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Postnatal Development of Nicotinamide Adenine Dinucleotide Phosphate-Diaphorase-Positive Neurons in the Retina of the Golden Hamster

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ABSTRACT

The histochemical method was used to investigate the postnatal development of nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d)-positive neurons in retinas of the golden hamster. NADPH-d-positive neurons were discernible in the retina at postnatal day (P)1. From P4 onward to adulthood, when the retina acquired its laminated characteristics, NADPH-d-positive neurons were observed in the inner nuclear layer (INL) and the ganglion cell layer (GCL). Results showed that NADPH-d-positive neurons in INL and GCL followed different time courses and patterns in their development. NADPH-d-positive neurons in INL underwent a sharp increase from P4 to P8 (3.6-fold), followed by a decrease to 46% of the maximum at P12. This value was maintained relatively constant to the adult level. The mean diameters of NADPH-d-positive neurons in INL, which were smaller than those in the GCL for all ages, increased from P8 to P12 and from P20 to adulthood. As for neurons in the GCL, the increase in cell number was not so apparent for the earlier postnatal days until P20; thereafter, an obvious increase to the adult level was observed. The mean diameters of the NADPH-d-positive cell bodies in the GCL increased with age, except for P16–P20, during which time there was a slight and insignificant decrease. The tendency of changes in cell density was basically similar to that of the total number for both the INL and the GCL. Between P12 and P20, the density distribution map of the NADPH-d-positive neurons underwent dramatic changes: The highest density shifted from the upper central retina at the earlier postnatal days to the lower central retina in the adult. The two waves of increase in NADPH-d-positive neurons coincide with the process of axonal elongation and synaptogenesis and the acquisition of visual function and experience. It is suggested that these NADPH-d-positive neurons are related to these two developmental events. J. Comp. Neurol. 446:342–348, 2002.

Indexing terms: NADPH diaphorase neurons; nitric oxide synthase; development; displaced amacrine cells; retina; hamster

Over the last 10 years or so, nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d), an oxidative enzyme, has been the focus of intensive investigations, although it was found in neurons 4 decades ago (Thomas and Pearse, 1961). The interest in this enzyme is largely due to the findings that it is involved in a number of physiological (for review see Koistinaho and Sagar, 1995; Garthwaite, 1995; Goldstein et al., 1997; Llomovatte et al., 1997) and pathological (Koistinaho and Sagar, 1995; Huxlin, 1995; Iadecola, 1997) processes. This enzyme was later proved to be identical to nitric oxide synthase (NOS; Koistinaho and Sagar, 1995; Goureau et al., 1997), which produces nitric oxide (NO), a neurotransmitter or a neuronal messenger (Garthwaite, 1995; Koistinaho and Sagar, 1995; Goureau et al., 1997). NOS has also been implicated as playing a role in promoting axonal elongation and reg

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ulating advancement of growth cone (Van-Wagenen and Rehden, 1999; Renteria and Constaintine-Paton, 1999) as well as triggering cell death in the nervous system during development (Gally et al., 1990; Iadecola, 1997).

Among other preparations, the retina has proved to be a good model for investigations to address a number of questions, including development, because of its accessibility and relative ease of experimental manipulation. Research on the expression of NADPH-d in the developing retina of mammals has produced different results concerning the types and distribution of NADPH-d neurons, especially during the developmental period. In the rat, for example, the number of NADPH-d-positive amacrine and displaced amacrine cells increases from postnatal day (P)3 to peak on P11 (Mitrofanis, 1989). By contrast, in kitten retina, NADPH-d-positive neurons peak at P25 (Vaccaro et al., 1991), and, in rabbit, the increase of NADPH-d-positive amacrine cells occurs from postconception days 28 to 50, before declining to the adult value (Mitrofanis et al., 1992). However, Sharma and Perez (1996) reported that the NOS-immunoreactive cells in the rabbit retina appeared at embryonic day 29, and the number continued to increase until P11, when their number stabilized. The present study examined the expression of NADPH-d in the developing retina of hamsters. Because the gestation period of the hamster is relatively short (16 days; see Hsiao, 1984), its eyes and brain are immature for a longer period after birth compared with other rodents. By making comparison with other animals, we may obtain some insights regarding the possible roles of NOS in developing as well as in adult retinas.

MATERIALS AND METHODS

Twenty-four golden hamsters (*Mesocricetus auratus*) of various ages were used in this study. Three animals of each age ranging from the day of birth (P0) to P1, P4, P8, P12, P16, P20, and 2-month-old adults (AD) were sampled at each time point. All procedures carried out in these experiments conformed to the Animals (Control of Experiment) Ordinance (Cap. 340) issued by the Department of Health and the Government of the Hong Kong Special Administrative Region and approved by the University of Hong Kong Committee on the Use of Life Animals in Teaching and Research (CULATR 84-94).

Histology and histochemistry

All preparations of the retina were made at the same time of day to avoid an influence of circadian rhythms or photic state of the animal (Llomovatte et al., 1997). P0 and P1 pups were humanely killed following deep hypothermic anesthesia, whereas animals aged P4 to adulthood were anesthetized with an overdose of Sagatal (sodium pentobarbitone; 100 mg/kg body weight i.p.) prior to death. Under a dissecting microscope, a small incision was made along the line of the prospective eyelid of younger pups or along the lower eyelid of older animals, and the eye, including all pigmented fragments, was carefully dissected out with fine forceps. Both the left and the right retinas were dissected and placed in 2% paraformaldehyde (in 0.1 M phosphate buffer, pH 7.4). The whole retina was separated from vitreous body and pigmented epithelium. Four radial cuts were made in superior, inferior, temporal, and nasal parts of the retina. The retinas were put in the same fixative for 1 hour and then transferred to 0.1 M phosphate buffer (PB; pH 7.4) at 4°C until they were processed for NADPH-d histochemistry. The NADPH-d reaction was performed as described by Lau et al. (1994). In brief, the retinas were incubated in 0.1 M PB containing 0.3% Triton X-100, 0.1 mg/ml nitroblue tetrazolium, and 1 mg/ml b-NADPH at 37°C for 60–90 minutes. After thorough rinsing in PB, the left retinas were flat mounted onto chrome-alum-coated slides, air dried, dehydrated, and coverslipped with Permount. The right retinas were processed for paraffin embedding, and 10 μm transverse sections were made with a rotatory microtome. The sections were mounted on glass slides, dewaxed, and coverslipped with Permount.

Quantitative analysis of NADPH-d neurons

To estimate the number of NADPH-d neurons in both the ganglion cell layer (GCL) and the inner nuclear layer (INL) of the whole-mount retinas, we used the same counting method as that described by Lau et al. (1994). A series of counting areas, each covering $200 \times 200\ \mu m^2$ and arranged radially with 0.2 mm (P4–P20) or 0.3 mm (AD) intervals from the optic disc (OD), was selected in each of the four quadrants of the retina. The total number of sampling sites for the counting of NADPH-d neurons in GCL and INL ranged from 30 for early postnatal to 64 for adult retinas. The sampling method allowed us to construct an isodensity map of the NADPH-d neurons in the retina by averaging the number of neurons observed in corresponding quadrants and eccentricities of the three retinas at each age. The counting of the NADPH-d neurons was made visually under a Nikon microscope with a 40× objective and 10× eyepieces equipped with a square micrometer. Different focusing depths were adjusted to distinguish the NADPH-d neurons located in GCL from those in INL. The measurement of the diameter of NADPH-d cell bodies in flat-mounted retinas was carried out by using a PC-based image-analyzing system (Neurolucida) attached to the Nikon microscope. In P4–P8 retinas, 20 fields, each covering $100 \times 200\ \mu m^2$, were randomly selected for each retina, whereas 30 such fields were selected for P12–AD retinas. Representative areas of the retina were photographed with a cooled color CCD camera (Optronics) and digitally stored on a desktop computer.

RESULTS

NADPH-d expression was observed in developing as well as adult retinas. NADPH-d-positive neurons were found in the photoreceptor layer, the outer plexiform layer (OPL), the INL, the inner plexiform layer (IPL), and the GCL. However, NADPH-d reactivity was most conspicuous in the INL, GCL, and fiber terminal and synaptic zone between these two cellular layers, i.e., the IPL. The present study was, therefore, devoted mainly to observations of NADPH-d-positive neurons in the INL, GCL, and IPL.

Morphological features of NADPH-d neurons during development

The morphology of NADPH-d neurons in the adult hamster retina has previously been described and identified by Lau et al. (1994). We observed similar morphological fea-

tures in the present study. Many NADPH-d neurons with clearly delineated cell bodies were located in both INL and GCL and have previously been demonstrated to be amacrine cells and displaced amacrine cells, respectively (Lau et al., 1994). Neurons in both of these two layers were found to send their dendrites to the IPL (Fig. 1A–E). NADPH-d-positive neurons in both INL and GCL could be grouped into two morphological categories of either heavily stained neurons with dark cell bodies or lightly stained neurons (Fig. 1F–I). Neurons with dark prominent soma had more and distinctive long dendrites, whereas those with pale cell bodies had fewer or no discernible dendrites. Such observations were similar to those in the retina of adult hamsters and other animals (Lau et al., 1994; for review see Koistinaho and Sagar, 1995) and in other regions of the brain (Yan et al., 1994; Xiao, et al., 1996).

NADPH-d activity in the photoreceptor layer was first observed at P0 and that in OPL at P8. A few NADPH-d neurons, which could already be discerned as heavily and lightly stained neurons, were first observed at P1. At P4 NADPH-d-positive neurons located in both GCL and INL began to possess more dendrites, which stretched into the IPL (Fig. 1A–E). The stratification of the IPL could be observed as early as P4, when sublamina S_3 appeared. Sublamina S_5 was apparent at P8, and sublamina S_1 appeared later at P12 (Fig. 1A–C). Compared with the peripheral part of the whole-mounted retina, the NADPH-d-positive neurons in the central part possessed fewer and shorter dendrites for all ages investigated (Fig. 1F–I).

Stratification of IPL

In the radial (transverse) sections of adult retina of the hamster, the NADPH-d-positive neurons in the INL and GCL sent their dendrites to sublaminae 1, 3, and 5 of the IPL, termed separately as S_1, S_3, and S_5 (Lau et al., 1994). At P4, dendrites of some NADPH-d neurons of the INL and GCL were observed to form initially a thin discontinuous sublamina, i.e., S_3, in the IPL (Fig. 1A). At P8, the S_3 became continuous and thicker because of the accumulation of more dendrites. In addition, S_5 started to appear at P8 (Fig. 1B). At this time, processes of some NADPH-d neurons in the GCL stretched into the IPL and ran near the GCL parallel to S_3. However, it was not until P12 when a continuous layer S_5 was formed (Fig. 1C). At P12, processes of some NADPH-d neurons in the INL extended into the IPL and ran parallel to S_3 and S_5 near the INL to form the discontinuous S_1 (Fig. 1C,D). At P16, more dendrites from the INL joined the S_1, making it a discernible continuous sublayer in the IPL (Fig. 1E). Occasionally, dendrites of a NADPH-d neuron in the GCL were observed projecting to both S_3 and S_5 (Fig. 1D) or crossing S_5 and S_3 before joining S_1 (Fig. 1E). All sublaminae increased in thickness and became more prominent with postnatal time through adulthood.

Number of NADPH-d neurons

Changes in the number of NADPH-d neurons were the most conspicuous features during development. The total number of NADPH-d neurons in the retina underwent two sharp increases (Table 1). The earlier wave of increase was transient and peaked at about P8, followed by the second surge, which started around P20 and progressed to reach the final adult value. However, when NADPH-d-positive neurons in INL and GCL were assessed separately, differences in the mean cell number, developmental time courses, and distribution patterns were manifested (Fig. 2). In the INL, there was an earlier sharp increase from 2,285 ± 842 (mean ± SD; n = 3) at P4 to 8,110 ± 702 at P8 (3.6 times). This was followed by a sharp decrease to 3,710 ± 576 at P12 (46% of the maximum), which stayed relatively constant to approximate the adult level of 5,666 ± 1,368. *t*-Tests of paired data points showed that there were no significant differences between the numbers at P12, P16, and P20, and of the adult ($P = 0.01$; n = 3). In the GCL, the number of NADPH-d-positive neurons increased from 1,577 ± 352 at P4 to 4,164 ± 820 at P12 (*t*-test, $P = 0.01$; n = 3). This value remained relatively constant until P20. In the adult retina, 8,349 ± 308 NADPH-d neurons were estimated (*t*-test, $P = 0.01$; n = 3). The appearance of the curves for the average density as well as that for the cell number of NADPH-d neurons over the period of investigation was similar in both the INL and the GCL (Fig. 2B,C). The initial sharp, transient change in both the cell density and the number was more conspicuous in INL than that in GCL (*t*-test, $P = 0.01$; n = 3). However, in the later period of development, changes in GCL became more pronounced (*t*-test, $P = 0.01$; n = 3).

Different developmental time courses of NADPH-d neurons between the INL and the GCL are further demonstrated by the change of the ratio of GCL neurons to INL neurons (GCL/INL; Fig. 2D). At P4–P8, the numbers of NADPH-d neurons in the GCL were lower than those in the INL, resulting in a ratio of 0.7 and 0.4, respectively. At P12 and P16 the numbers in the GCL and INL were about the same, resulting in a ratio of 1.1 and 1.0, respectively. For the P20 and adult retinas, however, NADPH-d neurons in the GCL were more numerous than in the INL; the ratios were 1.2 and 1.5, respectively.

Taken together, Figure 2A–D shows that the earlier increase in the total number of the NADPH-d neurons was a consequence of increases both in cell density of the INL and in net area of the retina, whereas the later increase resulted from the increases of both the cell density in GCL and the retinal area.

Soma diameters of NADPH-d neurons

In terms of the distribution of soma diameters, there was a substantial amount of overlap in the range of sizes between NADPH-d neurons in the GCL and those in the INL (Fig. 3). The mean diameter in the GCL was larger than that in the INL for all ages (*t*-test, $P = 0.01$). With the increase of postnatal time and the expansion of soma size of neurons in both GCL and INL, the range of distribution was shifted correspondingly toward the larger cells. However, the relative size distribution of more large cells in GCL than in INL was maintained. The mean diameters of NADPH-d cell bodies in INL increased from 8.4 ± 0.86 μm at P4 to 10.4 ± 1.38 μm at P12 and then remained almost constant to the adult level at 10.8 ± 1.59 μm. On the other hand, the mean diameters of the NADPH-d cell bodies in the GCL increased from 8.9 ± 0.89 μm at P4 to 12.8 ± 2.40 μm for the adult, except for P20, at which a slightly but insignificantly reduced value of 11.2 ± 1.86 μm was obtained (*t*-test, $P = 0.01$). There seemed to be a slight reduction in the diameter of NADPH-d neurons in P20 retinae for both INL and GCL. This observation was similar to the report of that in the developing rat retina, where the mean diameter of these

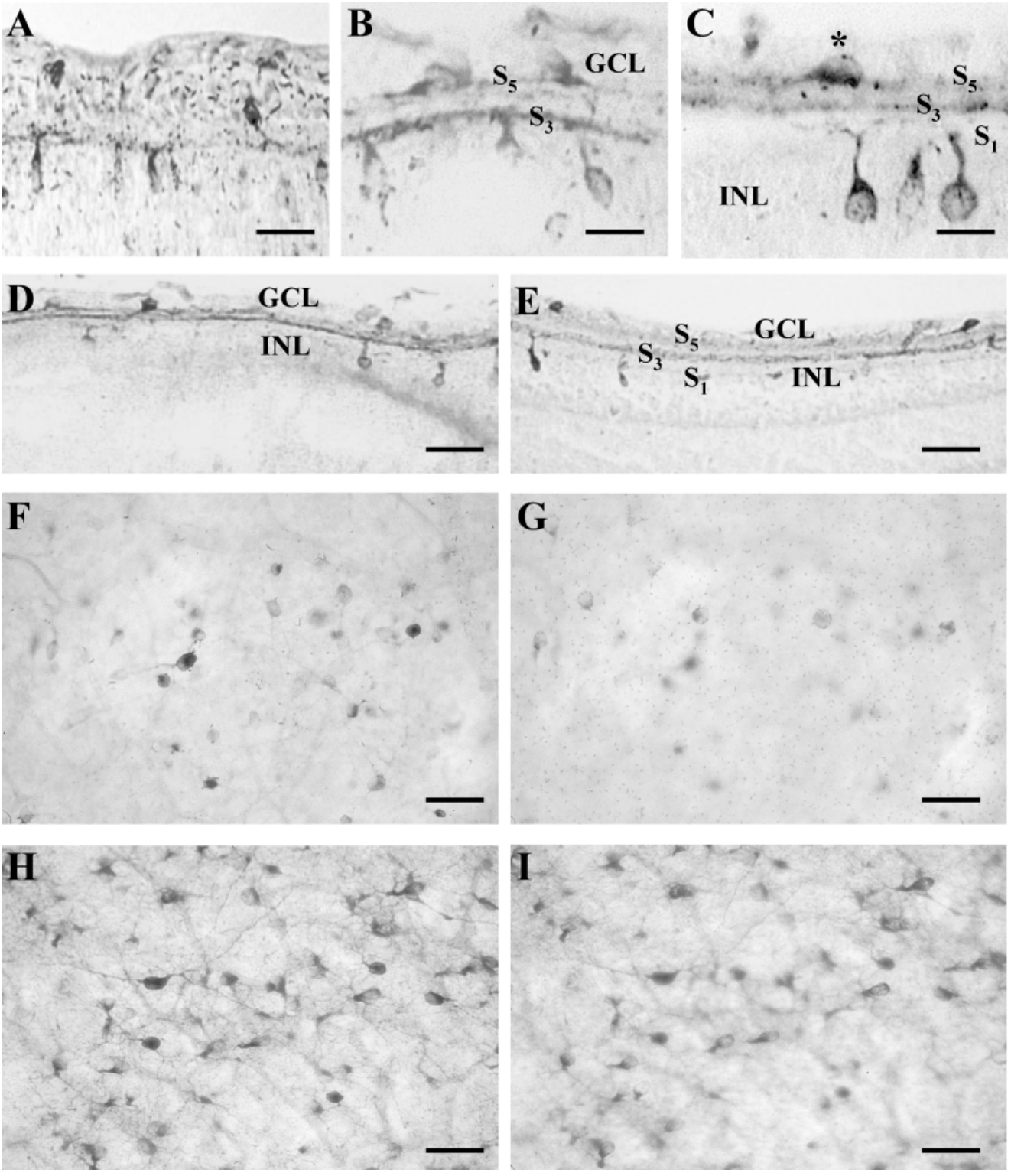

Fig. 1. Photomicrographs of NADPH-diaphorase expression in the developing retinas of the golden hamster. **A:** P4 retina. Sublamina 3 (S_3) of inner plexiform layer (IPL) is discernible. **B:** P8 retina. Sublamina 5 (S_5) starts to appear near the ganglion cell layer (GCL) and S_3. **C:** P12 retina. Dendrites of NADPH-d-positive amacrine neurons in the inner nuclear layer (INL) stretch out and run parallel to S_3 and S_5, forming sublamina 1 (S_1). Asterisk indicates a displaced amacrine cell. **D:** P12 retina. Dendrites of one NADPH-d neuron in the GCL projecting to both S_3 and S_5, S_1. **E:** P20 retina. NADPH-d expressing neurons in GCL and INL with dendrites extending to form S_1, S_3, and S_5. **F,G:** P12 retina. NADPH-d-positive neurons in the GCL (F) and INL (G) of the central area. **H,I:** P12 retina. NADPH-d neurons in the GCL (H) and INL (I) of the peripheral area. Note that, in the central retina, neurons are more lightly stained, and dendrites are fewer and shorter in comparison with those in the peripheral retina. A–E, radial section; F–I, whole-mounted retina. For details see text. Scale bars = 50 μm in A–C, 20 μm in D–I.

TABLE 1. Total Number of NADPH-d Neurons Estimated for Various Postnatal Days

	Age					
	P4	P8	P12	P16	P20	AD
INL + GCL	3,861	11,234	7,874	7,772	5,757	14,014
SD	668	1,401	1,131	791	946	1,302
Change		Increase	Decrease	No change	Decrease	Increase
t-Test A(n = 3)		P4–P8	P8–P12	P12–P16	P16–P20	P20–AD
P		0.001	0.031	0.912	0.047	0.001

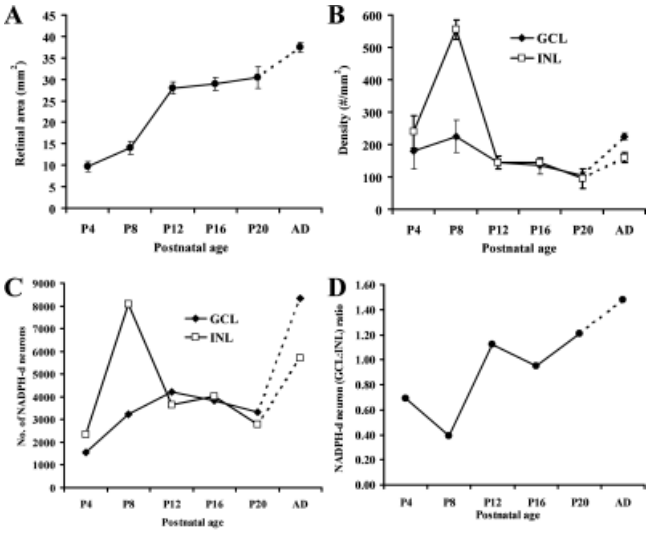

Fig. 2. **A:** Changes of the retinal areas at different ages from P4 (P4–P20; postnatal days 4–20) to adulthood (AD). **B:** Changes of number of NADPH-d neurons in GCL and INL of the retina at different ages from P4 to AD. **C:** Changes of density of NADPH-d neurons in the GCL and INL of the retina at different ages from P4 to AD. **D:** Changes of the ratio of number of NADPH-d neurons (GCL/INL) at ages from P4 to AD. The value at each age is the average from three whole-mounted retinas from three hamsters.

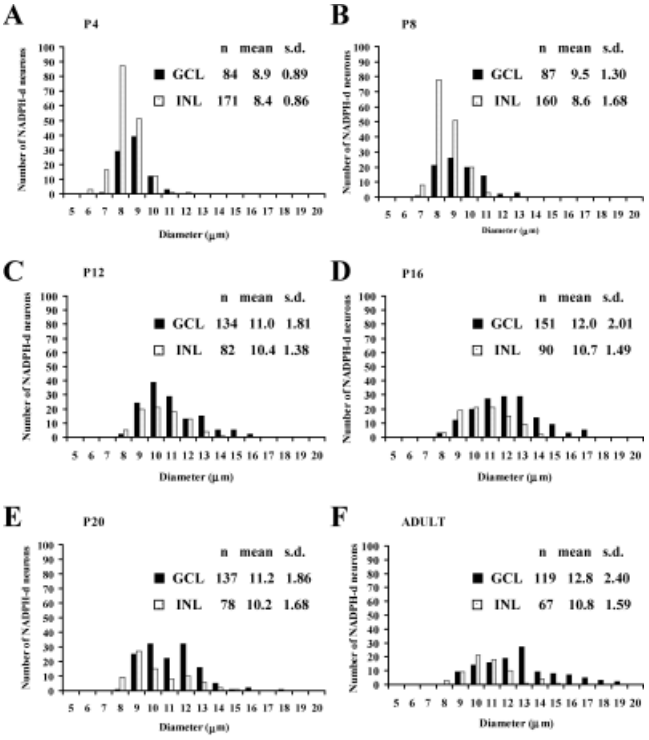

Fig. 3. Histograms showing the distributions of diameters of NADPH-d neurons in the GCL and INL at different postnatal ages. Measurements were taken randomly in two retinas at each age.

neurons was lower at P25 compared with diameters found at earlier developmental ages (Mitrofanis, 1989).

Isodensity map

From the sampling sites, the average density of the NADPH-d-positive neurons was utilized to construct isodensity maps for developing and adult retinas (Fig. 4A,B). For all ages investigated, the lowest density was always located in the far peripheral retina. At P4–P8, high densities were located toward the upper central retina. During this developmental period, the number of NADPH-d neurons in INL was much higher than that in the GCL. With the increase in postnatal time, the density of NADPH-d neurons in central retina was greatly reduced, whereas that in the vast region between the central and the peripheral retina showed no substantial change. However, in the adult, the region of higher densities was located in the lower central retina, where the number of NADPH-d-positive neurons in GCL exceeded that in INL (Fig. 4A,B).

DISCUSSION

The present study shows that NADPH-d expression in the retina underwent dramatic changes during a relative long period of postnatal development in golden hamsters and that the development of NADPH-d neurons in the INL and the GCL followed different time courses and patterns. Two aspects of the developmental events were most clearly demonstrated. The first is the changes in total number of NADPH-d neurons, these being an earlier, sharp transient increase dominated by cells in the INL and a later, steady increase to reach the adult level dominated by cells in the GCL. The second is the changes in density distribution, among which the highest density changed from the upper central retina at the earlier postnatal days to the lower central retina in the adult. In the meantime, the cell size and the synaptic connection zone also underwent developmental changes.

Classification of cell types

All NADPH-d-positive neurons found in the brain are interneurons, such as those in the superior colliculus (Vercelli et al., 2000), lateral geniculate nucleus (Vercelli et al., 2000; Wienchen and Casagrande, 2000), and visual cortex (Xiao et al., 1996; Wienchen and Casagrande, 2000). However, NADPH-d-positive neurons in the retina similar to those observed in the present study have been interpreted as amacrine cells and displaced amacrine cells in rabbit (Sagar, 1986; Mitrofanis et al., 1992), guinea pig (Cobcroft et al., 1989), and rat (Mitrofanis, 1989; Darius et al., 1995) but also as ganglion cells in rats (see Huxlin, 1995) and cat (Wassel et al., 1987; Vaccaro et al., 1991). Using both retrograde fluorescent labeling and degeneration techniques, Lau et al. (1994) have convincingly shown that in the retina of adult hamsters no ganglion cells are NADPH-d positive. We, therefore, suggest that in the present study the NADPH-d-positive neurons in INL and

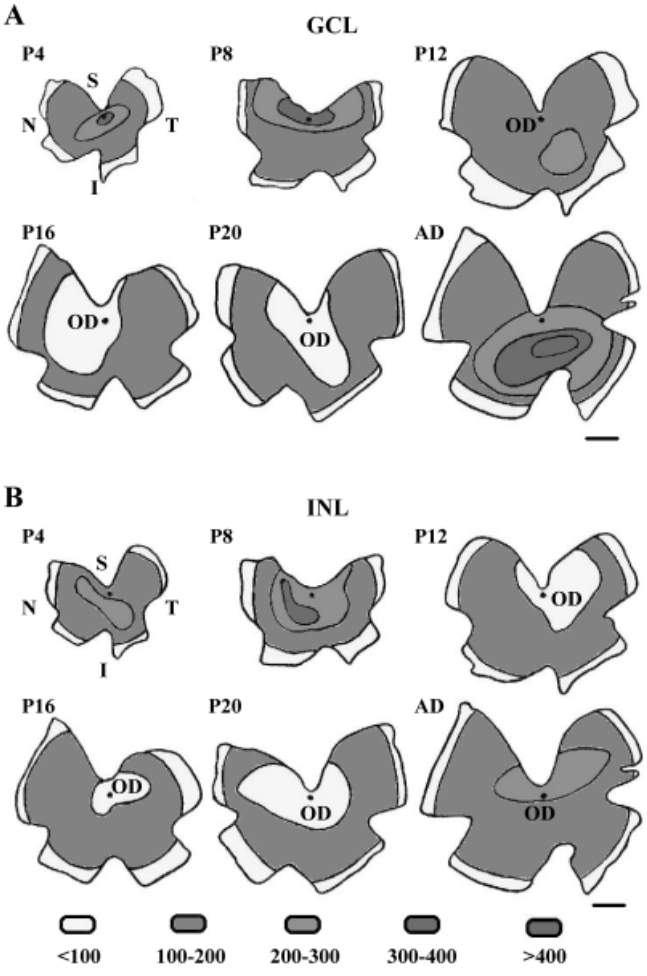

Fig. 4. Isodensity maps of NADPH-d neurons in the GCL (**A**) and INL (**B**). Data were collected from three whole-mounted retinas at each age. Density is given in number per square millimeter. AD and OD denote adult and optic disc, respectively. S, superior; I, inferior; T, Temporal; N, nasal quadrant of the retina. Scale bars = 1 mm.

GCL of the hamster retina are amacrine and displaced amacrine cells, respectively.

Methodological considerations

The method for distinguishing the GCL neurons from the INL neurons in the whole-mount retina, i.e., by focusing the microscope at a different depth in this study, is reproducible and reliable. The reliability of this method is further confirmed by the following two facts. The first comes from counts taken from the radial sections of the retina. In total, 330 sample sections, each 235 μm long, were selected randomly from two P12 right retinas. The numbers of neurons counted were 1,023 in GCL and 870 in INL, with a GCL neurons to INL neurons ratio of 1.18, which is similar to the average ratio of 1.12 obtained from three P12 left whole-mount retinas (see Fig. 2D). The other fact is that the average total number of NADPH-d-positive neurons in GCL, the average total number of NADPH-d-positive neurons in INL, and the ratio of these two numbers of the adult hamster are 8,349, 5,665, and 1.5, respectively. These values are comparable with those obtained by Lau et al. (1994): 8,033, 5,051, and 1.6 for the three respective groups.

Comparison with other animals

Transient increase of NADPH-d expression in the developing retina has been reported in several mammalian species. However, the time courses and patterns of the expression of NADPH-d reactivities were quite different among different species. The present study in hamsters showed that the first increase peaked on P8. This is similar to the case in the rat, in which the transient increase of numbers of NADPH-d neurons occurred at ages from P3 to P11 (Mitrofanis, 1989). In cat, NADPH-d-positive retinal neurons were first detected at embryonic day 58, and at birth there were twice as many as those observed in adult animals (Vaccaro et al., 1991). There is also another example showing a very early increase of the NADPH-d neurons in the rabbit retina. In this species, the peak of total number of NADPH-d neurons occurred from postconception day 28 to postconception day 50, followed by a decline to the adult value (Mitrofanis et al., 1992). On the other hand, Sharma and Perez (1996) reported that the NOS-immunoreactive cells in the rabbit retina appeared at embryonic day 29, and the number increased continuously until P11, when their number stabilized. This observation resembled the second increase of NADPH-d neurons in the hamster seen in this study. An interesting proposition is that the development of these neurons undergoes two periods of changes, one before or soon after birth during the period of the establishment of synaptic connections with the visual targets and the second on P14/15 after the opening of the eye, when the animals are experiencing light stimulation and acquiring visual function. Because the hamster is still immature when it is born, both episodes of increase in the expression of NADPH-d reactivity occur postnatally.

Implications of the developmental events

Two lines of facts should be kept in mind for comprehension of the implication of the present results. The first is that the generation of the retinal components does not necessarily follow the developmental sequence of generation of retinal ganglion cells in the vertebrate retina preceding (Austin et al., 1995), at least, the generation of amacrine cells (Hinds and Hinds, 1983). A synaptic reorganization or refinement must be taking place during development to ensure that appropriate connections are established (Campello-Costa et al., 2000). The second is that NADPH-d neurons are identical to the NO-synthesizing neurons. NO synthesized by NOS is diffusible across the cell membrane, and it acts as an intracellular messenger essential for the modulation of excitability of the cells and for the establishment of activity-dependent neuronal connections (Garthwaite, 1995; Koistinaho and Sagar, 1995; Goureau et al., 1997). On the other hand, NO has free radical properties and, therefore, is repellant and may cause cell death (Gally et al., 1990; Iadecola, 1997) if overproduced.

Changes in the number of NADPH-d neurons in the retina might be attributed to two main developmental events. First, transient increase of cell numbers during certain periods of development in the brain is probably a common process, for example, increase of the retinal ganglion cells (Sengelaub et al., 1986; Tay et al., 1986) and callosal neurons (Chow et al., 1981; Miller and Vogt, 1984; Sefton et al., 1991). The early increase of the NADPH-d-positive neurons in the retina of hamsters from P4 (prob-

ably earlier) to P8 seen in the present study coincides with the period when the elongating retinal axons are searching and reaching out to establish appropriate synaptic connections in the target sites. This is in agreement with suggestions that NO promotes axonal elongation and regulates advancement of growth cones (Van-Wagenen and Rehden, 1999; Renteria and Constaintine-Paton, 1999). The active establishment of synaptic contacts between the invading retinal axons and the visual targets may require interactions of these NADPH-d neurons in the retina. By P8, the formation of retinogeniculate pathway is fully established (So et al., 1984, 1990), and fewer interactions from the amacrine cells are, therefore, required, suggesting that NO is necessary for the process of synaptogenesis. Thus, the number of NADPH-d-expressing neurons would decrease, as was observed in the present investigation. We cannot rule out, however, that some of the displaced amacrine cells may have changed their NADPH-d reactivity from positive to negative. This change may be related to further functional differentiations, such as the final shaping of the neuronal circuitry of the ganglion cells. During this period, density distribution of the NADPH-d neurons underwent a dramatic change. The densest area located in the upper central retina at the earlier postnatal days was shifted to the lower central retina to coincide with the distribution of the ganglion cells in the adult hamster (Tiao and Blakemore, 1976).

Second, the change in histochemical reactivity of retinal cells during development observed in the present study may be the consequence of eye opening, which triggers another surge of NADPH-d activity in response to light stimulation in the visual targets and the acquisition of functional visual experience. We, therefore, suggest that NADPH-d is necessary for processing of visual function, and experiments are in progress to investigate the role of NADPH-d in visual activities.

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LITERATURE CITED

Austin CP, Feldman DE, Ida JA Jr, Cepko CL. 1995. Vertebrate retinal ganglion cells are selected from competent progenitors by the action of Notch. Development 121:3637–3650.

Campello-Costa P, Fosse AM Jr, Ribeiro JC, Paes-De-Carvalho R, Serfaty CA. 2000. Acute blockade of nitric oxide synthesis induces disorganization and amplifies lesion-induced plasticity in the rat retinotectal projection. J Neurobiol 44:371–381.

Chow KL, Baumbach HD, Lawson R. 1981. Callosal projections of the striate cortex in the neonatal rabbit. Exp Brain Res 42:122–126.

Cobcroft M, Vaccaro T, Mitrofanis J. 1989. Distinct patterns of distribution among NADPH-diaphorase neurons of the guinea pig retina. Neurosci Lett 103:1–7.

Darius S, Wolf G, Huang PL, Fishman MC. 1995. Localization of NADPH-diaphorase/nitric oxide synthase in the rat retina: an electron microscopic study. Brain Res 690:231–235.

Gally JA, Montague PR, Reeke GN Jr, Edelman GM. 1990. The NO hypothesis: possible effects of a short-lived, rapidly diffusible signal in the development and function of the nervous system. Proc Natl Acad Sci USA 87:3547–3551.

Garthwaite J. 1995. Neuronal nitric oxide signaling. Trends Neurosci 18:51–52.

Goldstein J, Lopez-Costa JJ, Saavedra JP. 1997. Changes in NADPH diaphorase reactivity and neuronal nitric oxide synthase in the rat retina following constant illumination. Neurosci Lett 231:45–48.

Goureau O, Regnier-Recard F, Jonet L, Jeanny JC, Courtois Y, Chany-Founier F. 1997. Developmental expression of nitric oxide synthase isoform I and III in chick retina. J Neurosci Res 50:104–113.

Hinds JW, Hinds PL. 1983. Development of retinal amacrine cells in the mouse embryo: evidence for two models of formation. J Comp Neurol 213:1–23.

Hsiao K. 1984. Bilateral branching contributes minimally to the enhanced ipsilateral projection in monocular Syrian golden hamsters. J Neurosci 4:368–373.

Huxlin KR. 1995. NADPH-diaphorase expression in neurons and glia of the normal adult rat retina. Brain Res 692:195–206.

Iadecola C. 1997. Bright and dark sides of nitric oxide in ischemic brain injury. TINS 20:132–139.

Koistinaho J, Sagar SM. 1995. NADPH-diaphorase-reactive neurons in the retina. Progr Ret Eye Res 15:69–87.

Lau KC, So K-F, Tay D, Leung MCP. 1994. NADPH-diaphorase neurons in the retina of the hamster. J Comp Neurol 350:550–558.

Llomovatte DW, Lacoste FF, Zotter C, Sarmiento MI, Rosenstein RE. 1997. Photic control of nitric oxide synthase activity in golden hamster retina. Neuroreport 8:3763–3766.

Miller MW, Vogt BA. 1984. The postnatal growth of the callosal connections of primary and secondary visual cortex in the rat. Dev Brain Res 14:304–309.

Mitrofanis J. 1989. Development of NADPH-diaphorase cells in the rat's retina. Neurosci Lett 102:165–172.

Mitrofanis J, Robinson S, Ashwell K. 1992. Development of catecholaminergic, indoleamine-accumulating, and NADPH-diaphorase amacrine cells in rabbit retina. J Comp Neurol 319:560–585.

Renteria RC, Constantine-Paton M. 1999. Nitric oxide in the retinotectal system: a signal but not a retrograde messenger during map refinement and segregation. J Neurosci 19:7066–7076.

Sagar SM. 1986. NADPH-diaphorase histochemistry in the rabbit retina. Brain Res 373:153–158.

Sefton AJ, Dreher B, Lim WL. 1991. Interactions between callosal, thalamic, and associational projections to the visual cortex of the developing rat. Exp Brain Res 84:142–158.

Sengelaub DR, Dolan RP, Finlay BL. 1986. Cell generation, death, and retinal growth in the development of the hamster retinal ganglion cell layer. J Comp Neurol 246:527–543.

Sharma RK, Perez MTR. 1996. Nitric oxide synthase in developing and transplanted rabbit retinas. Exp Eye Res 63(Supp 1):398.

So K-F, Woo HH, Jen LS. 1984. The normal and abnormal postnatal development of retinogeniculaate projections in golden hamsters: an anterograde horseradish peroxidase tracing study. Dev Brain Res 12:191–205.

So K-F, Campbell G, Lieberman AR. 1990. Development of the mammalian retinogeniculate pathways: target finding, transient synapses and binocular segregation. J Exp Biol 153:85–104.

Tay D, So K-F, Jen LS, Lau KC. 1986. The postnatal development of the optic nerve in hamsters: an electron microscopic study. Dev Brain Res 30:268–273.

Thomas E, Pearse AGE. 1961. The fine localization of dehydrogenase in the nervous system. Histochemie 2:266–282.

Tiao YC, Blakemore C. 1976. Regional specialization in the golden hamster's retina. J Comp Neurol 168:439–458.

Vaccaro TM, Cobcroft MD, Provis JM, Mitrofanis J. 1991. NADPH-diaphorase reactivity in adult and developing cat retinae. Cell Tissue Res 265:371–379.

Van-Wagenen S, Rehder V. 1999. Regulation of neuronal growth cone filopodia by nitric oxide. J Neurobiol 39:168–185.

Vercelli A, Garbossa D, Biasiol S, Repici M, Jhaveri S. 2000. NOS inhbition during postnatal development leads to increased ipsilateral retinocollicular and retinogeniculate projections in rats. Eur J Neurosci 12:473–490.

Wassle H, Chun MH, Muller F. 1987. Amacrine cells in the ganglion cell layer of the cat retina. J Comp Neurol 265:391–408.

Wiencken AE, Casagrande VA. 2000. The distribution of NADPH diaphorase and nitric oxide synthatase (NOS) in relation to the functional compartments of areas V1 and V2 of primate visual cortex. Cereb Cortex 10:499–511.

Xiao YM, Diao YC, So K-F. 1996. A morphological study of neurons expressing NADPH diaphorase activity in the visual cortex of the golden hamster. Brain Behav Evol 48:221–230.

Yan XX, Garey LJ, Jen LS. 1994. Development of NADPH-diaphorase activity in the rat neocortex. Dev Brain Res 79:29–38.