Neurotransmitters in the Vertebrate Retina

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After several decades of intensive research in many laboratories, it appears that the principal neurotransmitters in the vertebrate retina are all amino acids, namely L-glutamic acid (glutamate), \( \tau \)-aminobutyric acid (GABA), and glycine. Glutamate is the transmitter for most photoreceptors, bipolar cells, and probably ganglion cells.1–4 GABA is used by subpopulations of horizontal cells and amacrine cells, and glycine is the transmitter for certain amacrine cells and interplexiform cells.5,6,8–10 Glutamatergic neurons generally constitute the "center" pathways, responsible for fast, direct signal transmission from photoreceptors to the brain.2,3,5,6 GABAergic and glycinergic neurons, on the other hand, are predominantly responsible for the "surround" pathways, which are involved in mediating the antagonistic surrounds of retinal receptive fields, network adaptation, feedback control of synaptic signals, and motion sensitivity.2,3,6–13 Although these generalizations serve as a useful framework on which to build our understanding of retinal neurotransmission, it should be recognized that there are at least several exceptions, such as GABA-containing bipolar cells and glycinergic All amacrine cells, that may be more involved in the center pathway.

IDENTIFICATION OF NEUROACTIVE SUBSTANCES

The approaches used to match retinal cell types with their neurotransmitters and to examine their mechanisms of action have evolved over time, and they have proven consistent and complementary. The first was based on the hypothesis that neurons using an amino acid as a transmitter possess a selective high-affinity uptake mechanism for clearance of the transmitter. Accordingly, retinas were incubated with different \([H] \)-labeled transmitter candidates, including GABA, glycine, or glutamate, and autoradiography was used to visualize the cells that accumulate these substances.1–3,7 This method has been successful in mapping putative GABAergic and glycinergic neurons and their pathways but not glutamatergic neurons, in part because of strong spatial buffering by glutamate transporters on Müller cells. With the availability of specific antibodies against glutaraldehyde-conjugated glutamate, GABA, or glycine, respectively,2–6,11 or against the enzymes that synthesize GABA (glutamate decarboxylase) or glutamate (aspartate aminotransferase),1 immunocytochemistry was used to explore transmitter-specific cell types and their circuitries. These studies were particularly valuable in demonstrating the glutamatergic natures of photoreceptors, bipolar cells, and ganglion cells.

A number of other conventional transmitters, such as acetylcholine, serotonin, and dopamine, as well as neuroactive peptides, such as enkephalin, somatostatin, neurotensin, glucagon, and vasointestinal peptide have been found in the retina.2–3,14–16 Although some of these substances may act like conventional neurotransmitters in their ability to gate postsynaptic channels, they also may have functions or mechanisms of action more related to cellular modulation, regulation, or metabolism. Moreover, virtually all these transmitters coexist with GABA; they never appear alone.

To compare and contrast the roles played by different neuroactive substances in retinal synaptic transmission, it is proposed that these substances be divided into two classes, with the amino acid transmitters acting as the primary neurotransmitters, and the others as secondary neurotransmitters, for lack of better names. Under this scenario, conventional neurotransmission in the retina would be accomplished largely by glutamate as the fast, excitatory neurotransmitter responsible for vertical or center transmission cascade from photoreceptors to bipolar cells to ganglion cells, whereas GABA and glycine act fast as inhibitory neurotransmitters of horizontal and amacrine cells and form the lateral or surround pathways. Furthermore, as others have previously proposed,2,15 the amino acid transmitters would involve information processing mainly at a direct and proximal or local level. In contrast, the functions of the secondary transmitters might include
the participation or consolidation of neurotransmission at a more global (many soma lengths) or diffused level, a role analogous to that described as neuromodulatory or hormonal; the regulation of neurotransmission indirectly through metabolic pathways, such as second messengers; or the regulation of release, reuptake, or both of the primary transmitters.

Part of the rationale for this classification arises from the discovery that many retinal neurons, particularly amacrine cells, contain an amino acid as the primary transmitter in addition to one or more other neuroactive substances. For instance, certain chicken amacrine cells contain GABA and enkephalin, whereas some contain glycine and enkephalin and yet others contain different combinations of primary and secondary transmitters. Although its functional significance is unknown, these co-localizations greatly increase the number of cell types that can be represented uniquely and neurochemically for a fixed number of neuroactive substances, making it a valuable tool for more refined transmitter-specific circuitry analyses. In this context, we have previously proposed a signature hypothesis, which postulates that within such a class of neurons, each morphologically and physiologically distinct cell typed may be identified uniquely and categorized by the neuroactive substances it contains.

**POSTSYNAPTIC ACTIONS OF NEUROTRANSMITTERS**

The postsynaptic receptors for neurotransmitters represent the second half of the cascade in chemical neurotransmission. At least five global types of glutamate receptors have been discovered in the brain; all of them have been found in retinal synapses. Three of these (kainate, AMPA, and NMDA) are ionotropic receptors, whereas the other two (L-AP4 and ACPD) are metabotropic receptors. It is of interest that each of these pharmacologic varieties exists in further molecular forms with differential ionic or biophysical features. Kainate receptors, AMPA receptors, or both mediate synaptic transmission from photoreceptors to horizontal cells, off-center bipolar cells, and ganglion cells. NMDA receptors are located primarily between bipolar cells and ganglion cells, although they have also been found in catfish horizontal cells. These ionotropic receptors give rise to sign-preserving postsynaptic signals. The L-AP4 receptors, found only in the synapses between photoreceptors and the on-center bipolar cells, mediate a sign-inverting postsynaptic signal. This type of metabotropic receptor is coupled to cyclic guanosine monophosphate (cGMP)-gated cation channels, and the activation of these receptors results in a decrease of cytosolic cGMP and closure of cation channels. ACPD receptors have been found in several types of retinal neurons, but their function in the retina is unknown.

Three types of GABA receptors have been found in the brain and retina. The GABA<sub>A</sub> receptors are coupled with chloride channels, and they can be blocked by bicuculline. This type of GABA receptor has been found in cone photoreceptors, bipolar cells, and ganglion cells, where they probably mediate the feedback synapses between horizontal cells and cones, and they have been found in amacrine-ganglion cell synapses. The GABA<sub>B</sub> receptors are metabotropic and regulate intracellular messengers and neuronal function. These receptors have been found in bipolar cell and photoreceptor terminals as well as in ganglion cells, but their function is unclear. The GABA<sub>C</sub> receptors have been identified and cloned recently. Similar to GABA<sub>A</sub> receptors, they also gate chloride channels but are bicuculline insensitive. GABA<sub>C</sub> receptors have been found in retinal horizontal cells and bipolar cell axon terminals and are involved in mediating GABAAergic synaptic functions in the outer and inner retinas. GABA<sub>B</sub> receptors have different dose–response features and participate over different parts of the inhibitory range.

Glycine gates chloride channels, and its action can be blocked by strychnine. In the retina, glycineergic actions have been observed in horizontal, bipolar, amacrine, and ganglion cells. It is unclear, however, whether all glycineergic synapses are mediated by a single type or multiple types of glycine receptors. In addition to the amino acid receptors, receptors for some of the secondary transmitters have been reported. Among these, the most well understood might be the dopamine and acetylcholine receptors.

**FUTURE DIRECTIONS**

Where does one go from here? In my opinion, this represents the most exciting period for consolidating the massive amount of physiological, anatomic, and neurochemical information gathered during the past four decades into a unified understanding of how, at a molecular level, light and images arriving at the photoreceptors result in the sometimes complex representations we call receptive fields by way of the ganglion cells. In this regard, retinal physiological studies of Kuffler and others have long demonstrated that the receptive fields of certain ganglion cells can be every bit as complex and sophisticated as those for the most advanced visual cortical cells recorded by Hubel, Wiesel, and others. It is, therefore, comforting to know that indeed the retina is not only an approachable, but also a relevant, part of the brain for studying central nervous system function.

Here, then, in no particular order of priority, is
my top 10 list for future studies in retinal neurotransmission. This list is by no means novel; a number of these projects are already well in progress in many laboratories. Rather, it is intended to serve as a guideline, especially for the nonvisual scientists who are thinking about entering this field.

1. Identify the primary and secondary neurotransmitters used by the remaining retinal cell types. It is important, particularly for circuitry analyses, to know the chemical signatures for each retinal cell type.

2. Examine the key ingredients of neurotransmission (for example, transporters, receptors, second messengers) and their regulations at identified single cell levels. In this regard, the retina is superior to the brain; the retina can be dissociated into single, identified, viable cells whose molecular biology can now be studied because of the availability of methodologies such as polymerase chain reaction and monoclonal antibodies.

3. Continue to investigate the neurophysiology and pharmacology of isolated, identified cells or membranes thereof. This still may be the most direct way to study, for example, receptors, transporters, ion channels of a known cell type.

4. Study the influences of external factors (molecules or other cells) on, and their interactions with, a single identified type of cell in transient or established cultures. This may be a productive way of understanding the roles of trophic and growth factors, as well as the mechanisms of cellular interactions, synapse formation, and so on.

5. Use preparations such as retinal slice, isolated retina, eye cup, or in vivo to identify the combined transmitter-specific circuitry and electrophysiological profiles in a single cell. Much information already has been obtained using this technically demanding combination of well-established techniques, such as Golgi, cell type-specific histochemistry, autoradiography, immunocytochemistry, in situ hybridization, dye injection and intracellular recording.

6. Examine the patterns of neuronal activities in the retina under different conditions by means of novel techniques, such as optical imaging or multiple recording arrays. These methods might generate new insights into the way visual images are formed in the retina as well as into the physiological roles played by certain cell populations or neuroactive substances, especially those relating to longer-term phenomena such as adaptation, neuromodulation, and spatial interactions.

7. Use some of the aforesaid techniques to study the functional significance of transmitter coexistence: Does co-localization correlate with co-release or co-transmission, or both? 

8. Use some of the same techniques to study retinal development. It already is known that different transmitters and transmitter-specific properties develop at different times. Development may be one way of selectively isolating certain circuitries or cellular interactions.

9. Use some of these techniques to study retinal regeneration. Most animals, during development or as adults, have the ability to regenerate their retinas, an interesting process that could have clinical significance.

10. Perhaps the most challenging work is for theoretically minded scientists to consolidate all the experimental information into a rigorous working hypothesis on how visual information, including form, intensity, color and motion, might be analyzed and presented.

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