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 \([\text{3H}]\) glycine, \([\text{1H}]\) GABA or \([\text{3H}]\) 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 & Satí, 1976) 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 \(\mu\)M) 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-[\text{3H}]hydroxytryptamine (Fig. 2) and these cells can be distinguished from other neurons, which have the capacity to take up \([\text{3H}]\) glycine, \([\text{3H}]\) dopaminine, \([\text{3H}]\) GABA or \([\text{3H}]\) aspartate. An enriched population (3-fold increase) of the 5-[\text{3H}]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 \([\text{14C}]\) dansyl(5-dimethylaminonaphthalene-1-sulphonyl)chloride (Neal, Osborne 1974) to determine simultaneously the total and \([\text{3H}]\)-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

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In the rabbit retina \(\text{ACH}^*\) 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 sublaminar-A of the inner plexiform layer. The other half of these cells are displaced amacrine cells and ramify in sublaminar-B.

In our experiments, we have loaded the cholinergic amacrine cells with \([\text{1H}]\)choline and measured the release of \([\text{1H}]\)ACH from the retinal eye-cup preparation as described previously (Cunningham & Neal, 1983). We have used the light-evoked release of \([\text{1H}]\)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 \([\text{1H}]\)ACH. The effects of glycine and GABA were selectively blocked by strychnine and bicuculline respectively. Strychnine itself had no effect on 1983.
Fig. 1. Effect of aspartate (a), glutamate (b), quisqualate (c) and kainate (d) on the light-evoked (●) and spontaneous resting (□) release of $[^1]H$ACh and on the b-wave of the electroretinogram (□).

The results are expressed as the percentage of control values obtained in the absence of drugs and are means ± S.E.M. of four separate rabbit experiments.

Fig. 2. Diagram showing possible sites of transmitter and drug interaction with cholinergic neurons in the retina.

In the dark, transmitter (Glu/Asp?) is released continuously from the photoreceptors resulting in hyperpolarization of the depolarizing bipolar cells (DPBC) and depolarization of the hyperpolarizing bipolar cells (HPBC). Light reduces photoreceptor transmitter release and thus causes depolarization of DPBC and hyperpolarization of the HPBC. APB mimics the photoreceptor transmitter, hyperpolarizing the DPBC. This abolishes the electroretinogram b-wave and the light-evoked release of $[^1]H$ACh. The cholinergic amacrine cells probably possess receptors for GABA and glycine and a tonic GABAergic input resulting from afferent impulses from a HPBC is shown. The glycineergic feedback system cannot be as simple as the one illustrated because strychnine does not have the same effect on ACh release as atropine. Inputs to only the displaced cholinergic amacrine cells are shown.
ACh release but bicuculline markedly increased the spontaneous resting release, suggesting a tonic GABA-ergic input to the cholinergic amacrine cells (Cunningham & Neal, 1983).

The GABA analogues muscimol and 3-APS also reduced the light-evoked release of ACh but were several thousand times more potent than GABA itself. The remarkably high relative potencies of the analogues might be due to the uptake processes for GABA in the retina.

**Cholinergic drugs**

In other areas of the central nervous system, atropine increases the release of ACh. This effect is believed to be caused by the block of inhibitory feedback systems. However, there has been much discussion on whether the feedback system involves recurrent neuronal loops or ‘autoreceptors’ present on the cholinergic cells themselves.

In the retina we have found that atropine in the presence of eserine doubles the light-evoked release of ACh. Conversely, muscarine reduces the evoked release of ACh by at least 50%, an effect blocked by atropine. The nicotinic antagonists hexamethonium, pentidine and gallamine have no effect on ACh release. Thus the cholinergic amacrine cells appear to possess an inhibitory feedback system similar to that described in other areas of the brain. However, this retinal feedback system apparently does not involve muscarinic autoreceptors, since the actions of both atropine and muscarine are abolished by strychnine. This result implies that the feedback system involves an inhibitory loop containing a glycine-ergic amacrine cell (Cunningham et al., 1983).

**Excitatory amino acids**

There is increasing evidence that glutamate and/or aspartate are the excitatory transmitters released from the photoreceptors and probably from some bipolar cells as well. Slaughter & Miller (1981) found that APB, an excitatory amino acid antagonist in the spinal cord and cerebral cortex, mimicked the action of the photoreceptor transmitter on depolarizing bipolar cells of the mudpuppy. We confirmed this in the rabbit retina and found that APB almost abolished the light-evoked release of ACh. This result indicated that under our experimental conditions the bipolar cell input to the cholinergic amacrine cells involved mainly the depolarizing rather than the hyperpolarizing bipolar cells. The suggestion that APB was mimicking the photoreceptor transmitter rather than acting as an antagonist was supported by structure-activity studies. These revealed that the L(+)-isomer of APB was much more potent than the D(−)-form and that APB was more potent that other compounds in the same series, such as APV (Neal et al., 1981). The effects of glutamate, aspartate, quisqualate and kainate on the spontaneous resting release and light-evoked release of ACh are shown in Fig. 1. The main effects of all these compounds were a progressive decrease in the light-evoked release of ACh coupled with an increase in the resting release.

It is impossible in these experiments to determine the exact sites of action but as the electrotetrogram b-wave was not significantly reduced it is probable that the drugs act directly on the cholinergic amacrine cells. Kainate was unusual in that at low concentrations it actually increased the light-evoked ACh release.

The antagonists PDA and DGG abolished the light-evoked release of ACh and also the effects of kainate on the resting release. Quisqualate was not antagonized by these drugs. Glutamate diethyl ester had no effect on the retina. Since PDA did not affect the b-wave it must block the light-evoked ACh release by antagonizing the transmitter released from the depolarizing bipolar cells, a conclusion supported by recent electrophysiological findings in the mudpuppy retina (Slaughter & Miller, 1983).

A diagram of the cholinergic amacrine cell and some possible inputs deduced from the results outlined above is shown in Fig. 2.

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**Physiology of neuroactive peptides in vertebrate retina**

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**Introduction**

The number of putative transmitters in the central nervous system, including the retina, has increased substantially in recent years with the discovery of neuroactive peptides (for reviews, see, e.g., Hökfelt et al., 1980; Karten & Brecha, 1982). In vertebrate retinæ, peptides are almost exclusively contained in amacrine cells, a type of interneuron that receives synaptic inputs from bipolar, interplexiform and other amacrine cells, and whose outputs are directed to bipolar, interplexiform, other amacrine and ganglion cells (Dowling and Werblin, 1969; Dowling & Ehinger, 1975; Karten & Brecha, 1982; Stell et al., 1980). Retinal amacrine cells appear to contain peptides in a mutually exclusive manner, and also lack co-existence with conventional transmitters (Karten & Brecha, 1982). The main aim of the present paper is to seek an answer(s) to the question: what is the physiological role of neuroactive peptides in vertebrate retina?

First, we assume that peptides must have some functional role since they are contained so consistently in amacrine cells. A strong possibility is that peptides function as 'chemical messengers' mediating amacrine cell outputs. It is now generally accepted that there are three distinct modes of neuronal communication: neurotransmission (i.e. conventional synaptic transmission), neuromodulation and neurohormonal communication (Barker & Smith, 1980; Osborne, 1981). Neurohormonal communication appears to involve relatively long distances, and since the vertebrate retina, and the inner plexiform layer within it (where amacrine cell interactions take place), are only some 300 μm and 25 μm in thickness respectively in fish, retinal peptides are unlikely to function as neurohormones. Thus amacrine cells may use peptides as transmitters and/or modulators.

**Electrophysiological effects of peptides on ganglion cells**

If amacrine cells use peptides as transmitters/modulators, then exogenously applied peptides should influence the electrophysiological activities of those neurons to which amacrine cells provide synaptic input, i.e. bipolar, interplexiform, other amacrine and ganglion cells. Of the latter, ganglion-cell responses can be recorded extracellularly with relative ease, and consequently a number of studies have been undertaken to test