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The Monoamine-Containing Cell in the Gustatory Epithelium of Some Vertebrates

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Summary. Fluorescence histochemistry has revealed that a specific formaldehyde-induced yellow fluorescence, suggesting the presence of serotonin-like monoamines, was regularly observed in a group of cells (monoamine-containing cells: MC cells) in the normal gustatory epithelia of the carp, catfish, frog and rabbit.

The present report was also concerned with the ultrastructural features of the MC cell in the rabbit's foliate bud. The MC cells were found to have synaptic contacts with the intragemmal nerves. The synaptic morphology showed that the neural transmission may take place from the cell to the nerve. Two types of vesicles were encountered in the cytoplasm of MC cells. One was large cored vesicles (100–150 nm) suggesting storage of the serotonin-like monoamine. They were localized not only at the presynaptic area, but also in the infra- and supra-nuclear cytoplasm. The other was small vesicles (45–80 nm) containing variable amounts of dark material. They were similar in distribution to the large cored vesicles.

The functional significance of the MC cells was discussed.

Since a new cell type containing monoamine was discovered by REUTTER (1971) in the terminal bud of the catfish's barbel, there have been a few reports concerning the existence of this cell type in the gustatory epithelia (DEHAN and GRAZIADEI, 1973; GOOSSENS and VANDENBERGHE, 1974; HIRATA and NADA, 1975; NADA and HIRATA, 1975a). In this paper morphological and functional aspects of the monoamine-containing (MC) cells in the different gustatory epithelia of vertebrate species will be briefly reviewed, along with our recent findings by means of fluorescence and electron microscopy.

1. Fluorescence microscopy

The monoamine fluorescence of the gustatory epithelia is fairly weak and fades out rapidly upon UV-irradiation. It is known that an analogue of serotonin, that is, 5, 6-dihydroxytryptamine (5, 6-DHT), is selectively taken up in the MC cells and temporarily enhances their fluorescence intensity during the first few hours following the drug injection (NADA and HIRATA, 1975b). Thus, the overall morphology of MC cells in several gustatory epithelia can be detected by the use of this drug.

Carp (Fig. 1 a, b, 4): Just one MC cell is situated just above the basal lamina of the terminal bud and six or more cytoplasmic extensions emerge from the cell body, thus forming a characteristic radial pattern. The peripheral ends are considerably dilated and studded with tiny twigs.

Catfish (Fig. 2 a, b, 4): The terminal bud of this animal usually possesses three, but sometimes four, MC cells at the base. They are flat and are oval or polygonal in shape, with short spiny processes. The basal part of the bud is to a large extent occupied by these MC cells. This is revealed clearly in specimens sectioned horizontally through the basal lamina (REUTTER, 1971).

Fig. 1. Fluorescence micrograph of terminal buds on the barbel of the carp pretreated with 5,6-DHT. **a.** Transverse section of the bud. $\times 300$ **b.** Horizontal section through the base of the gustatory epithelium. $\times 600$

Fig. 2. Fluorescence micrograph of terminal buds on the barbel of the catfish pretreated with 5,6-DHT. **a.** Transverse section of the bud. $\times 300$ **b.** Horizontal section through the base of the gustatory epithelium. $\times 600$

Frog (Fig. 4): The MC cells in the fungiform papilla are found just above the basal lamina and their perikarya are always found at the peripheral region of the sensory disc. The cytoplasmic process stretches toward the center of the disc and runs horizontally just above the basal lamina. The process may well be only a direct extension of the perikaryon of a MC cell, as it does not give off any collateral branches. However, the inner end of the MC cell processes is thick and irregularly contoured, showing some evidence of ramification. As a whole, the arrangement of the MC cells in a sensory disc reminds us of the spokes of a wheel (GOOSSENS and VANDENBERGHE, 1974; HIRATA and NADA, 1977).

Fig. 3. Fluorescence micrograph of transverse section of the mouse's vallate papilla pretreated with 5-HTP. $\times 300$

Mouse (Fig. 3): No fluorescent cells can be detected in the vallate bud either of normal animals or of the animal pretreated with nialamide. However, the fluorescent cells are seen only when the animals have been treated with L-dopa (GEERDINK and DRUKKER, 1973) or 5-HTP. They are elongated, spindle in shape and extending from the basal lamina to the taste pore.

Rabbit (Fig. 4): In normal animals the monoamine fluorescence is demonstrated only in the foliate bud, though the fluorescence intensity is

Fig. 4. Schematic drawings of the cytoarchitecture of MC cells (solid black) in gustatory epithelia of some vertebrates. The open circle in the cells represents the nucleus.

very weak (NADA and HIRATA, 1975a). The fluorophore is mainly localized in the infra- and supra-nuclear cytoplasm of the MC cells. Following the administration of 5-HTP, 5-HT or 5, 6-DHT, fluorescent cells appear in the vallate bud, but not in the fungiform bud (NADA and HIRATA, 1976).

2. Electron microscopy

General features: The ultrastructure of the rabbit's foliate bud has been extensively studied by MURRAY and his co-workers (1969). The MC cell is identical with the type III cell designated by them.

The MC cells of the rabbit's foliate bud are spindle-shaped and extend from the basal lamina to the taste pore. Their nuclei are round or oval and often deeply indented. The chromatin is condensed, principally along the inner surface of the nuclear envelope (Fig. 5). The cytoplasm is rich in organelles. Small groups of ribosomes are abundant and scattered throughout the cytoplasm. Tubular elements of rough-surfaced endoplasmic reticulum are present in moderate numbers as are mitochondria. The smooth-surfaced endoplasmic reticulum shows a somewhat dilated and irregular outline (Fig. 7, 9).

Two types of cytoplasmic vesicles: The cytoplasm of MC cells is characterized by the presence of dense-cored vesicles, ranging from 100 to 150 nm in diameter (Fig. 5-9). Their distribution may vary from cell to cell and from one part of a cell to another. These vesicles are mainly localized in the perinuclear cytoplasm and in the vicinity of the synapse between the cell and nerve ending. They have an electron-opaque core separated from the irregular outlined limiting membrane by a relatively translucent halo of variable width. The central cores exhibit a moderate to dense granular appearance (Fig. 8, 10). Numerous studies on amine-containing tissues have implicated dense-cored vesicles as the storing site of amines. The serotonin-like monoamine revealed by fluorescence histochemistry may probably be located in this large type of vesicles. The most outstanding feature of the presynaptic area is the

Fig. 5. Survey view of a taste bud. Three profiles of MC cells (MC), characterized by the presence of electron dense granules in their cytoplasm, are visible in the micrograph. The plexus of unmyelinated nerve fibers can be seen just beneath the basal lamina. $\times 2,900$. **Insert** shows a nerve ending on the perikaryonal region of an MC cell. Large and small types of vesicles are clustered in the vicinity of the presynaptic membrane. The nerve ending of the postsynaptic side contains mitochondria and synaptic vesicles. $\times 20,000$

conspicuous presence of small, relatively clear vesicles (45–80 nm) along with dense-cored vesicles (Fig. 5, 6, 9, 11). These small vesicles are also clustered in the cytoplasm away from the presynaptic membrane (Fig. 9). The membrane of some vesicles stains asymmetrically, with its inner leaflet stained more densely than its outer one. The others contain variable amounts of a dark material and/or a minute dot-like core (Fig. 8, 10, 11). Thus, they are different from synaptic vesicles in the cholinergic nerve terminal which are characterized by a clear core.

These small vesicles have been designated as synaptic vesicles by MURRAY and his co-workers (1969) or synaptic-like vesicles by PARAN and his co-workers (1975). Although MURRAY (1973) in his review speculated that the synaptic vesicles might play a role in the generation of nerve impulses, no definite statement can be made as to the origin and nature of these small vesicles.

Synapse: The intragemmal nerve fibers vary considerably in size and form. The preterminal portions are narrow and have neurotubules, filaments, small mitochondria and synaptic vesicles. The terminal portions are dilated and form bulbous endings with a few or no neurotubules. These nerve endings are commonly observed on the perinuclear and infranuclear regions of MC cells (Fig. 5, 6). A single axon is traced in longitudinal sections for ten or more micrometers to form one or more synaptic contacts with the same MC cell (Fig. 6).

All of the synapses between the MC cells and nerve endings show morphological characteristics of an afferent nature (GRAY, 1959). The two types of cytoplasmic vesicles are located on the presynaptic side in the cytoplasm of the MC cell, while the postsynaptic structures (i.e. bulbous nerve endings) contain only several profiles of synaptic vesicles (Fig. 5, 6, 9). The regions of specialized membrane

Fig. 6. Infranuclear part of an MC cell, which forms synapses with at least three bulbous nerve endings (N_1 , N_2 , N_3). $\times 11,600$

Fig. 7. Supranuclear cytoplasm of an MC cell, occupied by numerous, large cored vesicles. At the supranuclear portion of a sustentacular cell, there are secretory granules which are often found in close association with Golgi apparatus. *MN* MC cell nucleus, *SN* sustentacular cell nucleus, *LY* lysosome. $\times 9,250$

Fig. 8. Supranuclear cytoplasm of an MC cell, showing details of large and small vesicles. Some of small vesicles (arrows) contain more or less dense material, or a dot-like core. *CV* coated vesicle. $\times 50,000$

Fig. 9. Infranuclear cytoplasm of an MC cell and a large nerve ending. Large and small vesicles are abundant in the MC cell cytoplasm. As seen at the middle left margin, small vesicles are often accumulated in an area away from the presynaptic membrane. A coated invagination of the presynaptic membrane is observed (an arrow). Profiles of smooth ER are somewhat irregular. $\times 21,300$

Fig. 10. Details of small vesicles which are clustered at the MC cell cytoplasm in an area away from the presynaptic membrane. The majority of vesicles have varying amounts of dense material, especially along the inner leaflet of the limiting membrane. Some vesicles (arrows) contain considerable amounts of dense material. $\times 77,500$

Fig. 11. Details of the synaptic contact between an MC cell and a nerve ending. Many small vesicles are confined to the presynaptic membrane of the MC cell. Some of them are filled with dense material (arrows), while others are devoid of visible content. Several clear synaptic vesicles are seen dispersed throughout the postsynaptic nerve ending. CV coated vesicle, M mitochondrion.
 × 77,500

contact between the cell and nerve ending are characterized by an increase in membrane thickness on both sides. At the thickened regions the pre- and post-synaptic membranes lie parallel to each other, about 15–20 nm apart. In the clear zone between synaptic membranes there is an intermediate band, showing variation in density along its length (Fig. 11).

3. Function of the MC cells

There are several conflicting reports on the chemical nature of the transmitter involved in the impulse transmission from taste cells to sensory nerve endings. On the basis of the physiological experiments on taste buds, two kinds of chemical substances have been proposed as the transmitter. One is acetylcholine (Ach) and the other noradrenaline. LANDGREN and his co-workers (1954) recorded the response of taste receptors to gustatory stimuli from the lingual nerve following application of Ach, cholinesterase inhibitors and the related compounds of Ach to the surface of the frog's tongue. Ach in appropriate doses increased the sensitivity to tap water and sodium chloride, and similar effects were obtained with prostigmine, DFP and succinylcholine. These findings were interpreted as an indication that Ach is related to the initiation of impulses in taste receptor. This idea was supported by SAKAI (1965), who observed

that the gustatory effect of sour and salty substances was enhanced remarkably by inhibition of cholinesterase. On the other hand, DUNCAN (1964) questioned this role of Ach in taste receptors, because the effect of cholinesterase inhibitors was obtainable only by a very high concentration of drugs. Recently, MORIMOTO and SATO (1975) stated that noradrenaline is a most likely transmitter involved in the taste cell to sensory nerve terminal transmission in frog's taste buds, since the impulse discharges were elicited by perfusion of noradrenaline through the lingual artery. However, the existence of noradrenaline fluorophore has not been demonstrated in the frog's gustatory epithelium by fluorescence histochemistry. Monoamines of gustatory epithelia in the frog and rabbit display yellow fluorescence with an emission maximum at 520 μ m, and hence, are identified as serotonin-like monoamines (HIRATA

and NADA, 1975; NADA and HIRATA, 1975a, b, c, 1976). The same monoamine has been found in the gustatory epithelia of the catfish (REUTTER, 1971) and the carp.

MURRAY and his co-workers (1969) were the first to describe the presence of a granule-containing cell (type III cell) in the foliate bud of the rabbit. They have referred to this cell as being the gustatory cell, since it makes synaptic contact with afferent nerves. In this case, the serotonin-like monoamine may be a good candidate for being the chemical transmitter which takes part in gustatory processes.

The MC cells other than those of the rabbit's foliate bud have not been studied extensively and their function remains enigmatic. REUTTER (1971) mentioned that the MC cell (basal cell) of the catfish's terminal bud is an interneuron interposed between the sensory cell and the afferent nerve. DEHAN and GRAZIADEI (1973) reported that in the frog's fungiform papilla the gustatory cell contains monoamines which may be involved in taste sensation as transmitter substances. This view was formerly supported by our fluorescence histochemical and ultrastructural study of the frog's fungiform papilla (HIRATA and NADA, 1975). However, the MC cells of the frog, as well as those of the carp and catfish, do not have any apical processes reaching the free surface. On the other hand, DÜRING and ANDRES (1975) have referred to this MC cell as being mechanoreceptor like Merkel cell. Our recent results make this contention rather unlikely, since during the early developmental stage of the fungiform papilla the MC cells are located at the surface of the epithelium and have a cytoplasmic extension stretching toward the basal lamina. An elucidation of the ultrastructural details of the frog's fungiform papilla is essential before reaching definitive conclusions as to the function of the MC cell. As for the MC cell of the carp, we have as yet no other available information, except for evidence by electron microscopy that an afferent synapse is found in the taste bud (UGA and HAMA, 1967).

各種動物の味覚上皮におけるモノアミン含有細胞

灘 修身と平田和穂

従来、ナマズ、カエルおよびウサギの味覚上皮内にはセロトニン様のモノアミンを含む細胞が蛍光組織化学的に見出されているが、コイにも同様の細胞が存在することを確認し、さらにこれらの動物についてモノアミン含有細胞の形態ならびに味覚上皮内の配列について比較検討を行なった。

またウサギの葉状乳頭味蕾を電子顕微鏡的に観察し、次の所見を得た。(1)モノアミン含有細胞は味蕾内の神経との間に求心性のシナプスを形成している。(2)細胞質にはこの細胞に特有な2種類の小胞が存在する。(3)その一つはモノアミンの貯蔵が示唆される大型の有芯小胞(100-150nm)であり、シナプス前域のみならず核上部、核下部にも多数観察される。他は暗調物質を少量に含む小型小胞(45-80nm)であり、その分布は前者のそれに類似している。

さらに諸種動物の味覚上皮内にみられるモノアミン含有細胞の機能的役割について考察を行なった。

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