Difference in PNA Label Intensity Between Short- and Middle-Wavelength Sensitive Cones in the Ground Squirrel Retina

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Purpose. Peanut agglutinin lectin (PNA) is known for its selective binding to cone cells and to the cone domains of the interphotoreceptor matrix. In the current study, the authors investigated whether there is any difference in PNA binding between color-specific cones of the cone-dominant ground squirrel.

Methods. Consecutive serial sections of the retina of Spermophilus tridecemlineatus were reacted alternately with PNA and antivisual pigment antibodies. The PNA labels associated with short- and middle-wavelength-sensitive cones (S-cones and M-cones, respectively) were compared with fluorescent lectin cytochemistry.

Results. Although all rod-like cells were left unstained, the cones exhibited a specific lectin label. There was, however, a significant difference between the two cone types; the intensity of the ring-like PNA label in the matrix sheath around S-cones significantly exceeded that of the M-cones.

Conclusions. The difference in PNA label intensity indicates a difference in the composition of the matrix sheaths surrounding the two respective cone types. To the authors' knowledge, this is the first report on lectin-cytochemical discrimination of cone matrix sheaths and the first lectin study in the ground squirrel retina leading to the observation that PNA can distinguish the three characteristic photoreceptor types in this animal. In this respect, the rod-like cells of the ground squirrel retina were shown to be no different from rod cells of other species. Invest Ophthalmol Vis Sci. 1993;34:3641-3645.

It has been known that rods and cones can be distinguished on the basis of their differential binding of lectins. Among these, peanut agglutinin (PNA), specific to galactose–galactosamine disaccharide linkages, proved to be highly selective to cones.1 The PNA binding to cones results from at least two components, a cone-specific domain of the interphotoreceptor matrix (cone matrix sheath) and a trypsin-resistant component in the cone outer segment itself (see Hageman and Johnson for review). On the basis of different glycoprotein molecules in the photoreceptor membrane, [3H]-fucose can distinguish between cone classes in the goldfish retina.3 In the pig retina, the PNA label was found only in the outer segments of M-cones.4 No report has been published, however, about a difference in lectin label between the matrix sheaths of various cone subpopulations.

Rods also are ensheathed by a specific interphotoreceptor matrix domain that shows a different carbohydrate composition; it binds wheat germ agglutinin lectin but not PNA.5–7

As in most nonprimate mammals, the cone-dominant ground squirrel retina contains two cone types in addition to the rods.8 The middle-wavelength–sensitive (M- or G) cones are dominating, constituting 90% of the total photoreceptor cell number. The more rarely (5%) occurring cone type (S- or B) is short wave sensitive. Although these two cone types show morphologic differences, the use of special histologic meth-
ods or immunocytochemistry with cone-specific antibodies makes the identification unequivocal.

The antirhodopsin-positive photoreceptors exhibit both rod- and cone-like traits in their morphologic characteristics and synaptic organization and are described as rod-like cells.

A previous study showed that the M-cones in the European ground squirrel (Spermophilus citellus or Citellus citellus) retina are recognized by COS-1, an antibody specific to the middle to long wave-sensitive visual pigment. The S-cones are labeled by OS-2, a short wave-sensitive cone pigment-specific antibody. It is interesting that the rod-like cells also are stained by this cone-specific antibody (von Schantz et al, unpublished data). To our knowledge, lectins have not been used previously to study photoreceptors in the ground squirrel.

MATERIALS AND METHODS

Adult ground squirrels (Spermophilus tridecemlineatus) were trapped by commercial vendors in Illinois and Wisconsin and kept in the laboratory on a grain-nut diet. Light- and dark-adapted eyes were enucleated after carbon dioxide anesthesia. The anterior segment was removed, and the eyecups were immersed in fixative (4% paraformaldehyde dissolved in 0.1 M Sorensen's phosphate buffer, pH 7.25). After 18 hours of fixation, the central part of the eye was cut into small pieces, dehydrated, and embedded in Durcupan ACM (Fluka, Buchs, Switzerland). Tangential sections at the outer segment level were cut 1 um thick on an ultramicrotome. Consecutive sections were reacted with a PNA-fluorescein isothiocyanate conjugate (Vector Laboratories, Burlingame, CA; 200 µg/ml), the monoclonal antibody OS-2 (1:10,000), or the polyclonal antibody AO (1:10,000). OS-2 recognizes the short wave-sensitive cone pigment, and AO is a rat antirhodopsin antibody. After 2 hours of incubation in the dark with the lectin, coverslips were mounted with glycerol-phosphate-buffered saline and sealed with nail polish. The other sections were incubated for 12 hours in primary antibody and processed by the avidin-biotin peroxidase method with antimouse or antirat secondary antibodies (Vectastain, Vector). After the reaction was visualized with hydrogen peroxide and diaminobenzidine, the sections were dehydrated and coverslips were mounted with Permount (Fisher Scientific, Nepean, Ontario).

Lectin specificity was tested with the appropriate competing hapten sugar, D-galactose (0.3 M). To discriminate between the outer segment and matrix sheath label, some sections were treated with trypsin (0.2%, 30 seconds, 22°C) to remove the sheaths before incubation with the lectin.

Slides processed for lectin cytochemical studies were examined with an Axiophot photomicroscope (Zeiss, Oberkochen, Germany) with the appropriate filter for fluorescein isothiocyanate. The immunoreacted sections were studied with the same microscope with Nomarski differential interference optics. Photographs were taken of identical areas of consecutive sections.

All experimental procedures conformed with the ARVO Resolution on the Use of Animals in Research.

RESULTS

PNA stained most photoreceptor profiles, and only a few visual cells lacked the lectin label. There was signifi-

FIGURE 1. Tangential section of the ground squirrel retina cut at the outer segment level. The PNA cytochemical study shows lectin label associated with most photoreceptors. A small group of the PNA-positive elements are stained more intensely than the others. The lectin label in general is apparently weaker at the upper margin of the photograph because the sectioning plane arrives at the level of the outer segment tips. Magnification X960.
cant heterogeneity among the labeled elements; a few—distributed relatively evenly—were stained more intensely than the others. Those with the more intense PNA label were all hollow, ring-like profiles, in contrast to the less intensely stained ones that generally appeared as filled circles (Fig. 1). The lectin label was abolished completely in sections incubated in the presence of galactose (not shown).

The antirhodopsin antibody recognized the rod-like cells, which constitute a minority of the photoreceptors and have a small photoreceptor domain (Fig. 2A). OS-2 stained the rarely occurring S-cones, which have a relatively larger diameter, and also produced a very weak staining of the rod-like cells (Fig. 2B). The remaining unstained broad elements represented the M-cones. When the immunoreactions were compared with PNA cytochemical results (Fig. 2C) on consecutive sections, the PNA-negative elements could be identified readily as the rod outer segments stained by antirhodopsin. The brightly stained hollow profiles corresponded to the cones that were stained by OS-2 but not AO. The profiles that showed less PNA label corresponded to those cones that were not stained with OS-2 or AO (Fig. 2D).

In sections treated with trypsin before the PNA label, the rings had disappeared from around the cone outer segments, and the individual cone cells were represented only by small fluorescing dots (Fig. 3B). Although most cones showed a relatively strong lectin label, a few of them were unlabeled, and some showed only very weak fluorescence. Trypsinized (Fig. 3B) and untreated (Fig. 3A) consecutive sections showed that the outer segments with the lightly stained dots corresponded to the cones with the brighter PNA-positive matrix sheaths (arrows). The remaining strongly stained outer segments could be matched with the filled, circular profiles showing a less intense lectin label. The rod outer segments and their matrix sheaths had negative results in both sections.

Although the described difference in the PNA label intensity of the matrix sheaths also could be observed in radial sections (not shown), it was much less obvious because of the obscuring effect of the retinal pigment epithelium. Furthermore, in radial sections, the individual cones were sectioned in different planes relative to their long axes, resulting in a gradient of label intensity rather than distinct subclasses of uniformly cut circular profiles.

**DISCUSSION**

It appears that the matrix sheaths of the S-cone cells show very high affinity to PNA in the ground squirrel retina. The sheaths of the M-cones, which constitute most of the ground squirrel photoreceptors, are recognized unambiguously but stained less intensively by the lectin.

PNA lectin is specific to the galactose residues Galβ1-GalNAc. Obviously, both cone types are similar in containing this sugar component in their matrix sheaths, rendering PNA a suitable marker for cones. There must be a subtle but conspicuous difference,
FIGURE 3. PNA label on adjacent sections of the ground squirrel retina without (A) and with (B) trypsin pretreatment. Notice that the trypsinization completely abolished the lectin label of the matrix sheath. After trypsinization, the S-cones were devoid of their distinctive ring-like staining, and the remaining outer segments were stained very weakly. Therefore, it is obvious that different interphotoreceptor matrix domains around the two color-specific cone types are responsible for the observed cone-sheath labeling pattern.

Furthermore, our results indicate that the two PNA-binding components in cones show reciprocal affinity to the lectin, and this is opposite in the two cone types. PNA is highly selective for M-cone outer segments in the pig and ground squirrel retina (this study) but reacts only weakly with the outer segments of the S-cones; however, the matrix sheath of the M-cones is only lightly reactive with the lectin, whereas that of the S-cones exhibits a strikingly high affinity for PNA. Our results also show that the rod-like cells of the ground squirrel retina are quite similar to rods in other species in their lack of affinity for PNA.

Additional studies are needed to elucidate the difference in the organization of the sugar components of the nonsoluble cone matrices that ensheath various cone subtypes in other mammalian retinas.

Key Words
lectin cytochemistry, cone matrix sheath, peanut agglutinin lectin, immunocytochemistry, interphotoreceptor matrix

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References


