in the cytoplasm of the reactive cells, and the reaction was distributed over the cytoplasmic matrix (Fig. 2).

Before specifying the types of the immunoreactive cells, the ultrastructural features of the taste bud cells in the guinea pig will be described concisely (for detail, see YOSHIE et al., 1990). The intrinsic constituents of the taste bud are basal, Type I, Type II, and Type III cells. The basal cell resides at the basolateral region of the taste bud without reaching the taste pit apically, whereas all other types are spindle-shaped extending apically to the taste pit. The basal cell is roundish in profile and includes a relatively large nucleus. The cytoplasm contains fair amounts of free ribosomes mostly in the form of polysomes but is poor in its development of other organelles such as rough endoplasmic reticulum and a Golgi apparatus. The basal cells may exhibit mitotic phases.

The Type I cell is characterized by an accumula-
Figs. 2-5. Legends on the opposite page.
tion of membrane-bound, dense granules (mean diameter: 300 nm) towards the apical cytoplasm. The supranuclear cytoplasm is occupied by rough endoplasmic reticulum and a Golgi apparatus. The apical granules are partly found close to the Golgi apparatus. Although the Type I cell often envelopes the intragemmal nerve fibers, no synaptic specialization is recognizable between the cell and the nerve.

The Type II cell, electron-lucent in appearance, supranucleally contains characteristic smooth endoplasmic reticulum arranged concentrically with its parallel lamellae. The Type II cells make direct contact with the swellings of nerve fibers, and the cytoplasm reveals a subsurface cistern along and close to the nerve-contacting area.

The Type III cell is characterized by fair numbers of dense-cored vesicles and a vast amount of intermediate filaments. The nucleus often shows a deep indentation. The cytoplasm contains round vesicles measuring 90 nm in diameter and filled with a moderately electron-dense material. Some of the vesicles are localized close to the Golgi apparatus, while others are dispersed in the cytoplasm mainly around the nuclei. A conspicuous feature of this cell type is its making synapses on the intragemmal nerve fibers. The dense-cored vesicles gather along and close to the cell membrane at the synapses. Based on these ultrastructural features of the Type III cell, it has been proposed that this type cell is primarily responsible for gustatory sensation (YOSHIE et al., 1990).

Immunocytochemical detection under the electron microscope revealed that the immunoreactivity for spot 35 protein was localized in part of the taste bud cells and distributed diffusely over the cytoplasm (Fig. 2). More precisely, the reaction was restricted to the cytoplasmic matrix and was not observed inside the nuclei, vesicles or other organelles. These immunostained cells all showed characteristics of the Type III cell, containing a deeply indented nucleus (Fig. 2) and dense-cored vesicles in the vicinity of the nerve-contacting area (Fig. 3).

In contrast, the cells specified as basal, Type I (Fig. 4) and Type II (Fig. 5) were completely immunonegative for spot 35 protein. These data prove that the Type III (i.e., gustatory) cells in the guinea pig taste buds are exclusively immunoreactive for this substance.

**DISCUSSION**

As far as the immunohistochemical distribution of spot 35 protein in various tissues or organs is concerned, such immunoreactivity has extensively been localized in the sensory and endocrine paraneurons including cochlear inner and outer hair cells and olfactory cells of guinea pigs (IWANAGA et al., 1983), chemosensory cells of frog taste organs (KURAMOTO, 1988), certain cells of the rat and guinea pig taste buds (YOSHIE et al., 1988, 1989), and mammalian endocrine cells located in the anterior hypophysis, carotid body (chief cells), thyroid gland (parafollicular cells), gastro-entero-pancreatic system (HOZUMI et al., 1986), and adrenal medulla (KONDO et al., 1985; HOZUMI et al., 1986). Although most of these previous reports have been done at the light-microscopic level, KONDO et al. (1985) applied both light and electron-microscopic immunocytochemistry to the rat adrenal medulla, detecting the immunoreactivity in all the noradrenaline-chromaffin cells, certain ganglion cells, and a few nerve fibers derived from immunostained ganglion cells. The ganglion cells showed a diffuse immunostaining of the cytoplasmic matrix, whereas in noradrenaline cells immunoreactive staining was found not only in the cytosol but also was demonstrated in the chromaffin granules (KONDO et al., 1985).

In the present study on the guinea pig taste bud, however, the immunoreactivity was localized in the cytoplasmic matrix, and did not appear in those vesicles which were believed to contain a transmitter or transmitters towards nerves. The subcellular localization pattern of the spot 35 protein immunoreactivity in the taste bud cells agrees with the fact that this protein was originally isolated from the cytosol of the Purkinje cells (YOSHIDA and TAKAHASHI, 1980; YAMAKUNI et al., 1984).

As was shown in our previous paper regarding the guinea pig taste buds (YOSHIE et al., 1988), the immunoreactivity for NSE was localized to all the bud cells immunostained for spot 35 protein. Likewise, all of the spot 35 protein-immunoreactive cells proved to be NSE-immunoreactive in the rat taste bud (YOSHIE et al., 1989). Immunoreactivity for NSE was further reported in certain taste bud cells of other mammalian species including man (FUJITA et al., 1983; HIRATA and KANASEKI, 1989; YOSHIE et al., 1989). As for sensory paraneurons, NSE seems to be distributed more widely: the immunoreactivity has been localized in the Merkel cell (GU et al., 1981), olfactory cell (TAKAHASHI et al., 1984), cochlear hair cell (ALTSCHELER et al., 1985), and frog taste organ (KURAMOTO, 1988; TOYOSHIMA and SHIMAMURA, 1988).

The extensive distributions of these neuron-specific proteins in the sensory paraneurons including the taste bud may reflect their physiologic and metabolic features common to neurons. The data obtained here demonstrate that the Type III cell is exclusively...
immunostained for spot 35 protein, and positively give credence to the gustatory function of this type of cell. However, the functional significance of both substances in the gustatory cell remains to be ascertained.

So far as we are aware, the present study is the first to reveal that a specific type of cell identified by electron microscopy displays immunoreactivity for a neuron-specific protein. This finding would be useful for reevaluating some notions concerning the lineage of the taste bud cells, for example. There are two hypotheses arguing whether the various types of cells differentiate from a single cell line or from separate cell lines (see DELAY et al., 1986). We are now in the process of exploring this question using spot 35 protein immunocytochemistry in combination with autoradiography. This combined technique is also expected to determine the cell-renewal rate of the taste bud cells (see BEIDLER and SMALLMAN, 1965). When applied to the developing animal, immunocytochemical staining of spot 35 protein may also serve to determine at what stage of differentiation the cells of the taste buds become functional (see HIRATA and KANASEKI, 1989).

REFERENCES


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