II General introduction

1 Development and cellular organization of the retina

The vertebrate retina, like other regions of the central nervous system (CNS), developmentally derives from the neural tube at the end of the neurular stage of embryogenesis (Fig. 1A). In early embryonic life, the neural tube evaginates to form two optic vesicles in the parts of the presumptive interbrain. Each optic vesicle subsequently invaginates to form an optic cup. Initially, the optic cup is composed of the two walls of neuroepithelium (Fig. 1B). The outer wall of the optic cup differentiates into a monolayered retinal pigment epithelium (RPE) that lines the back of the neural retina. The neuroepithelial cells on the inner wall of the optic cup proliferate actively and become a multi-layered neural retina (Fig. 1C). A schematic diagram of the horizontal view of the adult vertebrate eye is shown in Figure 1D. The eyes of all vertebrates consist essentially of three layers. The outermost layer is a protective tissue, termed the sclera that is transparent in front (cornea). The middle coat is a highly pigmented layer, termed the choroid, which consists of a dense meshwork of blood vessels and other supportive tissues. The anterior side of the choroid becomes a pigmented epithelial layer, termed the iris. The pupil is the hole in the iris. The innermost layer is composed of the retinal pigment epithelium, and the neural retina which is transparent except for the photopigments in the photoreceptor cells.

RPE cells not only play a crucial role in retinal ionic homeostasis and transport of nutrients, as glial cells also do, but also phagocytoze the shed photoreceptor outer segment discs and reduce possible scatter of stray light (for reviews, see Zinn and Marmor, 1979; Dowling, 1987;

Tombran-Tink et al.,1992). The neural retina is specialized for the absorption and transduction of light energy from the visual image of the environment, and for the generation and integration of neural responses (for reviews, see Dowling, 1987; Rodieck, 1998).

The adult vertebrate retina shows a laminar organization comparable to that of the cerebral cortex of the brain (Ramòn y Cajal, 1892; Dowling, 1987; Rodieck, 1998). All vertebrate retinas consist of at least five basic types of neuron (photoreceptor cells, bipolar cells, horizontal cells, amacrine cells, and ganglion cells) and non-neuronal glial cells (Fig. 2). The constituent cells are arranged in a penta-laminar array; three nuclear and two synaptic layers. The outer nuclear layer (ONL) contains the somata of photoreceptor cells. Photoreceptor cells absorb light energy by their outer segments, convert this to an electrophysiological response and transmit the visual signals to second-order neurons, bipolar and horizontal cells. The region of the synapses formed by photoreceptor, bipolar and horizontal cells is the first synaptic zone of the retina, termed the outer plexiform layer (OPL). The inner nuclear layer (INL) contains the somata of bipolar, horizontal and amacrine cells. The bipolar cells receive input from photoreceptor cells and transmit electrical signals to ganglion cells whose somata are located in the ganglion cell layer (GCL). The region of the synapses between bipolar, amacrine and ganglion cells is the second synaptic zone, termed inner plexiform layer (IPL). Ganglion cells integrate the different aspects of the visual information, encode them as frequencies of action potentials, and transmit them into the brain via the optic nerve that is composed of ganglion cell axons.

2 Regeneration of the retina

Most vertebrates can regenerate their neural retina following damage of the original retina. However, there is considerable variability in this capacity among species. In birds and mammals, for example, retinal regeneration is restricted to early embryonic life (Stroeva, 1960; Coulombre and Coulombre, 1965, 1970; Machemer and Norton, 1968; Anderson et al., 1981; Fisher et al., 1991). In anuran amphibians, this ability persists up to metamorphosis (Lopashov and Sologub, 1972; Reh et al., 1987). On the other hand, certain species of fish and urodele amphibians retain the ability to regenerate a functional retina even in adult life. In adult goldfish, for example, two cellular sources of the regenerated retina have been described: (1) Intrinsic progenitor cells existing at the retinal margin; and (2) progenitor cells within the ONL, termed rod precursor cells (Raymond and Rivlin, 1987; Raymond et al., 1988; for review, see Hitchcock and Raymond, 1992). Following surgical removal of the original retina, progenitor cells remaining at the retinal margin give rise to new retinal cells that migrate to their appropriate positions, differentiate into various retinal neurons and restore the neural circuitry to some extent. However, this is not true regeneration, because the same cells provide for normal growth of the retina throughout life (Johns, 1977; Negishi et al., 1990). The rod precursor cells give rise exclusively to new rod photoreceptor cells during normal retinal growth (Sandy and Blaxter, 1980; Johns and Fernald, 1981; Johns, 1982). When the retina is damaged, they proliferate actively and produce all classes of retinal neurons and finally give rise to the regenerated retina (Raymond and Rivlin, 1987; Raymond et al., 1988; for review, see Hitchcock and Raymond, 1992).

The process of retinal regeneration in adult newts and salamanders has been well studied morphologically (Wachs, 1920; Stone, 1950a, b; Hasegawa, 1958). These investigations have put forward some conflicting theories of the cellular sources of retinal regeneration involving either RPE cells or intrinsic progenitor cells at the retinal margin. From electron microscopic analysis coupled with autoradiography in adult newts (Keefe, 1973a, b), the consensus of most investigators now is that the central part of the neural retina is regenerated mainly from RPE cells, whilst the peripheral part is regenerated from progenitor cells existing at the retinal margin.

Retinal regeneration in the vertebrate eye has been reviewed by Reyer (1977), Hitchcock and Raymond (1992), Mitashov (1996), Raymond and Hitchcock (1997).

3 Neurochemical differentiation of the retina

Our understanding of retinal development and regeneration includes studies of appearance and maturation of chemical transmission of signals at synapses (neurotransmitter/receptor systems). Neurons can communicate with target cells through the release of neurotransmitters. Neurotransmitters that are released from the presynaptic cells act on postsynaptic cells (possibly by directly altering membrane permeability to one or more ions) and produce depolarizing or hyperpolarizing voltage changes in the postsynaptic cell membrane (Fig. 3). In the CNS, two main classes of synaptic pathways are generally recognized because neurotransmitters released from presynaptic terminals at chemical synapses can generate excitatory or inhibitory effects (for reviews, see Scheller and Hall, 1992). The main excitatory interactions appear to be mediated

principally by two neurotransmitters: An amino acid (probably L-glutamate) and acetylcholine. In the vertebrate retina, L-glutamate has been identified at the terminals of photoreceptor cells and bipolar cells; acetylcholine has been identified in a subpopulation of amacrine cells (cholinergic amacrine cells). The inhibitory pathways in the retina appear to be mediated mainly by GABA (γ-aminobutyric acid) and by glycine. GABA has been identified at the terminals in horizontal cells (GABAergic horizontal cells) and a subpopulation of amacrine cells (GABAergic amacrine cells), and glycine has been identified in a subpopulation of amacrine cells (glycinergic amacrine cells) (Vaughn et al., 1981; Ball, 1987; Yang and Yazulla, 1988; Araki and Kimura, 1991; Pourcho and Owczarzak, 1991).

It is important to study what types of neurotransmitters and their receptors are first expressed during development and regeneration, and whether the maturation of one neurotransmitter system is linked to another. Recently, it has been reported that neurotransmitters affect a diversity of developmental processes long before they mediate synaptic transmission in the mature nervous system. For example, L-glutamate may regulate the survival of neuronal cells and their patterns of neurite outgrowth in cell culture, or may promote synaptogenesis during development in vivo (for reviews, see Mattson, 1988; Hanley, 1989; Lipton and Kater, 1989; McDonald and Johnston, 1990; Lauder, 1993).

4 Aims of the present study

As described above, adult newts possess the ability to regenerate a functional retina following the complete removal or destruction of the original retina. My overall research interest is to understand the

process of retinal regeneration from a functional perspective, including genesis of neurotransmitter systems, and of voltage- or ligand-gated ion channels. The acetylcholine system (cholinergic system) is one of the important neurotransmitter systems in the vertebrate retina. However, the cholinergic system of the adult newt retina has not previously been systematically investigated. In the present study, I have investigated first the organization of the cholinergic system in the mature newt retina using immunocytochemistry and histochemistry (Chapter I). Second, on the basis of the results of Chapter I, I have investigated the time course of the appearance and maturation of the cholinergic system during retinal development (Chapter II). Third, I have investigated the same aspects of the cholinergic system during retinal regeneration (Chapter III). In the general discussion, I have compared the appearance and maturation of the cholinergic system during regeneration with those of the cholinergic system during development.

The results obtained indicate 1) that the cholinergic system is present in the adult newt retina, and 2) that acetylcholinesterase (hydrolyzing enzyme of acetylcholine) and muscarinic acetylcholine receptors appear earlier than choline acetyltransferase (catalyzing the synthesis of acetylcholine) during development and regeneration. On the basis of the latter point, I further discuss whether genesis of the cholinergic system during regeneration has the same time-course as that of development, and whether acetylcholinesterase and muscarinic acetylcholine receptors play functions which are unrelated to the establishment of cholinergic neurotransmission during retinal development and regeneration.