Abstract

Goldfish retina grows throughout the animal's life by adding new cells of all types from multipotent progenitor cells that are clustered at the peripheral edge -- the "marginal region" -- of the retina. Therefore, cells in the peripheral retina are spatially ordered with respect to cellular development, multipotent progenitor cells being most peripheral and differentiated retinal cells being most central. I took advantage of this spatial arrangement of progenitor and mature cells at the peripheral retina, to investigate the morphological and functional differentiation of retinal neurons. For this purpose, I have developed living slice preparations of the peripheral retina of adult goldfish. The peripheral retina was divided into three broad regions ("marginal", "intermediate" and "mature") on the basis of their morphological development. In this paper, I investigated two main problems in this developing system, electrical membrane properties of marginal progenitor cells, and appearance of voltage-gated Na^+ channels as a possible marker of ganglion cells. Whole-cell patch-clamp recordings were performed in ruptured-patch mode. Cells from which currents were recorded were identified by Lucifer Yellow and/or biocytin.

All progenitor cells examined (n = 37) were slender shape. When cells were voltage-clamped near resting potential, and when K^+ and Ca^{2+} channels in the cell membrane were suppressed, large amounts of passively flowing currents were recorded at both hyperpolarizing or depolarizing test potentials. They did not exhibit any voltage-gated Na^+ currents. In 13 out of 37 cells, passively flowing currents declined in a voltage- and time-dependent manner, while the current in remaining 24 cells did not exhibit voltage dependency. The passively flowing current was suppressed by a gap junction channel blocker, halothane, and was not detected in mechanically isolated progenitor cells (n = 7). Taking account of all these results, it is possible to consider that the current is gap junctional current that is driven by

potential difference between the clamped cell and its neighbors. A decrease in extracellular pH reduced gap junctional currents and its increase enhanced them. Dopamine, cAMP and retinoic acid did not influence coupling currents. Injection of biocytin into single progenitor cells revealed strong tracer coupling which was restricted in the marginal region.

Intermediate cells closely located to the retinal margin, facing the vitreal side of the retina, exhibited voltage-gated Na^+ currents (n = 17). They did not reveal apparent tracer coupling. Morphology of these "intermediate cells" was round in shape and some of them had axon-like process which ran along the vitreal surface. Intermediate cells adjacent to the marginal region tended to have smaller Na^+ currents than intermediate cells closer to the mature region. On average, the maximum Na^+ current amplitude recorded from intermediate cells, that is, immature ganglion cells (257 pA) was roughly 6-fold smaller than that of mature ganglion cells (1,621 pA). In addition, the activation threshold of the Na^+ current in intermediate cells was nearly 14 mV more positive than that of mature ganglion cells. Cells located in the intermediate region, but further from the vitreous, exhibited neither voltage-gated Na^+ currents nor gap junctional couplings.

These results suggest that the intermediate cells closely located at the vitreal side may start to differentiate into ganglion cells soon after leaving a cluster of progenitor cells. These results also suggest that the differentiation of progenitor cells into retinal neurons may be controlled by environmental or positional cues. It remains unclear what the environmental factors are, or when and how their effects are exerted.