LOCALIZATION, CHROMATOGRAPHIC CHARACTERIZATION, AND DEVELOPMENT OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE GUINEA PIG RETINA¹

ARTHUR W. SPIRA,^{*,2} YOSHIKI SHIMIZU,^{*,3} AND O. P. RORSTAD[‡]

^{*}*Department of Anatomy and Lions' Sight Centre, and [‡]Department of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 4N1*

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Abstract

We have studied the anatomical localization, chromatographic properties, and development of somatostatin-like immunoreactivity (SLI) in the guinea pig retina. The majority of guinea pig retinal SLI in the adult and fetus eluted similarly to somatostatin-28 by gel filtration and high pressure liquid chromatography. This represents a higher ratio of somatostatin-28-like material to total SLI than had been observed in retinal extracts from most other animal species. Somatostatin-like-immunoreactive cells and fibers were localized using two antisera in cryostat-sectioned and flat mounted retinæ. Distribution of cells and fibers differed uniquely from that in other species previously reported. Reactive perikarya were located only in the far peripheral region: in the innermost layer of the inner nuclear layer (INL), in the inner plexiform layer (IPL), and in the ganglion cell layer. Reactive fibers were prominent in the IPL and nerve fiber layer (NFL) in both the peripheral and central retina. Less frequently, processes were observed between the NFL and IPL, between the IPL and INL, and, rarely, in the outer plexiform layer and outer nuclear layer. Small numbers of reactive fibers were found in the optic nerve and disc. These observations suggest that processes of intrinsic (amacrine or associational ganglion cells) and projection neurons (true ganglion cells or efferent fibers) containing SLI are intermingled in the guinea pig retina. SLI, quantified by radioimmunoassay, was present in the developing retina as early as the 6th week of gestation (full term is 10 weeks). Immunohistochemically detected somatostatin-like-immunoreactive elements were seen first at 2 weeks before birth, coincident with the onset of the period of most rapid increase in levels of assayed SLI. Somatostatin-like-immunoreactive peptides reached two-thirds of adult levels by birth.

Coincident with the application of radioimmunoassay (RIA) and immunohistochemical localization techniques to the study of retinal neurotransmitters has come the realization that the retina contains a considerable number of peptides familiar to the gastrointestinal system and to other regions of the brain (reviewed in Brecha and Karten, 1983). A small but steadily growing number of accounts indicates that some peptides have a demonstrable effect on retinal neuronal activity (Dick and Miller, 1981; Djamgoz et al., 1981, 1983; Glickman et al., 1982;

Stell et al., 1983). In the mammalian retina five or six of the peptides have been identified. These include somatostatin-like (somatostatin release-inhibiting factor) (reviewed by Rorstad et al., 1980), thyrotropin-releasing hormone-like (Schaeffer et al., 1977), substance P-like (Kanazawa and Jessell, 1976), vasoactive intestinal polypeptide-like (Loren et al., 1980), cholecystokinin-like (Eskay and Beinfeld, 1982), and possibly Met-enkephalin-like (Altschuler et al., 1982) peptides.

Somatostatin-like substances have been among the most intensively studied retinal neuropeptides. They were first extracted from the mammalian retina and assayed nearly concurrently by Rorstad et al. (1979), Shapiro et al. (1979), Yamada et al. (1980), and Lake and Patel (1980). They were found to have the same biological effect on cultured anterior pituitary cells as that derived from the hypothalamus, i.e., an inhibition of the secretion of growth hormone (Rorstad et al., 1979). Retinal somatostatin-like immunoreactivity (SLI) was reduced by chemical lesions of the inner retinal layers in the rat (Lake and Patel, 1980) but not by optic nerve section (Rorstad et al., 1979). Early localization studies in non-mammalian retinas indicated SLI in presumed amacrine cells of the inner nuclear layer (INL), in fibers in sublayers of the inner plexiform layer (IPL), and in cells of the ganglion cell layer (GCL), the general pattern being similar to that seen for other neuropeptides (Yamada et al., 1980; Buckerfield et al., 1981; Ishimoto et al.,

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² To whom correspondence should be addressed, at Department of Anatomy, The University of Calgary, 3330 Hospital Drive N. W., Calgary, Alberta, Canada T2N 4N1.

³ Present address: Department of Ophthalmology, Osaka University Medical School, Osaka, Japan.

Figure 1. Gel filtration chromatography of guinea pig retinal SLI using 0.1 M ammonium acetate/acetic acid buffer, pH 3.0, as the eluent. The shaded area indicates the detection limit of the RIA. The elution position of ^{125}I -labeled bovine serum albumin (Vo), somatostatin-28, somatostatin-14, and phenol red solution are shown. SRIF, somatostatin (somatotropin release-inhibiting factor).

Figure 2. HPLC of guinea pig retinal SLI. The shaded area refers to the detection limit of the assay. The dashed line indicates the percentage of concentration of acetonitrile in the eluent. The retention times of somatostatin-28 and somatostatin-14 are shown. SRIF, somatostatin (somatotropin release-inhibiting factor).

1982a). In contrast to submammalian orders, mammalian retinas appear to have a dearth of SLI in cell bodies. Tornqvist et al. (1982) observed somatostatin-like-immunoreactive somata in only 1 of 10 different mammalian species (the squirrel monkey). SLI in the rat seems to localize variably; it was found in fibers but not in cell bodies by Eriksen and Larsson (1981), in amacrine cells of the INL by Ishimoto et al. (1982a), in interplexiform cells by Sagar et al. (1983), and in amacrine, GCL, and horizontal cells by Krisch and Leonhardt (1979) using one antiserum, but not in any cells using a second antiserum. Possible sources for the differing accounts within a species are the obvious ones of antiserum specificity and sensitivity and the more subtle one of differences in tissue sampling and processing. None of the investigators, for example, has used flat mount preparations or indicated that they had surveyed both central and peripheral areas. Localization differences between species, on the other hand, surely exist and

might reflect chemical differences in the antigen as well as differences in apportionment among cell types and IPL sublaminae. All reactive cells may not necessarily contain the same molecular form of somatostatin. In the rat, SLI co-elutes predominantly with synthetic somatostatin-14 (Rorstad et al., 1979; Shapiro et al., 1979; Patel et al., 1981; Sagar et al., 1982). Elsewhere, retinal somatostatin-like-immunoreactive peptides exist in both SS-14- and SS-28-like forms, with SS-14 predominant in the goldfish (Yamada et al., 1980), frog (Yamada and Basinger, 1982), and rabbit (Sagar et al., 1982) and SS-28 the major form in the bovine retina (Marshak et al., 1983). The work of Yamada and Basinger (1982) suggests the presence of a precursor to somatostatin-14 in *in vitro* biosynthetic experiments using the frog retina. The molecular forms of somatostatin are of interest, as well, for the possibilities that the cells which contain them possess different functional properties and because the different forms may be of ontogenetic and phylogenetic significance.

In spite of the intense interest in retinal neuropeptides, few studies have been made of their occurrence during differentiation. The ontogenesis of SLI has been examined only in the rat (Kuwayama et al., 1981). With a relatively late postnatal course of retinal structural and functional maturation, the rat does not provide information that can be immediately extrapolated to species in which retinal development is nearly completed at birth, e.g., primates. The guinea pig lends itself well to developmental studies in retinæ which differentiate *in utero* (Spira, 1975). It is born with its eyes open and has a nearly mature electroretinographic response to light. Retinal samples may be obtained relatively economically and in significant numbers for meaningful quantification of SLI by RIA. Published assays of SLI in guinea pig retina (Eskay et al., 1980) indicate that amounts are large enough to permit characterization of its molecular forms in the adult as well as permitting its assay during development.

Materials and Methods

All experiments were performed on albino Hartley guinea pigs. Fetuses were obtained for microscopical examination and for RIA from time-bred animals between the 38th and 69th days (full term) of gestation. Material for chromatographic analysis was obtained from mature animals (more than 10 weeks old) and fetuses at 56 days of gestation. Enucleations were carried out on pentobarbital-anesthetized mature animals (10 weeks or older) prior to their sacrifice or on fetuses of Caesarian-exposed, halothane-anesthetized pregnant sows.

After excision of the anterior segment and careful removal of the vitreous body, the posterior part of the eye cup was placed in fixative

Figure 3. Somatostatin-like immunofluorescence in a transverse cryostat section of the guinea pig retina. SLI in cell bodies is located in three layers of the guinea pig retina: (a) the proximal INL, (b) the IPL, and (c) the GCL. Magnification $\times 600$.

Figure 4. a, SLI in a PAP-treated flat mount of the guinea pig retina. SLI is present in a multipolar cell body and in beaded processes in the peripheral retina close to the ora serrata (OS). Magnification $\times 500$. b, In a colchicine-treated animal immunofluorescence is enhanced in cells and processes viewed in flat mount. Magnification $\times 300$.

for immunohistochemical localization of SLI, or in 0.1 M HCl for extraction of somatostatin-like-immunoreactive substances.

Radioimmunoassay. RIA of retinal SLI was carried out by the method of Arnold et al. (1982), with the exception that ^{125}I -labeled *N*-Tyr-somatostatin-14 was used as the radioligand. This radioligand results in RIA specificity identical to that obtained with ^{125}I -labeled [Tyr¹] somatostatin-14 when used with the rabbit antiserum (batch M4) employed in this study (Rorstad, 1983). The RIA recognizes the central molecular region of the somatostatin molecule (Arnold et al., 1982; Rorstad, 1983), the portion most important for the biological actions of somatostatin (Vale et al., 1978). Somatostatin-28 is equipotent with somatostatin-14 on a molar basis in the RIA (Arnold et al., 1982; Rorstad, 1983). The RIA standard was somatostatin-14 (Beckman Instruments Inc., Palo Alto, CA).

Retinas used for RIA were obtained from a mean of six guinea pig fetuses at each of several ages. Guinea pig retinas were extracted in 0.1 M HCl by a previously reported method (Rorstad et al., 1979) with the following minor modifications. The retinal sample was heated at 100°C for 5 min in 0.5 ml of 0.1 M HCl, then cooled in an ice bath and homogenized. The homogenization apparatus was rinsed with 0.2 ml of 0.1 M HCl, and the rinse was added to the original volume. The homogenate was frozen at -20°C and thawed at room temperature before centrifugation. The supernatant was lyophilized and the residue was dissolved in 0.4 ml of 0.1 M sodium phosphate buffer, pH 7.4, prior to RIA. Each retinal extract was assayed at two dilutions. The inter-assay coefficient of variation for the retinal RIAs was 12.9%. Protein assay was done on a portion of the retinal homogenate by the Bio-Rad

Figure 5. Somatostatin-like immunofluorescence in a cryostat section of the guinea pig retina. SLI in fibers is observed in substrata of the IPL: lamina 1 (a), lamina 3 (b), lamina 5 (c), and in oblique fibers (d) which span the full thickness of the IPL. Magnification $\times 600$.

method which utilized Coomassie brilliant blue as the indicator reagent (Bio-Rad Laboratories, Richmond, CA). The reference protein was bovine serum albumin.

Chromatography. Gel filtration chromatography of guinea pig retinal SLI was carried out using two conditions: first, a 70×1 cm column of Bio-Gel P6, 100 to 200 mesh (Bio-Rad Laboratories), with 0.1 M ammonium acetate/acetic acid buffer, pH 3.0, at room temperature, and second, a 70×1 cm column of Bio-Gel P10, 200 to 400 mesh, with 8 M urea/50 mM sodium phosphate buffer, pH 7.4, at room temperature. Separate lyophilized extracts of 16 adult guinea pig retinas each were dissolved in 1.0 ml of the column eluents before chromatography. RIA was performed on each eluate fraction.

Immunoaffinity chromatography of adult guinea pig retinal SLI was done by a previously reported method (Rorstad et al., 1979) using sheep anti-somatostatin immunoglobulin coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Dorval, Canada) as the immuno-adsorbent. An extract of six guinea pig retinas was neutralized with 0.1 M sodium phosphate buffer, pH 7.4, and was applied to the column at 4°C . The column-washing buffer was 0.1 M sodium phosphate buffer, pH 7.4. The adsorbed SLI was eluted from the column by 0.1 M HCl. The fractions containing SLI were pooled and lyophilized.

Reversed phase high pressure liquid chromatography (HPLC) of crude guinea pig retinal extracts and of SLI purified by immunoaffinity chromatography of a guinea pig retinal extract was carried out using a Waters Associates apparatus with a model 441 absorbance detector, and a 0.4×30 cm μ Bondapak C18 column. The mobile phase consisted of 0.1% trifluoroacetic acid (TFA; Pierce Chemical Co., Rockford, IL) and acetonitrile (HPLC grade; Fisher Scientific Ltd., Fair Lawn, NJ). Lyophilized samples for chromatography were dissolved in 0.1% TFA prior to injection. The column was eluted isocratically at 0.1% TFA/20% acetonitrile for 10 min, followed by a linear gradient to 0.1% TFA/30% acetonitrile over 30 min at a flow rate of 1 ml/min for separation of the major species of SLI. Fractions of 1 ml were collected, evaporated to dryness, redissolved in RIA buffer, and assayed for SLI. Somatostatin-14 (Beckman) and somatostatin-28 (Peninsula Laboratories Inc.,

San Carlos, CA) were used as chromatography standardization markers.

Immunohistochemistry. A total of 35 fetal and mature animals were studied for SLI localization. Posterior eye cups were immersed in 2% paraformaldehyde containing 0.1 M DL-lysine and 0.1 M sodium periodate in 0.1 M phosphate buffer at pH 6.4 (McLean and Nakane, 1974) for 24 hr at 4°C and stored in 30% buffered sucrose. In three experiments, colchicine ($10 \mu\text{g}$ in $5 \mu\text{l}$) was injected intraocularly in 8-week-old animals, 24 to 30 hr prior to sacrifice. Eye cups were sectioned transversely in a cryostat at 10 to $18 \mu\text{m}$ or the retinae were dissected free. Flattened retinae or cryostat sections were mounted and dried on warmed gelatin-coated slides and washed in phosphate-buffered saline (PBS) prior to immunohistochemical treatment. Sections and flat mounts were reacted according to the indirect immunofluorescence method (Coons, 1958) or the peroxidase-antiperoxidase (PAP) method (Sternberger, 1979). For indirect immunofluorescence, cryostat sections and flat mounted retinae were incubated in primary rabbit antiserum to somatostatin diluted 1:400 (antiserum 1), or 1:20 to 1:50 (antiserum 2) in 0.02 M PBS containing 0.1% Triton X-100 for 12 to 24 hr at 4°C . Secondary antiserum was goat anti-rabbit IgG conjugated to fluorescein isothiocyanate (Miles Laboratories, Elkhart, IN) applied at a dilution of 1:500 in 0.02 M PBS containing 0.1% Triton X-100 for 30 min at 37°C in a humid atmosphere.

For immunoperoxidase, flat mounted specimens were incubated in primary antiserum to somatostatin, diluted 1:400 (antiserum 1) or 1:20 (antiserum 2) for 24 to 48 hr at 4°C . The tissues were washed twice for 15 min. Secondary incubations in goat anti-rabbit IgG, diluted 1:50, and then in rabbit PAP complex (Chappel), diluted 1:500, were each carried out for 12 to 14 hr at room temperature. Following the conventional diaminobenzidine reaction, cryostat sections and flat mounts were washed in 0.02 M PBS and coverslipped in 0.1 M phosphate buffer-glycerin mixture.

Primary antisera against somatostatin which were used for immunohistochemistry were obtained from two sources. Antiserum from ImmunoNuclear Corp. (Stillwater, MN; rabbit 7747, lot no. 31100)

Figure 6. Somatostatin-like immunofluorescence in transverse cryostat sections of the guinea pig retina. A somatostatin-like-immunoreactive fiber (arrowheads) in a through focus series (*a* to *c*) is continuous between the ONL and OPL (*a*), INL (*b*), and IPL (*c*). Fibers are present in the NFL (*d*) and are continuous between the NFL and IPL (*e*). Magnification $\times 600$.

shows central molecular specificity (residues Phe⁶ to Phe¹¹ of somatostatin-14) as does the Arnold batch M4 antiserum used for RIA (Rorstad, 1983). It recognizes both somatostatin-14 and somatostatin-28. The second antiserum, from Japan Immunoresearch Laboratory Co. Ltd. (Takasaki, Japan; lot no. JG-1) has not been fully characterized although cross-reactivities with a variety of peptides were previously determined to be absent (Inagaki et al., 1981). The immunohistochemical findings obtained with the lots of antisera did not differ.

Nonspecific immunoreactivity was ruled out by comparing tissues reacted with anti-somatostatin versus control serum. The latter was diluted antiserum preabsorbed with 5×10^{-6} M synthetic somatostatin-14 (Sigma Chemical Co., St. Louis, MO).

Results

Characterization: Chromatography of retinal SLI

Gel filtration chromatography, using acidic elution conditions, revealed three peaks of SLI eluting at volumes similar to those of somatostatin-14 and somatostatin-28 and at the column void volume (Fig. 1). The somatostatin-28-like peak accounted for 59% of the total eluted SLI. Gel filtration chromatography in 8 M urea (50 mM sodium phosphate buffer, pH 7.4) was carried out to assess whether the peaks of SLI which eluted in the void volume and the position of somatostatin-28 under acidic elution conditions would persist under conditions more certain to dissociate noncovalent bonds. The elution pattern of retinal SLI was substantially the same as that obtained using an acidic elution buffer.

HPLC of a crude adult guinea pig retinal extract demonstrated three peaks of SLI, of which two of the retention times corresponded to those of somatostatin-14 and somatostatin-28 (Fig. 2). The third peak of SLI eluted at a later retention time,

comparable to that of larger proteins, such as bovine serum albumin and the majority of retinal proteins. The somatostatin-28-like peak accounted for 63% of the total SLI eluted by HPLC. HPLC of adult guinea pig retinal SLI, partially purified by immunoaffinity chromatography, again revealed peaks of somatostatin-14- and somatostatin-28-like material, with the somatostatin-28-like peak representing 59% of the total eluted SLI.

Localization. Both immunofluorescence and immunoperoxidase techniques revealed the presence of SLI in cells and processes in the guinea pig retina in transverse cryostat sections and in flat mounts. SLI in cell bodies observed by each approach was located only in the peripheral retina, within 0.5 mm of the ora serrata. The cell density determined in flat mounts was approximately 35 cells/mm² in the peripheral retina with no prevalence in any particular retinal quadrant. Transverse sections of the peripheral retina revealed the presence of cells in three layers. Among the innermost row of the INL were widely scattered perikarya 10 to 12 μ m in diameter (Fig. 3*a*). Within the middle third of the IPL were elongated cells 9 $\times$ 14 μ m in diameter (Fig. 3*b*). Reactive cells within the GCL were 11 to 13 μ m in diameter with the strongest immunoreactivity in that portion of their perikaryal cytoplasm facing the IPL (Fig. 3*c*).

Somatostatin-like-immunoreactive cell processes viewed in flat mount preparations formed a dense network of beaded fibers in the peripheral retina (Fig. 4). Three or four primary dendrites branched from each cell body and ramified in an elongated field. Some processes extended a distance of 0.4 mm from the perikarya toward the central retina. Other peripherally directed fibers paralleled the ora serrata. Reactive fibers were present in all regions, although their numbers decreased cen-

Figure 7. SLI in a PAP-treated flat mount of the guinea pig retina. SLI is seen in fibers in the NFL radiating in the direction of the optic disc. P, far peripheral retina; MP, mid-peripheral retina. Magnification $\times 420$.

Figure 8. Somatostatin-like immunofluorescence in a cryostat section of the guinea pig optic disc. Fibers (arrowheads) are present in both the retina (R) and the optic disc (OD). The border between these regions is demarcated (dashed line). Magnification $\times 600$.

trally. Somatostatin-like-immunoreactive processes in the IPL, seen in transverse sections, formed bands adjacent to the INL (layer 1) (Fig. 5, *a* and *b*), in midlayer (layer 3) (Fig. 5*b*), and adjacent to the GCL (layer 5) (Fig. 5*c*). Other IPL processes commonly took long oblique courses spanning the thickness of the IPL (Fig. 5*d*). INL cells extended neurites mainly to layer 1 and perhaps to layers 3 and 5, although individual cells were not seen ramifying in all three layers. Processes of cells located in the IPL extended from opposite poles of the somata into IPL layer 3. Processes of cells in the GCL ramified in layer 5 of the IPL and possibly in layer 3. Pretreatment with colchicine failed to reveal the presence of immunoreactive somata or processes in locations not already visualized in the absence of treatment. However, its use did result in intensification of the reactions (Fig. 4*b*).

In addition to the conventional distribution of processes in the strata of the IPL noted above, SLI processes were uniquely distributed in several other retinal layers. These included some in the outer nuclear layer (ONL), the outer plexiform layer

(OPL), the INL, the GCL, and the nerve fiber layer (NFL). Fibers in the ONL extended one-third the distance through the layer (Fig. 6*a*). Processes in the OPL spread for short distances in the middle of the layer (Fig. 6*b*). Their cell bodies of origin were not seen; neither horizontal nor bipolar cells were immunoreactive. In fortuitous thick cryostat sections fibers could be followed between the IPL and ONL (Fig. 6, *a* to *c*). Most strikingly and not infrequently, beaded cell processes were observed spanning long distances within the NFL (Figs. 5*b* and 6*d*). Occasionally, these penetrated through the GCL to enter the IPL (Fig. 6*e*). In flat mounts some NFL fibers were seen to radiate toward the optic disc (Fig. 7). These sometimes crossed branching dendritic processes located at a deeper, more scleral, focal plane. Connections were not seen linking cell bodies in the GCL and processes in the NFL. In favorable sections through the optic disc and optic nerve (Fig. 8) beaded fibers were also observed, although they were in very small numbers.

Results of localization of retinal SLI in somata and processes are summarized schematically in Figure 9.

Figure 9. Schematic drawing summarizing somatostatin-like-immunoreactive structures present in the guinea pig retina in sectional view (A) and in flat mount view (B). OD, optic disc.

Figure 10. Concentration of SLI and protein in the prenatal guinea pig retina. Extraction and assays for SLI and protein were as described under "Materials and Methods." A, Retinal SLI expressed as femtomoles per milligram of retinal protein. B, Retinal SLI expressed as femtomoles per retina ($\circ$), and retinal protein expressed as milligrams per retina ($\square$). Each point represents the mean of data from 3 to 11 experimental samples. The vertical lines indicate the SEM of data from 4 to 11 experimental samples. The shaded bar in A indicates the detection limit of the RIA.

Development. SLI was detectable by RIA in the fetal guinea pig retina throughout the period of gestation studied, which ranged from 38 to 68 days after conception. The content of SLI per retina remained relatively low and stable from 38 to 52 days of gestation (Fig. 10B). From between 52 and 56 days of gestation to birth the content of SLI per retina increased considerably. In contrast, the protein content per retina increased progressively from 38 to 68 days after gestation. Expressed as retinal SLI per milligram of retinal protein, an initial decrease of SLI concentration occurred from days 38 to 52 of gestation, followed by an increase in SLI concentration contin-

uing until birth (Fig. 10A). The retina of pregnant female guinea pigs contained 140 ± 14.6 fmol of SLI/mg of protein (mean $\pm$ SEM, $n = 17$ animals).

HPLC of an extract of 24 fetal guinea pig retinas obtained at 56 days of gestation was performed to determine the predominant molecular form of somatostatin present during the early part of the period of rapid increase in fetal retinal SLI. As was the case in the adult animal, somatostatin-28-like immunoreactivity accounted for the majority of fetal retinal SLI, representing 55 and 60% of total SLI in two RIAs of the HPLC fractions.

Figure 11. Somatostatin-like immunofluorescence in the fetal retina at the 56th day of gestation. SLI is present in cell bodies in the GCL and the INL, and in some IPL fibers (arrowheads). Magnification $\times 450$.

Figure 12. Theoretical scheme of possible sources of SLI in fibers in the NFL and in other layers of the guinea pig retina. The cellular bases for observed SLI may reside in one or more of the following: 1, ordinary ganglion cells; 2, displaced ganglion cells; 3, biplexiform ganglion cells; 4, associational ganglion cells; 5, interplexiform cells; or 6, centrifugal fibers. Omitted are the several structural classes of amacrine cells.

Although SLI was indicated by RIA analyses at the 38th day of gestation, immunohistochemical localization in cryostat sections was only suggested in fibers in the IPL at 52 days and not clearly discernible until the 56th day. At 56 days SLI was present peripherally in cryosections (Fig. 11) in cells in the INL, IPL, and GCL. Fiber distributions in the IPL were like that in the adult retina. The earliest flat mounts examined at 59 days, like those of the adult retina, contained cell bodies only in the periphery.

Discussion

Characterization. Comparison of our chromatographic data with other published studies reveals a considerable interspecies variability in the proportion of different forms of retinal so-

matostatin-like-immunoreactive peptides. Under four chromatographic conditions (acidic and denaturing gel filtration, HPLC, and combined immunoaffinity chromatography and HPLC), the predominant form of SLI present in extracts of guinea pig retinas co-eluted with somatostatin-28. This finding contrasts with observations on rat retinal extracts, that more than 95% of total SLI co-eluted with somatostatin-14 (Rorstad et al., 1979; Shapiro et al., 1979; Patel et al., 1981; Sagar et al., 1982). No chromatographic peak corresponding to somatostatin-28 was observed in these studies. Interestingly, similar investigations of SLI in non-retinal regions of the rat central nervous system have suggested that a somatostatin-28-like peptide constitutes from 12% to 25% of total brain SLI (Spiess and Vale, 1980; Patel et al., 1981; Trent and Weir, 1981). Sagar

et al. (1982) found that a somatostatin-28-like peptide accounts for 15 to 17% of rabbit retinal SLI. Goldfish and frog retinas contain substantial amounts of a somatostatin-like peptide larger than somatostatin-14 (Yamada et al., 1980; Yamada and Basinger, 1982). The highest proportion of somatostatin-28 relative to somatostatin-14-like immunoreactivity in the retina has been observed in studies of the bovine retina, in which 93% of the SLI co-eluted with somatostatin-28 (Marshak et al., 1983). In the same study structural characterization indicated that the predominant somatostatin in the bovine retina was almost certainly somatostatin-28. The peak of guinea pig retinal SLI, which eluted at the void volume of gel filtration chromatography and which had a longer retention time on HPLC, may be similar to the large form of somatostatin in rat nervous tissue reported as having a molecular weight of from 12,000 to 14,000 (Spiess and Vale, 1980; Patel et al., 1981; Trent and Weir, 1981). A comparable peak of large SLI eluting earlier than somatostatin-28 on gel filtration chromatography had previously been observed in low concentration in extracts of rat (Rorstad et al., 1979; Patel et al., 1981) and rabbit retinas (Sagar et al., 1982).

Localization. Confidence in the validity of the specificity of localization of SLI is enhanced in this study by the concurrence between recognition sites and molecular specificity in the antiserum used for RIA and at least one of those employed for immunohistochemistry. The second antiserum used for immunohistochemistry, although not extensively characterized, resulted in localizations comparable to those of the first. Few descriptions of SLI in the mammalian retina have uncovered the presence of cell bodies as presented here. In a preliminary report (Spira et al., 1982), SLI was reported to be absent from perikarya of the guinea pig's retina although it was readily evident in fibers, findings in essential agreement with those of Tornqvist et al. (1982). The failure to observe cells previously in the guinea pig is probably due to their peripheral distribution and scant numbers. With respect to the negative findings of others, it should be pointed out that positive results are less likely to be obtained with the use of sections as opposed to flat mounts. Re-examination of other species might well reveal the presence of perikarya if peripheral retinal sections or flat mounted specimens were used. Since colchicine pretreatment failed to reveal newly reactive structures centrally, although it did enhance the intensity of reactions, the conclusion that somatostatin-like-immunoreactive cells are limited to the retinal periphery seems justified. Although the possibility of a different preservation of immunoreactivity in the central as opposed to the peripheral retina cannot be excluded, our care in removing vitreous to enhance fixative penetration and the localization of other peptides in cells in contiguous sections of the central retina (Shimizu et al., 1983) diminish the likelihood of an artifactual pattern of cell distribution. The peripheral distribution of SLI cells contrasts to the general dispersal of cells containing conventional neurotransmitters. Like the somatostatin-like-immunoreactive cells in the guinea pig and rat, substance P-immunoreactive cells in the chicken are also limited to a peripheral location (Ishimoto et al., 1982b).

Somatostatin-like-immunoreactive cells in the guinea pig retina appear to include two or three types of peripherally located amacrine cells. Some are ordinary amacrine cells with processes distributed to one or more layers of the IPL. The presence of fibers between the ONL and IPL suggests that some reactive cells in the innermost layer of the INL could be interplexiform cells (Boycott et al., 1975). Recent re-investigation of the rat retina provides some evidence of somatostatin-like-immunoreactive interplexiform cells (Sagar et al., 1983), but we have not observed reactive cells in the INL giving rise to fibers in the two plexiform layers of the guinea pig. Cells present within the IPL appear similar to the interstitial ama-

crine cells described by Ramón y Cajal (1972) in the ox. Their association with SLI has not previously been shown, although Brecha et al. (1982) localized substance P immunoreactivity to similar cells in the monkey retina. Cells in the GCL could be either displaced amacrine cells or ganglion cells. Indirect support of their identification as amacrine cells was the absence of axonal processes emanating from their cell bodies. However, the failure to see a direct connection between stained fibers in the NFL and cells in the GCL could have been due to technical problems or to an origin of axons from dendrites.

The somatostatin-like-immunoreactive fibers in the NFL, optic disc, and optic nerve could all be part of the axonal projections of typical ganglion cells. If so, this would represent a unique finding of a peptide-like immunoreactivity within projection fibers from the retina to the brain. However, serious consideration should be given to the possibility that the fibers seen in the optic disc and NFL are part of an efferent system with a cell origin located more centrally within the brain. It is widely accepted that a centrifugal fiber system exists in birds and submammalian orders. Munz et al. (1982), Ball and Stell (1983), and Stell et al. (1984) have reported the existence of an efferent neuropeptide-containing (luteinizing hormone-releasing hormone and FMRF-amide (molluscan cardioexcitatory polypeptide-like peptides) system in several teleost fishes. The teleostean efferent fibers course from the NFL obliquely through the IPL and the INL to reach the OPL and ONL. There is controversy over the existence of a comparable centrifugal system in mammals. Itaya (1980), however, succeeded in retrograde labeling of pretectal cells in the rat by intraocular horseradish peroxidase injection in well controlled experiments. The retinal course of the guinea pig somatostatin-like-immunoreactive fibers bears a similarity to the course of the teleostean efferents, although it still remains to be proven that the processes in the guinea pig which extend from the NFL to the IPL are continuous with those extending from the IPL to the ONL. Ultimately, surgical lesions applied to the optic nerve may help to ascertain whether any of the SLI fibers in the guinea pig are extraretinal in origin.

Should the fibers in the NFL prove not to be part of a centrifugal system, it will be necessary to explain the cellular basis for the presence of fibers (1) between the IPL and ONL and (2) joining the NFL and IPL. In each case there are alternative possibilities for their origins. A simple explanation for the occurrence of the IPL-to-ONL set of fibers is that they are derived from a new variety of interplexiform cells, one which extends processes into the ONL, although, as discussed, we have no evidence of reactive interplexiform cells. A second possible explanation for the observed distribution of these fibers is that they are dendritic processes of biplexiform ganglion cells, described in Golgi preparations of the squirrel monkey retina by Mariani (1982) as ganglion cells with some dendritic processes extending to the base of the rod photoreceptors in the OPL and others terminating in the IPL. There are also two possible sources for fibers passing between the NFL and the IPL. Some of the presumed amacrine cells in the INL may in fact be displaced ganglion cells whose axons pass through the IPL to reach the NFL. However, as was the case for cells in the GCL, no continuity has been seen between INL-reactive cells and these fibers. Alternatively, there is the intriguing possibility that NFL-to-IPL fibers are processes of "associational" ganglion cells, as suggested by Krisch and Leonhardt (1979) in the rat. These have been described, in the dog and human retina in Golgi preparations, as ganglion cells which project intraretinally with axons passing through the NFL and GCL to terminate 0.5 to 6 mm distant on primary dendrites of other ganglion cells (Gallego and Cruz, 1965). The perikarya of associational ganglion cells are present only in the peripheral retina and thus match in distribution the somatostatin-like-

immunoreactive cells in the guinea pig. The theoretical sources for SLI in fibers are summarized in Figure 12. Not all need to be present to account for the observed distributions; e.g., the combination of a somatostatin-like-immunoreactive centrifugal fiber system and several classes of amacrine cells or, alternatively, of interplexiform, amacrine, and projecting and associational ganglion cells would account for all of the somatostatin-like-immunoreactive cells and processes observed.

Development. The development of somatostatin-like-immunoreactive peptide in the guinea pig retina occurs in three phases: prior to 56 days of gestation, 56 days to birth, and postnatally. Activity is first detected during the 6th week of gestation with SLI per milligram of retinal protein declining significantly ($p < 0.01$) between the 38th and 50th days of gestation. Somatostatin content per retina remains constant during this period in the face of rising protein levels. It is not clear why localization of somatostatin-like-immunoreactive structures is absent at this early stage. Its absence suggests that SLI at this age either is lost during tissue processing, is inaccessible to immunohistochemical recognition, or is present in concentrations too low to be visualized. During this initial period in the fetal guinea pig amacrine cell synapses in the IPL are just beginning to form (Spira, 1975). The initial presence of somatostatin-like-immunoreactive neurons in the rat (Kuwayama et al., 1981) also coincides with the postnatal development of its synapses (Weidman and Kuwabara, 1968). SLI in its early forms may have an ontogenetic role distinct from that of neurotransmission, a possibility first raised by Takatsuki et al. (1981) for the prenatal SLI they found in the brainstem of the rat. The second fetal phase in the guinea pig is marked by a rapid, real accumulation of SLI and an apparent enhancement of somatostatin gene expression. A somatostatin-28-like peptide is the predominant molecular form of retinal SLI during the early part of this development phase, as is the case in the adult animal. This second phase is accompanied by the onset of immunohistochemically detectable activity. During these late fetal stages, photoreceptors have well developed outer segments, and amacrine cell synapse formation is at its peak (Fry and Spira, 1982). At these developmental stages various neurotransmitter systems are making or have already made their appearance in the guinea pig. For example, acetylcholinesterase activity is detectable by 43 days and by 54 days is extensive in the IPL (Spira, 1976). Dopamine begins to accumulate at 45 days and dopaminergic cells are detectable microscopically by 50 days (Patten et al., 1982; Parkinson et al., 1985). Among the neuropeptides, substance P is detectable considerably earlier than somatostatin (unpublished observation). At the outset of the third stage of SLI accumulation, at birth, SLI levels are two-thirds of the pregnant adult values.

Preliminary results indicate that the onset of patterned visual stimulation at birth (eyelids are open) does not appear to lead to an immediate change in the balance between the rates of synthesis and turnover of somatostatin-like peptides. SLI levels in the neonate appear similar to those in the term fetus. Several neurotransmitters are known to be synthesized and turned over more rapidly in response to light. For example, in both the adult and newborn guinea pig, dopamine *in vivo* synthesis and *in vitro* turnover are both stimulated by light (Parkinson et al., 1984). Somatostatin turnover, however, is considered to be slow (Yamada and Basinger, 1982), but the extent to which it might be stimulated by light is not known. Lake and Patel (1979) failed to detect differences in SLI at different times of the day. In contrast, at least two other neuropeptides, substance P and thyrotropin-releasing hormone, are responsive to illumination levels. Substance P release from the isolated perfused rabbit retina is enhanced by illumination (Schenker and Leeman, 1981). Thyrotropin-releasing hormone immunoreactivity in the developing rat is low at the 8th postnatal day and high at 38

days, but the increase does not occur in dark-reared pups (Martino et al., 1980). Similar experiments with dark-reared guinea pigs would reveal whether SLI is also responsive to long-term changes in illumination.

The present study reveals a species specificity of SLI with respect to biochemical form, localization pattern, and appearance in relation to synaptogenesis. Although the specific function of retinal somatostatin-like peptides is unclear at present, they may have important roles in both short- and long-range intra- and extraretinal neuronal communication.

References

- Altschuler, R. A., J. L. Mosinger, D. W. Hoffman, and M. H. Parakkal (1982) Immunocytochemical localization of enkephalin-like immunoreactivity in the retina of the guinea pig. *Proc. Natl. Acad. Sci. U. S. A.* 79: 2398-2400.
- Arnold, M. A., S. M. Reppert, O. P. Rorstad, S. M. Sagar, H. T. Keutmann, M. J. Perlow, and J. B. Martin (1982) Temporal patterns of somatostatin immunoreactivity in the cerebrospinal fluid of the rhesus monkey: Effect of environmental lighting. *J. Neurosci.* 2: 674-680.
- Ball, A. K., and W. K. Stell (1983) Structure of LHRH- and FMRFamide-like immunoreactive fibres in the goldfish retina. *Invest. Ophthalmol. Vis. Sci. Suppl.* 24: 66.
- Boycott, B. B., J. E. Dowling, S. K. Fisher, H. Kolb, and A. M. Laties (1975) Interplexiform cells of the mammalian retina and their comparison with catecholamine-containing retinal cells. *Proc. R. Soc. Lond. (Biol.)* 191: 353-368.
- Brecha, N. C., and H. J. Karten (1983) Identification and localization of neuropeptides in the vertebrate retina. In *Brain Peptides*, D. Kreiger, M. Brownstein, and J. Martin, eds., pp. 437-462, John Wiley & Sons, Inc., New York.
- Brecha, N., A. Hendrickson, I. Floren, and H. J. Karten (1982) Localization of substance P-like immunoreactivity within the monkey retina. *Invest. Ophthalmol. Vis. Sci.* 23: 147-153.
- Buckerfield, J., J. Oliver, I. W. Chubb, and I. G. Morgan (1981) Somatostatin-like immunoreactivity in amacrine cells of the chicken retina. *Neuroscience* 6: 689-695.
- Coons, A. H. (1958) Fluorescent antibody methods. In *General Cytochemical Methods*, J. F. Danielli, ed., pp. 399-422, Academic Press, Inc., New York.
- Dick, E., and R. F. Miller (1981) Peptides influence ganglion cells. *Neurosci. Lett.* 26: 131-135.
- Djamgoz, M. B. A., W. K. Stell, C. -A. Chin, and D. M. -K. Lam (1981) An opiate system in the goldfish retina. *Nature* 292: 620-623.
- Djamgoz, M. B. A., J. E. G. Downing, and D. J. Prince (1983) Physiology of neuroactive peptides in vertebrate retina. *Biochem. Soc. Trans.* 11: 686-689.
- Eriksen, E. F., and L. -I. Larsson (1981) Neuropeptides in the retina: Evidence for differential topographical localization. *Peptides* 2: 153-157.
- Eskay, R. L., and M. C. Beinfeld (1982) HPLC and RIA of cholecystokinin peptides in the vertebrate neural retina. *Brain Res.* 246: 315-318.
- Eskay, R. L., R. T. Long, and P. M. Iuvone (1980) Evidence that TRH, somatostatin and substance P are present in neurosecretory elements of the vertebrate retina. *Brain Res.* 196: 554-559.
- Fry, K. R., and A. W. Spira (1982) The development of synaptic arrays in the inner plexiform layer of the guinea pig retina. *Invest. Ophthalmol. Vis. Sci. Suppl.* 22: 247.
- Galleo, A., and J. Cruz (1965) Mammalian retina: Associational nerve cells in ganglion cell layer. *Science* 150: 1313-1314.
- Glickman, R. D., A. R. Adolph, and J. E. Dowling (1982) Inner plexiform circuits in the carp retina: Effects of cholinergic agonists, GABA and substance P on the ganglion cells. *Brain Res.* 234: 81-99.
- Inagaki, S., S. Shiosaka, K. Takatsuki, M. Sakanaka, H. Takagi, E. Senba, T. Matsuzaki, and M. Tohyama (1981) Distribution of somatostatin in the frog brain, *Rana catesbeiana*, in relation to location of catecholamine-containing neuron system. *J. Comp. Neurol.* 202: 89-101.
- Ishimoto, I., S. Shiosaka, Y. Kuwayama, M. Fukuda, S. Inagaki, H. Takagi, M. Sakanaka, K. Takatsuki, E. Senba, and M. Tohyama (1982a) Phylogenetical development of somatostatin-containing cells

- in the retina from teleosts to mammals: Immunohistochemical analysis. *J. Hirnforsch.* 23: 123–132.
- Ishimoto, I., S. Shiosaka, Y. Kuwayama, M. Fukuda, S. Inagaki, H. Takagi, M. Sakanaka, K. Takatsuki, E. Senba, and M. Tohyama (1982b) Two types of substance P-containing cells and their uneven distribution in the chicken retina: An immunohistochemical analysis with flat-mounts. *Brain Res.* 240: 171–174.
- Itaya, S. K. (1980) Retinal efferents from the pretectal area in the rat. *Brain Res.* 201: 436–441.
- Kanazawa, I., and T. Jessell (1976) Post mortem changes and regional distribution of substance P in the rat and mouse nervous system. *Brain Res.* 117: 362–367.
- Krisch, B., and H. Leonhardt (1979) Demonstration of a somatostatin-like activity in retinal cells of the rat. *Cell Tissue Res.* 204: 127–140.
- Kuwayama, Y., M. Fukuda, S. Shiosaka, I. Ishimoto, Y. Shimizu, H. Takagi, E. Senba, M. Sakanaka, S. Inagaki, and M. Tohyama (1981) Ontogeny of somatostatin in relation to the catecholamine-containing system. *Folia Ophthalmol. Jpn.* 32: 541–547.
- Lake, N., and Y. C. Patel (1979) Somatostatin in the rat retina. *Invest. Ophthalmol. Vis. Sci. Suppl.* 19: 65.
- Lake, N., and Y. C. Patel (1980) Neurotoxic agents reduce retinal somatostatin. *Brain Res.* 181: 234–236.
- Loren, K., K. Tornqvist, and J. Alumets (1980) VIP (vasoactive intestinal polypeptide)-immunoreactive neurons in the retina of the rat. *Cell Tissue Res.* 210: 167–170.
- Mariani, A. P. (1982) Biplexiform cells: Ganglion cells of the primate retina that contact photoreceptors. *Science* 216: 1134–1136.
- Marshak, D. W., J. R. Reeve, J. E. Shively, D. Hawke, M. S. Takami, and T. Yamada (1983) Structure of somatostatin isolated from bovine retina. *J. Neurochem.* 41: 601–606.
- Martino, E., H. Seo, A. Lernmark, and S. Refetoff (1980) Ontogenetic pattern of thyrotropin-releasing hormone-like material in rat hypothalamus, pancreas and retina: Selective effect of light deprivation. *Proc. Natl. Acad. Sci. U. S. A.* 77: 4345–4348.
- McLean, I. W., and P. K. Nakane (1974) Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. *J. Histochem. Cytochem.* 22: 1077–1083.
- Munz, H., B. Claas, W. E. Stumpf, and L. Jennes (1982) Centrifugal innervation of the retina by luteinizing hormone releasing hormone (LHRH)-immunoreactive telencephalic neurons in teleostean fishes. *Cell Tissue Res.* 222: 313–323.
- Parkinson, D., M. Patten, and A. Spira (1984) Effect of dark-rearing on retinal dopamine metabolism in the guinea pig. *Invest. Ophthalmol. Vis. Sci. Suppl.* 25: 285.
- Parkinson, D., A. Spira, J. P. Wyse, and M. Patten (1985) The ontogenesis of the dopaminergic cell in the pre- and postnatal guinea pig. *Int. J. Dev. Neurosci.*, in press.
- Patel, Y., T. Wheatley, and C. Ning (1981) Multiple forms of immunoreactive somatostatin: Comparison of distribution in neural and nonneural tissues and portal plasma of the rat. *Endocrinology* 109: 1943–1949.
- Patten, M., D. Parkinson, P. Wyse, and A. Spira (1982) Ontogenesis of dopaminergic neurons in the fetal and postnatal guinea pig retina. *Invest. Ophthalmol. Vis. Sci. Suppl.* 22: 114.
- Ramón y Cajal, S. (1972) *The Retina of Vertebrates*, S. A. Thorpe and M. Glickstein, eds., pp. 119–120, Charles C Thomas, Publisher Springfield, IL.
- Rorstad, O. P. (1983) Effects of different radioligands on the antigen binding specificity of somatostatin antisera. *J. Immunoassay* 4: 49–63.
- Rorstad, O. P., M. J. Brownstein, and J. B. Martin (1979) Immunoreactive and biologically active somatostatin-like material in rat retina. *Proc. Natl. Acad. Sci. U. S. A.* 76: 3019–3023.
- Rorstad, O. P., J. B. Martin, and L. C. Terry (1980) Somatostatin and the nervous system. In *The Role of Peptides in Neuronal Function*, J. L. Barker and T. G. Smith, Jr., eds., pp. 573–614, Marcel Dekker, New York.
- Sagar, S. M., O. P. Rorstad, D. M. D. Landis, M. A. Arnold, and J. B. Martin (1982) Somatostatin-like immunoreactive material in the rabbit retina. *Brain Res.* 244: 91–99.
- Sagar, S. M., P. E. Marshall, and D. M. D. Landis (1983) Light microscopic immunocytochemistry of somatostatin in the rat retina. *Soc. Neurosci. Abstr.* 9: 802.
- Schaeffer, J. M., M. J. Brownstein, and J. Axelrod (1977) Thyrotropin-releasing hormone-like material in the rat retina: Changes due to environmental lighting. *Proc. Natl. Acad. Sci. U. S. A.* 74: 3579–3581.
- Schenker, C., and S. E. Leeman (1981) Light stimulated release of substance P from the isolated perfused rabbit retina. *Soc. Neurosci. Abstr.* 7: 60.
- Shapiro, B., S. Kronheim, and B. Pimstone (1979) The presence of immunoreactive somatostatin in rat retina. *Horm. Metab. Res.* 11: 79–80.
- Shimizu, Y., W. K. Stell, and A. Spira (1983) Localization of somatostatin, substance P and vasoactive intestinal polypeptide-like immunoreactivity in the guinea pig retina. *Invest. Ophthalmol. Vis. Sci. Suppl.* 24: 223.
- Spies, J., and W. Vale (1980) Multiple forms of somatostatin-like activity in rat hypothalamus. *Biochemistry* 19: 2861–2866.
- Spira, A. W. (1975) *In utero* development and maturation of the retina of a non-primate mammal: A light and electron microscopic study of the guinea pig. *Anat. Embryol.* 146: 279–300.
- Spira, A. W. (1976) The localization of cholinesterase in the retina of the fetal and newborn guinea pig. *J. Comp. Neurol.* 169: 393–408.
- Spira, A., M. Patten, and O. Rorstad (1982) Ontogenesis of somatostatin-like immunoreactivity in the guinea pig retina. *Invest. Ophthalmol. Vis. Sci. Suppl.* 22: 246.
- Stell, W. K., S. E. Walker, K. S. Chohan, and A. K. Ball (1984) The goldfish nervus terminalis: An LHRH and FRMFamide-immunoreactive olfactory pathway. *Proc. Natl. Acad. Sci. U. S. A.* 81: 940–944.
- Sternberger, L. A. (1979) *Immunocytochemistry*, Ed. 2, pp. 104–169, John Wiley & Sons, Inc., New York.
- Takatsuki, K., S. Shiosaka, M. Sakanaka, S. Inagaki, E. Senba, H. Takagi, and M. Tohyama (1981) Somatostatin in the auditory system of the rat. *Brain Res.* 213: 211–216.
- Tornqvist, K., R. Uddman, F. Sundler, and B. Ehinger (1982) Somatostatin and VIP neurons in the retina of different species. *Histochemistry* 76: 137–152.
- Trent, D. F., and G. C. Weir (1981) Heterogeneity of somatostatin-like peptides in rat brain, pancreas and gastrointestinal tract. *Endocrinology* 108: 2033–2038.
- Vale, W., J. Rivier, N. Ling, and M. Brown (1978) Biologic and immunologic activities and applications of somatostatin analogs. *Metabolism* 27 (Suppl. 1): 1391–1402.
- Weidman, T. A., and T. Kuwabara (1968) Postnatal development of the rat retina. An electron microscopic study. *Arch. Ophthalmol.* 79: 470–484.
- Yamada, T., and S. Basinger (1982) Biosynthesis of somatostatin-like immunoreactivity by frog retinas *in vitro*. *J. Neurochem.* 39: 1539–1546.
- Yamada, T., D. Marshak, S. Basinger, J. Walsh, J. Morley, and W. Stell (1980) Somatostatin-like immunoreactivity in the retina. *Proc. Natl. Acad. Sci. U. S. A.* 77: 1691–1695.