

Neurotransmitter Properties of the Newborn Human Retina

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Human retinal tissue from a newborn was examined autoradiographically for the presence of high-affinity uptake and localization of the following putative neurotransmitters: dopamine, glycine, GABA, aspartate, and glutamate. In addition, the dopamine content of this newborn retina was measured by high pressure liquid chromatography. Our study reveals that specific uptake mechanisms for ^3H -glycine, ^3H -dopamine, and ^3H -GABA are present at birth. However, the number and distribution of cells labeled with each of these ^3H -transmitters are not identical to those observed in adult human retinas. Furthermore, the amount of endogenous dopamine in the newborn retina is approximately $1/20$ the adult level. Photoreceptor-specific uptake of ^3H -glutamate and ^3H -aspartate are not observed. These findings indicate that, while some neurotransmitter-specific properties are present at birth, significant maturation of neurotransmitter systems occurs postnatally. Invest Ophthalmol Vis Sci 24:893-897, 1983

Several amino acids, such as gamma aminobutyric acid (GABA), aspartic acid, glutamic acid, and glycine, as well as the catecholamine, dopamine, have been implicated as possible neurotransmitters in the vertebrate retina. We have recently extended neurotransmitter studies from animal retinas to the adult human retina by documenting the uptake, localization, synthesis, content, and release properties of these compounds.¹⁻³ Integral to these studies is the ability of neurons to take up their specific neurotransmitter from the extracellular compartment via high-affinity uptake systems. By incubating isolated retinas for short intervals in ^3H -transmitter candidates, neurons that use a specific transmitter accumulate that ^3H -transmitter and can later be visualized in standard histologic sections following autoradiography. Here we present new data on the state of differentiation of the high-affinity uptake systems of several neurotransmitter candidates in the newborn human retina, as well as an analysis of dopamine content.

Methods and Results

The pair of eyes used in these studies was obtained through the cooperation of the Lions Eyes of Texas

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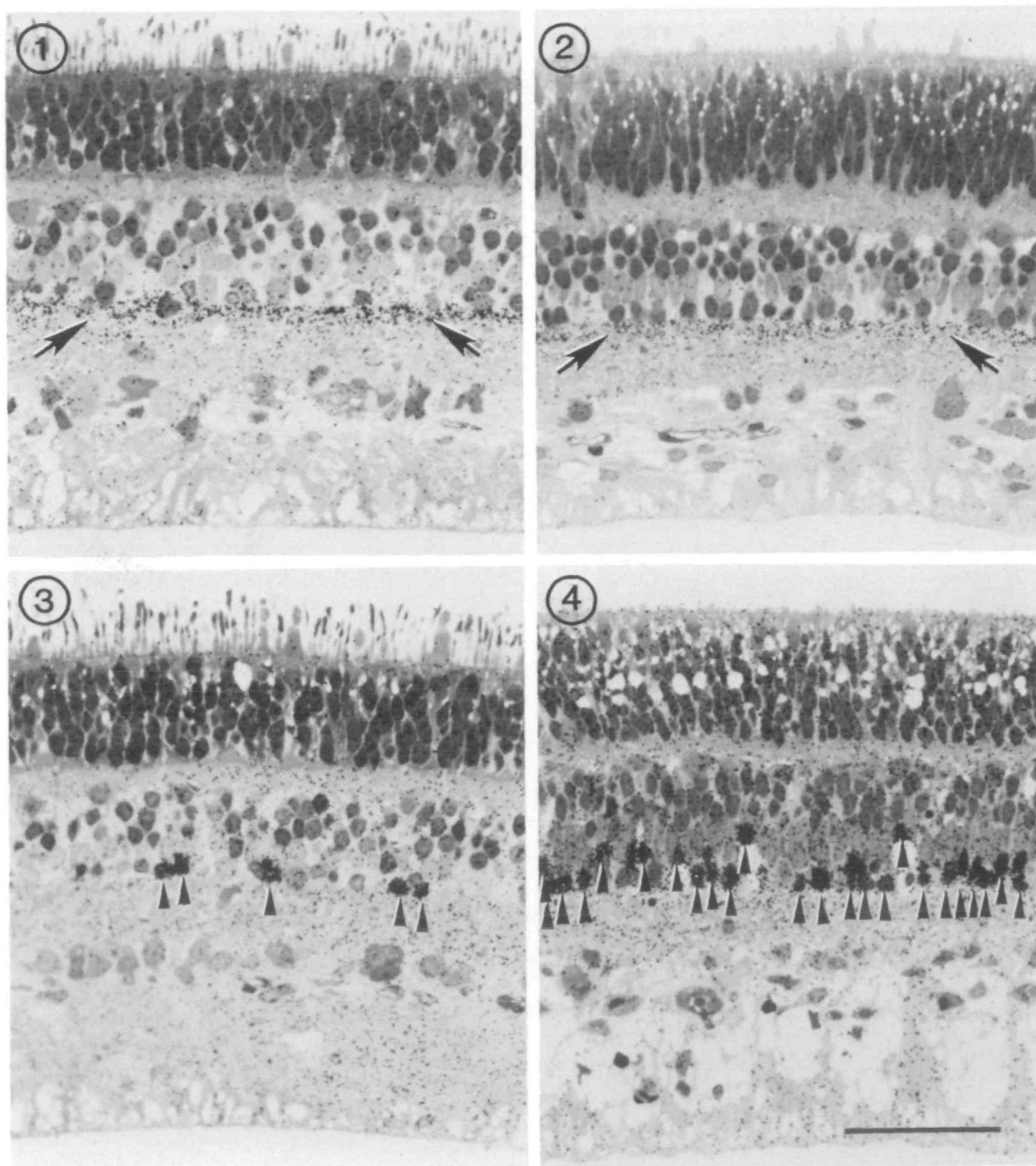
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Eye Bank in the Cullen Eye Institute (Houston). The eyes measured 1.47 cm in diameter and were obtained from a full-term hydrocephalic infant that died shortly after parturition. They were placed on ice following enucleation and reached the laboratory within 20 min after death. The anterior segment was removed and the retina isolated from the vitreous humor and posterior segment. The retinas were cut into small strips along the central to peripheral axis, and these radial strips were further divided so that representative retinal expanses from the center, mid-, and far-periphery could be identified.

Retinal fragments were incubated for 10 min in oxygenated mammalian Ringer's solution, supplemented with 5% fetal calf serum and 5 mM Tris-HCl (pH 7.35), and containing 20 uCi per 400 μl of either L- ^3H -aspartic acid (10.7 Ci/mmol), L- ^3H -glutamic acid (18.8 Ci/mmol), D- ^3H -aspartic acid (16.0 Ci/mmol), ^3H -muscimol (9.3 Ci/mmol), gamma- ^3H -aminobutyric acid (25.0 Ci/mmol), ^3H -glycine (15.0 Ci/mmol), or ^3H -dopamine (33.5 Ci/mmol), all supplied by New England Nuclear. After a brief wash in fresh media, the retinal fragments were fixed in 1% formaldehyde and 2% glutaraldehyde in 58 mM Sorenson's phosphate buffer (pH 7.4). Following dehydration and embedding in Epon, the tissues were prepared for light microscope autoradiography by methods previously described.¹ Autoradiographs were exposed for 4-12 days prior to developing.

The retina showed distinct lamination of outer nuclear, inner nuclear, and ganglion cell layers, separated by interposed neuropile throughout the entire retinal expanse. The fovea contained few cells and was virtually identical in morphology to the newborn

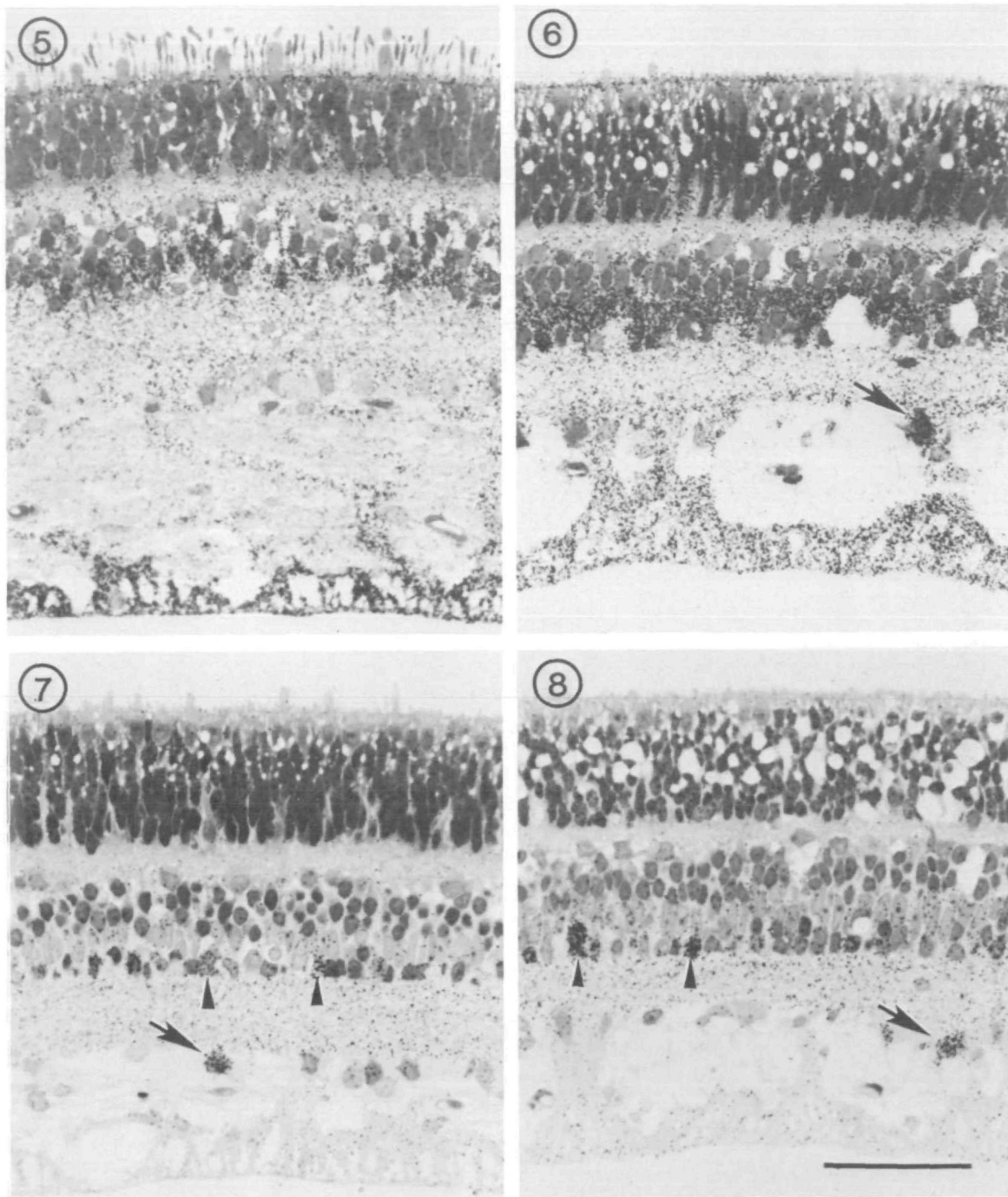


Figs. 1-2. Newborn retina from central (Fig. 1) and from peripheral (Fig. 2) regions incubated with ^3H -dopamine. Note the dense band of radioactivity at the border of the inner nuclear and plexiform layers (arrows). Compare photoreceptor development in Figures 1 and 2. **Figures 3-4.** Newborn retina incubated with ^3H -glycine. Five labeled cell bodies (arrowheads) in the inner nuclear layer are present in the central retina (Fig. 3) whereas over 20 labeled cells are present in the peripheral region (Fig. 4). Bar in lower right represents 50 μm .

fovea recently described.⁴ In extrafoveal regions, the photoreceptor layer contained large numbers of cells that approached adult density. Although retinal stratification was complete throughout the retina, photoreceptor differentiation was more advanced in the central retina than in the periphery (Figs. 1-8).

Retinal fragments incubated with ^3H -dopamine showed a distinct band of radioactivity over the most

distal region of the inner plexiform layer (sublamina 1). Localization of label to this position (Figs. 1-2), is identical to the location of ^3H -dopamine uptake in the inner plexiform layer in the adult. Only one labeled cell body was observed in over 11 mm of retinal expanse examined. Labeled processes of interplexiform cells were not observed traversing the inner nuclear layer nor were punctate terminals observed in



Figs. 5-6. Newborn retina from the central (Fig. 5) and peripheral (Fig. 6) regions incubated with ^3H -GABA. Note extensive labeling of glial cell processes with grain distributions at both the inner and outer limiting membranes. Numerous perikarya are labeled in the inner nuclear layer, and are predominantly situated adjacent to the inner plexiform layer. Arrow points to a labeled perikaryon in the ganglion cell layer. **Figs. 7-8.** Retinas incubated in the presence of ^3H -muscimol. Mid-peripheral (Fig. 7) and far-peripheral (Fig. 8) regions show labeled cells in the inner nuclear layer (arrowheads) and in the ganglion cell layer (arrows). Bars in lower right represents 50 μm . Figures 5-8 photographed and printed to identical magnifications.

the outer plexiform layer. The absence of interplexiform cell labeling with ^3H -dopamine in the newborn differs from our findings in the adult.³

Small fragments of the newborn retina were also extracted and analyzed by HPLC-EC as previously

described³ to determine endogenous dopamine content. The level measured was 0.93 pmol/mg protein, a value significantly lower than the mean value measured in mature retinas (19.0 ± 8.6 pmol/mg protein).

Retinas incubated in the presence of ^3H -glycine

showed uptake of the label by cells located in the inner region of the inner nuclear layer in positions consistent with the location of ^3H -glycine labeled cells in the adult (Figs. 3–4). However, the density of these cells varied from center to periphery. The number of cells in the central retina was 20 ± 1.4 cells per mm linear retinal expanse (mean \pm SD), which is approximately half the number present in the adult,¹ whereas in the peripheral retina, the number of glycine labeling cells was 73 ± 12 , approximately twice the density present in the adult.

Retinal tissues incubated with ^3H -GABA showed extensive uptake in glial processes in all regions, ie, extensive labeling was present from inner to outer limiting membranes (Figs. 5–6). Additionally, large numbers of labeled cells were also present in the inner nuclear layer, and occasionally, labeled cells in the ganglion cell layer were observed (Fig. 7). This pattern of glial uptake of ^3H -GABA has been described in a variety of mammalian species. We had previously incubated human retinas with ^3H -muscimol and found that this analog of GABA was selectively accumulated by GABAergic neurons, but not by glia. Accordingly, we incubated the newborn human retina with ^3H -muscimol and observed the uptake of this GABA analog which is presumed to be exclusively neuronal (Figs. 7–8). ^3H -muscimol-labeled cells were present at the border of the inner nuclear and inner plexiform layer as well as in the ganglion cell layer. The density of the muscimol-labeled cells was 23 ± 9 cells per mm linear retinal expanse and was similar throughout all regions of the retina examined. This density of ^3H -muscimol-labeling in the newborn retina was virtually identical to that seen in adult human retina (22.1 cells per mm linear retinal expanse).¹

Retinas labeled with either D- or L- ^3H -aspartic acid or L- ^3H -glutamic acid showed extensive glial cell uptake in each preparation. Although we have reported that, among photoreceptors, rods selectively accumulate aspartic acid while both rod and cone photoreceptors take up glutamic acid,² extensive photoreceptor labeling was not observed with either ^3H -aspartate or ^3H -glutamate in the newborn retina.

Discussion

These observations indicate that while a number of neurotransmitter specific properties are present at birth, some characteristics of the adult retina have not developed by this time. Our observations with ^3H -dopamine clearly demonstrate the same inner plexiform layer labeling pattern present in adult retina, but fail to demonstrate the interplexiform cell terminals in the outer plexiform layer. In the adult human retina, processes of interplexiform cells ex-

tending to the outer retina take up ^3H -dopamine. Our analysis does not allow us to distinguish whether the interplexiform processes are entirely absent at birth or whether the interplexiform cell uptake properties are only immature at this time. It is also noteworthy that the dopamine content of the newborn retina is only 1/20 that present in the adult.³ A factor to be considered in a comparison of dopamine content between newborn and adult human retinas stems from an observation that tyrosine hydroxylase (TH) is activated by light in the neonatal rat. If TH in the human retina is also light sensitive, the low dopamine content at birth could in part be related to the absence of light stimulation in utero.

Another apparent difference from the adult is the variation in the number of ^3H -glycine-accumulating cells from central to peripheral retina. In the central retina of the newborn, the number of ^3H -glycine-labeling cells is approximately one-half that found in the adult whereas in the periphery the number of these cells is approximately twice the adult density. Postnatal maturation of the human retina may therefore involve redistribution of these glycinergic neurons. If mitotic activity has stopped in the central retina, it might be predicted that the large population of glycinergic neurons in the peripheral retina may contribute to the central glycinergic amacrine cell population. This could be accomplished by lateral migration from peripheral to central locations. Lateral migration of cells from the peripheral germinative zone through the inner nuclear layer has been demonstrated during maturation in the frog retina.⁶

The density of ^3H -muscimol-labeled neurons (GABAergic neurons) is similar to the number present in the adult retina in all regions examined. It should be emphasized that a normal density of GABAergic cells coexist in the newborn retina with populations of glycinergic cells that are below adult levels in the central retina, and above adult levels in the peripheral retina. Because of these differences it is likely that specific translocation of individual cells within the retina must occur in order to achieve the appropriate distribution of cells relative to each other. It is unlikely that simple stretching of the tissue at the periphery to reduce the number of cells per linear expanse would be sufficient to achieve the appropriate cell densities. On the other hand, a combination of stretching of the peripheral retina to reduce the density of glycinergic cells, coupled with the additional proliferation of GABAergic cells to increase their number in the periphery, might be a mechanism by which this could be accomplished. Additional studies that combine an analysis of the mitotic capabilities of the newborn retina along with uptake studies will be required to answer this question.

A recent study reports that the density of foveal

photoreceptors at birth is below the number present in the adult.⁴ Our studies on ³H-aspartate and ³H-glutamate uptake indicate that photoreceptors, foveal and extrafoveal, are immature at birth in that they have not yet developed specific uptake properties. Unlike the adult, photoreceptors in the newborn were relatively free of label, whereas heavy labeling over glial cells was comparable to that previously observed in mature retina.^{1,2}

The exact time during development when the uptake properties for glycine, dopamine, and GABA first emerge must await the analyses of retinas obtained from prenatal stages. We have studied the uptake of these three putative transmitters in a single retina from a 9-week aborted fetus. While diffuse labeling was present throughout this retina, no specific neuronal labeling was evident suggesting the absence of high-affinity uptake properties. On the other hand, studies using material from later (postnatal) times will be required in order to determine when the glutamate and aspartate uptake properties in photoreceptors first appear, as well as to determine the time of maturation of the dopaminergic interplexiform cells.

Key words: retinal development, human retina, newborn retina, autoradiography, neurotransmitters, glycine, dopamine, GABA, aspartate, glutamate

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