Characterization and isolation of mammalian retinal neurons

NEVILLE N. OSBORNE* and VOLKER NEUHOFF†


In mammalian retina the following groups of neurons are to be found: ganglion, amacrine, horizontal, bipolar, photoreceptor and interplexiform cells. Within each class of cells there is a great deal of heterogeneity, which Ramon y Cajal had recognized as far back as 1893. Initial morphological studies, later substantiated by the electrophysiologists, proved beyond doubt that there are subdivisions within each class of neuron. For example, within the cat retina at least three different types of ganglion cells (Boycott & Wässle, 1974), five different types of bipolar cells (McGuire et al., 1980) and 12 types of amacrine cells (Cajal, 1893) have been described. The subdivision of neuronal groups within the amacrine cells, in particular, has been extensively studied in recent years because of the introduction of immunohistochemical procedures to localize neurotransmitter-like substances (Osborne, 1982; Stell et al., 1981). Most retinal neurons (e.g. ganglion cells), except certain amacrine cells, do not stain positively for the battery of immunohistochemical procedures at present available to localize known neurotransmitter substances. One major finding from recent immunohistochemical studies is that no correlation exists

Fig. 1. Localisation of Thy-1 antigen by immunofluorescence on a cryostat section of adult rat retina (A), in a freshly dissociated retinal preparation (B) and in a fraction of the dissociated retinal cells recovered from a 30–40% metrazamide interface (E)

In (A) the arrow points to a ring of fluorescence encircling a large cell body in the ganglion cell layer (gc) and a complete lack of fluorescence is associated with the outer plexiform (op), outer nuclear (on) and inner nuclear (in) layers; moderate fluorescence is in the inner plexiform layer (ip) and brightly labelled bundles of axons in the ganglion cell layer (gc). A control section (B) is devoid of fluorescence. By comparing (C) and (E) with their respective phase-contrast pictures, (D) and (F), it can be seen that the fractionation of dissociated cells on a metrazamide gradient does increase the yield of Thy-1-positive cells (from Beale & Osborne, 1982; and Beale et al., 1983).
Fig. 2. Neurons in rabbit retinal tissue and in neonatal rabbit retinal cultures take up exogenous 5-hydroxytryptamine

(A) is a retinal section previously incubated in 0.1 μM 5-hydroxytryptamine and processed for the localization of the amine by immunofluorescence histochemistry. The 5-hydroxytryptamine is associated with amacrine perikarya (arrows) in the inner nuclear layer (in) and processes of these cells in the inner plexiform layer (ip). Retinal tissue only processed by immunofluorescence histochemistry did not reveal the presence of endogenous 5-hydroxytryptamine (B). Specific neurons in neonatal rabbit retinal cultures (C) accumulate exogenous 5-^3^H-hydroxytryptamine as revealed by autoradiography.

Other abbreviation used: gc, ganglion cell layer.

between the structure of amacrine neurons in different species and the transmitter (e.g. 5-hydroxytryptamine) they contain (see, e.g., Ehinger 1982; Osborne et al., 1982).

The characterization of retinal cell types in culture does offer the possibility of isolating specific neurons for subsequent biochemical studies. In recent years we have been using this technique in an attempt to isolate rat retinal ganglion cells (Beale & Osborne, 1982; Beale et al., 1983). The nature of the neurotransmitter(s) in mammalian ganglion cells is unknown and if populations of these neurons can be isolated and analysed valuable data might come to light. Rat brain Thy-1 antigen is a plasma membrane glycoprotein of mol. wt. 17,500 that is widely distributed in the central nervous system (see Williams et al., 1977). Immunofluorescent staining of cryostat sections of rat retina showed the Thy-1 antigen predominantly localized in the inner plexiform and ganglion cell layers (Fig. 1). The distribution would seem to support the idea that Thy-1 is mainly associated with ganglion cell axons, somata and dendrites. The optic nerve is Thy-1 positive (Morris & Ritter, 1980). When a similar immunofluorescence assay for Thy-1 was applied to living cells dissociated from 8 day-old retinas, a small percentage (0.3–0.6%) of cells was brightly labelled over their
surfaces (Fig. 1). Most cells were not labelled and could be compared with controls. The morphology of the Thy-1-labelled cells in culture was quite distinctive: they are the largest, with an eccentrically placed nucleus. Furthermore these cells do not accumulate [3H]glucose, [3H]GABA or D-[3H]aspartate as do other cells in the culture (see Beale & Osborne 1983). We therefore conclude that Thy-1 antigen is restricted to rat ganglion cells in culture as it appears to be in intact tissue. Thy-1 antibody was subsequently used as a probe for obtaining ‘enriched’ populations of ganglion cells. Cells dissociated from neonatal and adult rat retinas were separated by density-gradient centrifugation. The proportion of Thy-1-positive neonatal cells was increased from about 0.4% in the initial dissociates to about 8% in the most enriched fraction of a Percoll step gradient. Among adult cells initial 0.7% Thy-1-positive cells were increased to roughly 4% in the best fraction of a metrizamide step gradient. The presence of relatively large numbers of Thy-1-positive cells in other fractions suggests that it would be difficult to increase the proportion of rat ganglion cells (either neonatal or adult) by methods based on their sedimentation properties. We have, in fact, used a variety of other gradients in an attempt to increase yields, but to no avail. The degree of enrichment resulting could, nevertheless, be a useful preliminary step to other immunoselection procedures such as fluorescence-activated cell sorting, panning (Wysocki & Sati, 1978) or cell selection using antibody-coated magnetic beads (Meier et al., 1982), all of which also depend on cell-type specific antibodies.

One of the main arguments against 5-hydroxytryptamine being a transmitter in the mammalian retina is the fact that present procedures are unable to show that the endogenous amine is restricted to specific retinal neurons (Osborne, 1982; Ehinger, 1982). When rabbit retinas are incubated with exogenous 5-hydroxytryptamine (0.1 μM) at 37°C and then processed by immunohistochemistry to localize 5-hydroxytryptamine, specific amacrine neurons can be seen to have taken up the exogenous amine (Fig. 2). Clearly, if it is possible to isolate these ‘5-hydroxytryptamine accumulating cells’, then it would be possible to design experiments to establish whether these cells contain endogenous 5-hydroxytryptamine. Specific neurons from neonatal rabbit retinal cultures also take up exogenous 5-[3H]hydroxytryptamine (Fig. 2) and these cells can be distinguished from other neurons, which have the capacity to take up 3H[glucose, 3H]adiponate, 3H[GABA or 3H]aspartate. An enriched population (3-fold increase) of the 5H[hydroxytryptamine-accumulating cells from cultures using a Percoll step gradient (see Schaeffer, 1982) has been produced. It is still important, however, to improve on this enrichment before the cells can be analysed for meaningful data on their endogenous 5-hydroxytryptamine content. We propose to use [3H]dansyl(5-dimethylaminonaphthalene-1-sulphonylchloride (Ehinger, 1974) to determine simultaneously the total and 3H-labelled amount of 5-hydroxytryptamine in the different cell populations of a cell culture fractionated on a Percoll gradient. It may then be possible to establish whether mammalian retinal neurons that take up exogenous 5-hydroxytryptamine also contain low amounts of the amine. It has been suggested that neurons in the mammalian retina which take up exogenous 5-hydroxytryptamine actually do not contain the amine but utilize another chemical as a neurotransmitter (Ehinger & Floren, 1980; Floren & Hansson, 1980).

In summary, histological and electrophysiological studies have clearly confirmed the heterogeneity of neuron types in the mammalian retina. Although immunohistological studies have facilitated the chemical characterization of certain retinal neurons in intact tissues, the data are limited and restricted primarily to the amacrine cells. Attempts have been made to isolate specific groups of cells, e.g. ganglion cells, for biochemical analysis. Unfortunately, the populations of neurons so far produced are not of a purity sufficient for chemical analysis.

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Transmitter interactions in the rabbit retina

MICHAEL NEAL
Department of Pharmacology, School of Pharmacy, University of London, London WC1 IAX, U.K.

In the rabbit retina ACh* is a transmitter substance released from a small subpopulation of amacrine cells. About half of these cholinergic cells are located in the inner part of the inner nuclear layer and are believed to ramify in sublamina-A of the inner plexiform layer. The other half of these cells are displaced amacrine cells and ramify in sublamina-B.

In our experiments, we have loaded the cholinergic amacrine cells with 1H[Choline and measured the release of 1H[ACh from the retinal eye-cup preparation as described previously (Cunningham & Neal, 1983). We have used the light-evoked release of 1H[ACh as an indication of the activity of the cholinergic amacrine cells and have studied the effects on these cells of other putative transmitter substances and related drugs (for review on cholinergic mechanisms in the retina, see Neal, 1983).

Inhibitory amino acids

There is much evidence that GABA and glycine are both important inhibitory transmitters in the inner plexiform layer of the rabbit retina, being released from separate populations of amacrine cells. Both substances when applied locally to the retina reduced the light-evoked release of 1H[ACh. The effects of glycine and GABA were selectively blocked by strychnine and bicuculline respectively. Strychnine itself had no effect on