

I Abstract

The acetylcholine (ACh) system is regarded as one of the important neurotransmitter systems in the vertebrate retina. This system includes ACh as the neurotransmitter, its receptors (AChRs), choline acetyltransferase (ChAT) that catalyzes the synthesis of ACh, and acetylcholinesterase (AChE) that hydrolyzes ACh. In the present study, the existence of a cholinergic system has been demonstrated in the adult newt retina using immunocytochemical and histochemical techniques. Two types of ChAT-ir cells were identified in the mature retina. One cell type has somata closely apposed to the outer aspect of the inner plexiform layer (IPL) where conventional amacrine cells are located. The somata of the other type lie in the outermost region of the ganglion cell layer (GCL) where displaced amacrine cells are located. ChAT-ir conventional amacrine cells had their dendrites at relative depth of 0-15% within the IPL (defining the INL/IPL border as 0% and the IPL/GCL border as 100%). In contrast, ChAT-ir displaced amacrine cells sent their dendrites to lower-middle level of the IPL (45-60%). There was no ChAT-ir in the outer plexiform layer (OPL).

Three types of AChE-positive somata were distinguished according to their location in the retina. One type has somata close to the IPL where the ChAT-ir conventional amacrine cells are located. The somata a second cell type lie one or half cell body away from the IPL. A third AChE-positive cell type has somata in the GCL at the IPL/GCL border where the ChAT-ir displaced amacrine cells are located. An AChE-positive band occupied approximately 0-60% of the IPL width with an intense AChE-positive layer at a relative depth of 20-40%. The OPL was also AChE-positive.

Muscarinic acetylcholine receptor (mAChR)-ir somata were detected

in the GCL, especially at the most proximal level where ganglion cells are located. mAChR immunoreactivity was also detected within the IPL (at depths of 0-15% and 85-100%) and in the OPL. Three nicotinic acetylcholine receptor (nAChR) markers, α -bungarotoxin, and antibodies against $\alpha 3$ and $\alpha 8$ subunits were used for identifying and localizing nAChRs. These markers stained the IPL diffusely, but none of them stained somata of neurons.

To evaluate whether retinal regeneration follows the same sequence of cellular differentiation steps that are found in retinal development, the time course of appearance and maturation of the cholinergic system in developing and regenerating retinas were examined. ChAT-ir cells (cholinergic neurons) were first detected in the retina just before or at the beginning of the formation of the IPL in both developing and regenerating retinas. Also in both cases, AChE activity first became detectable in somata located at the most proximal level of the retina before the ChAT-ir cells could be detected and well before the IPL developed. During subsequent development and regeneration, the IPL, the OPL and somata close to either side of the IPL became AChE-positive.

The mAChR-immunoreactivity was first detected in somata located at the most proximal level of the retina before the appearance of the cholinergic neurons and well before the period of synaptogenesis in both developing and regenerating retinas. Interestingly, during subsequent development and regeneration, somata in the horizontal cell layer transiently became the mAChR-ir. nAChR-immunoreactivity was first detectable as IPL started to form. During subsequent development and regeneration, the nAChR-ir band became thicker in width and occupied the whole thickness of the IPL. None of three nAChR markers stained any somata of retinal neurons during

development and regeneration.

The fact that the time courses of the appearance of ChAT, AChE activity, and AChRs were similar during regeneration and development would suggest that common mechanisms may control both processes in the newt retina.

However, the localizations of ChAT, AChE, mAChRs and nAChRs were not exactly the same in the IPL in mature, developing and regenerating retinas. Furthermore, AChE activity and AChRs appeared before the appearance of cholinergic neurons and well before the period of synaptogenesis in both developing and regenerating retinas. These results would suggest possible non-cholinergic roles of AChE and mAChRs in the retina.