*Brain Research*, 522 (1990) 235–240
Elsevier



BRES 15682

# The binding of somatostatin to the mouse retina is altered by the pearl mutation

Malgorzota Kossut[1], Leslie B. Aldrich[2], Tadataka Yamada[2] and Lawrence H. Pinto[3]

[1]*Nencki Institute, Warsaw (Poland),* [2]*Department of Internal Medicine, Division of Gastroenterology, University of Michigan, Ann Arbor, MI 48109 (U.S.A.) and* [3]*Department of Neurobiology and Physiology, Hogan Hall, Northwestern University, Evanston, IL 60201 (U.S.A.)*

(Accepted 16 January 1990)

*Key words:* Somatostatin; Retina; Pearl mutant; Mouse; Binding

Pearl mutants have a night-blind phenotype and abnormal optokinetic nystagmus. Preliminary results from another study[14] showed that the light responses of retinal ganglion cells of pearl mutant mice were affected by bathing the isolated retina with low (<1 nM) concentrations of either somatostatin-14 or -28, whereas the responses in wild-type retinas were affected only by somatostatin-28. In order to test the possibility that these physiological differences resulted from alterations in receptor affinity, we studied the binding of $^{125}$I-[Tyr$^{11}$]-somatostatin-14 and $^{125}$I-[Leu$^{8}$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28 to frozen, unfixed sections of eyes of wild-type (C57BL/6J +/+) and congenic pearl mutant (C57BL/6J-pin $pe^{pin}/pe^{pin}$) mice. As found previously for wild-type mice, specific binding occurred in 3 maxima in pearl mutants: a broad band extending from the retinal ganglion cell to the inner nuclear layer, a narrow and inconstant band over the outer plexiform layer, and a band over the pigment epithelium and choroid. We quantified the label over the inner plexiform layer and found evidence for a single saturable site in both genotypes. However, several results indicate that somatostatin-14 binds more avidly to pearl mutant retinas than to wild-type retinas. In saturation binding studies, $K_d$ for $^{125}$I-[Tyr$^{11}$]-somatostatin-14 was 600 pM in pearl mutants vs 1.5 nM in wild-type; whereas, for $^{125}$I-[Leu$^{8}$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28, $K_d$ was nearly equal in the two genotypes (500 and 625 pM, respectively). $B_{max}$ was nearly equal for both ligands in both genotypes (63–69 fmol/mg protein). Somatostatin-(14) was also a more potent competitor in pearl mutant retinas (against $^{125}$I-[Tyr$^{11}$]-somatostatin-14, $K_d$ was 250 pM for pearl mutants and 1.12 nM for wild-type; against $^{125}$I-[Leu$^{8}$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28 these values were 640 pM and 3.77 nM, respectively). However, somatostatin-28 compared nearly equally in both genotypes (against $^{125}$I-[Tyr$^{11}$]-somatostatin-14, $K_d$ was 510 pM for pearl mutants and 420 pM for wild-type; against $^{125}$I-[Leu$^{8}$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28, $K_d$ was 1.28 and 1.20 nM, respectively). These results indicate that the greater affinity possessed by wild-type retinas for somatostatin-28 over somatostatin-14 is altered by the pearl mutation, elevating the affinity of the retina for somatostatin-14 is altered by the pearl mutation, elevating the affinity of the retina for somatostatin-14.

## INTRODUCTION

A variety of visually defective mutants of the mouse that do not have retinal degeneration have been identified[1]. One of these, the pearl mutant, results from a recessive mutation, and displays a night-blind phenotype when studied with recordings from the optic nerve fibers of anesthetized animals. Pearl mutants also show ultrastructural abnormalities of the basal membrane of the retinal pigment epithelium[16] and of the lamellae of the photoreceptor synapse[17]. Recordings of the light responses of on-center retinal ganglion cells in the isolated, superfused retina[14] have shown that the application of low (<10 nM) concentrations of somatostatin-(28), but not somatostatin-14, increases the amplitude of the responses to a small, centered light stimulus. However, the responses of pearl mutant mice are increased by both molecular forms of somatostatin. We undertook the present studies to examine whether the electrophysiological differences between wild-type and pearl mutant may result from differences in the affinity of retinal receptors for the two molecular forms of somatostatin. Quantitative autoradiography has been employed to study the binding of somatostatin to the retinas of wild-type mice. Both saturation binding and binding competition studies have shown that the dissociation constant for the binding of somatostatin-28 to the wild-type retina is lower than the dissociation constant for the binding[7] of somatostatin-14. In the present study we examined the binding of both molecular forms of somatostatin to the retinas of pearl mutant mice, using simultaneously prepared specimens of both wild-type and pearl mutant genotypes. We found that, in contrast to the binding found in wild-type retinas, somatostatin-14 binds more avidly than somatostatin-28 to pearl mutant retinas.

## MATERIALS AND METHODS

### *Quantitative autoradiography*

Mice from the pearl mutant strain (C57BL/6J-pin $pe^{pin}/pe^{pin}$), 3–7 months old, were maintained with a 12 h light/12 h dark cycle and

*Correspondence:* L.H. Pinto, Department of Neurobiology and Physiology, Hogan Hall, Northwestern University, Evanston, IL 60201, U.S.A.

killed by cervical dislocation 3–4 h after light onset. The eyes were frozen (−140 °C) and mounted side-by-side in a cryostat chuck with eyes from wild-type mice of the congenic C57BL/6J strain. These eyes were sectioned together and mounted together on microscope slides. All further treatments were identical for the eye sections from both genotypes. After coding the slides, the sections were incubated with $^{125}$I-labeled somatostatin analogs according to the procedure of Maurer and Reubi[11], rinsed and dried according to the procedure of Kossut et al.[7] and placed in X-ray cassettes with LKB ultrofilm. The $^{125}$I-[Leu$^{8}$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28 was produced using the chloramine-T method, as previously described[12,18]. The developed autoradiograms were superimposed upon the histological sections in order to identify the part of the autoradiogram that overlay the inner plexiform layer. The optical density of this part of the autoradiogram was measured with a microphotometer (60 $\mu$m aperture), and the distribution of density was measured with an Eikonix scanning microphotometer (7 $\mu$m pixel size). Calibration was accomplished using brain paste standards[15]. In a few cases we used emulsion autoradiography to confirm the localization of the label[7].

## RESULTS

### *Anatomical localization of binding in the retina*

The distribution of total binding in the eye of pearl mutant mice (Fig. 1A–C) was similar to that of wild-type mice (Fig. 1D–F). With both $^{125}$I-[Tyr$^{11}$]-somatostatin-(14) and $^{125}$I-[Leu$^{8}$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28, the maxima of binding occurred in the retinal pigment epithelium–choroid complex (RPE–choroid complex), in a thin and inconstant band over the outer plexiform layer, and in a broad band over the inner plexiform layer and the inner margin of the inner nuclear layer (see Fig. 1A,C). These maxima of total binding did not appear to have different intensities in the central and peripheral parts of the eye (Fig. 1A). Non-specific binding (Fig. 1B) was higher in the RPE–choroid complex than in the retina, but in the retina the non-specific binding was lower and more uniform than total binding. By restricting our photometric measurements to the innermost 60 $\mu$m of the broad band of label in the retina[7] we quantified the binding in the inner plexiform layer.

### *Saturation binding studies*

We measured the binding of $^{125}$I-[Tyr$^{11}$]-somatostatin-(14) to the inner plexiform layer using concentrations from 30 pM to 5 nM. Non-specific binding was determined in the presence of 10 $\mu$M somatostatin-14, and accounted for 30–70% of the total binding. Specific binding was consistently greater in each of 7 paired experiments in pearl mutant retinas than in wild-type retinas for concentrations of 2 nM and less (Fig. 2A). Scatchard analysis of the averaged specific binding data (Fig. 3A) was consistent with the presence of a single class of binding sites in pearl mutant retinas with a dissociation constant ($K_d$) of 600 pM. This dissociation constant is lower than that of wild-type retinas (1.48 nM, see ref. 7). We noted that the Scatchard plots of the averaged binding data for pearl mutant retinas (Fig. 3A), fell above the fitted line for a single site model for the 3 lowest values of bound ligand and below the fitted line for the next 3 higher values. We, therefore, compared the goodness-of-fit of a single site model with the goodness-of-fit of a two-site model, using the 'Ligand' program. For this comparison we used both the data from each of the 7 individual incubations and the averaged data from all incubations. The goodness-of-fit was only slightly

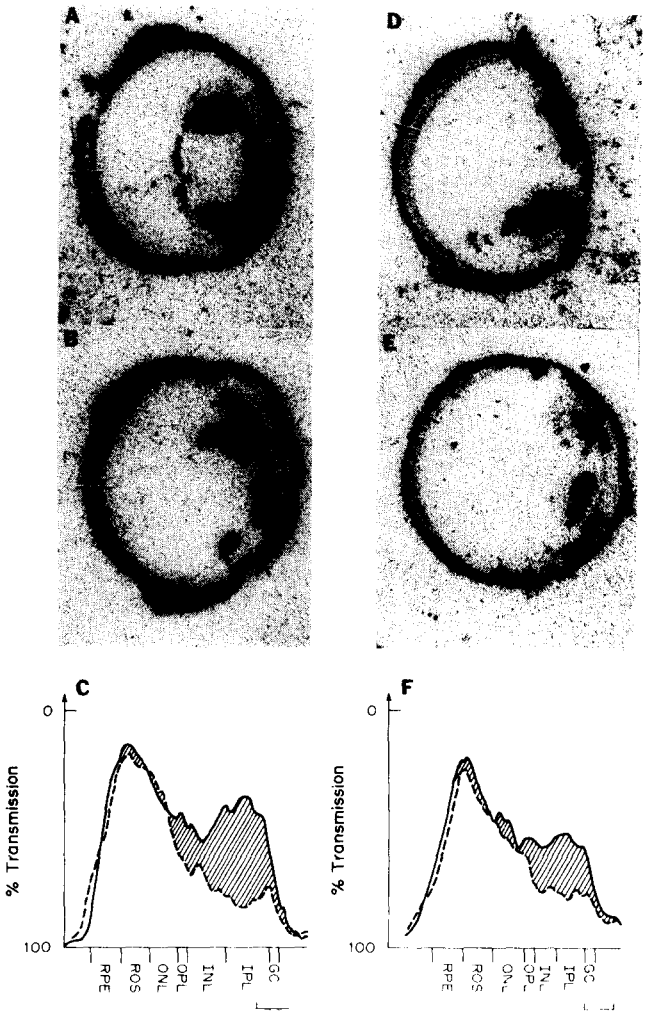


Fig. 1. Film autoradiograms (A, B, D and E) and the distribution of optical transmission (C and F) of sections of a pearl mutant eye (A–C) and a wild-type eye (D–F) that were incubated in $^{125}$I-[Tyr$^{11}$]-somatostatin-14. Total binding is shown in A and D. Non-specific binding (B and E) was obtained in the presence of 10 $\mu$M unlabeled somatostatin-14. The straight lines in the autoradiograms show the path along which optical transmission was measured. The layers of the retina that underlie the scan path were identified from the section and are indicated in the plots in C and F (RPE, retinal pigment epithelium and choroid; ROS, rod outer segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer and GC, ganglion cell layer). Note the high specific binding (hatched) in the inner plexiform layer. Scale bar, 60 $\mu$m.

better for the two-site model, and was, therefore, not considered further. The total number of binding sites ($B_{max}$) in pearl mutant retinas (69 fmol/mg protein) was similar to that found in wild-type retinas (68 fmol/mg protein).

We also measured the binding of $^{125}$I-[Leu$^8$,D-Trp$^{22}$, Tyr$^{25}$]-somatostatin-28 using concentrations from 50 pM to 2.5 nM. Non-specific binding was determined in the presence of 10 $\mu$M somatostatin-28, and accounted for 20–50% of total binding. Specific binding (Fig. 2B) was nearly equal in the two genotypes for each concentration of this ligand. Scatchard analysis (Fig. 3B) revealed the presence of a single class of binding sites with very similar properties in the two genotypes. For pearl mutant retinas the $K_d$ was 500 pM and the $B_{max}$ was 63 fmol/mg protein, values similar to those for wild-type retinas in which $K_d$ was 625 pM and $B_{max}$ was 69 fmol/mg protein[7].

Two-way analysis of variance of the specific binding of each of the two ligands was performed for concentrations of $^{125}$I-[Tyr$^{11}$]-somatostatin-14 of 1250 pM and lower. The amount of specifically bound ligand was greater for pearl mutant retinas than for wild-type retinas (Fig. 2A; ANOVA: $F = 67.5$; $P < 0.001$). No difference was seen between the amount of $^{125}$I-[Leu$^8$,D-Trp$^{22}$,Tyr$^{25}$]- somatostatin-28 specifically bound to pearl mutant retinas and wild-type retinas (Fig. 2B; ANOVA: $F = 0.77$; $P = 0.79$).

*Binding competition studies*

Somatostatin-(14) was a more potent competitor in pearl mutant retinas than in wild-type retinas against the binding of both $^{125}$I-[Tyr$^{11}$]-somatostatin-14 (Fig. 4A) and $^{125}$I-[Leu$^8$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28 (Fig. 4B). This greater potency was seen in each of five experiments with $^{125}$I-[Tyr$^{11}$]-somatostatin-14 and in both of two experiments with $^{125}$I-[Leu$^8$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-(28). The median inhibitory concentration ($IC_{50}$) for somatostatin-14 in pearl mutant retinas was 250 pM against $^{125}$I-[Tyr$^{11}$]-somatostatin-14 and was 1.12 nM against $^{125}$I-[Leu$^8$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28. The corresponding values for $IC_{50}$ in wild-type retinas were 2–3 fold higher, 640 pM and 3.77 nM, respectively[7].

Unlike somatostatin-14, somatostatin-28 was nearly equally potent in competing against both ligands (Fig. 5A,B) in pearl mutant and wild-type retinas. We found

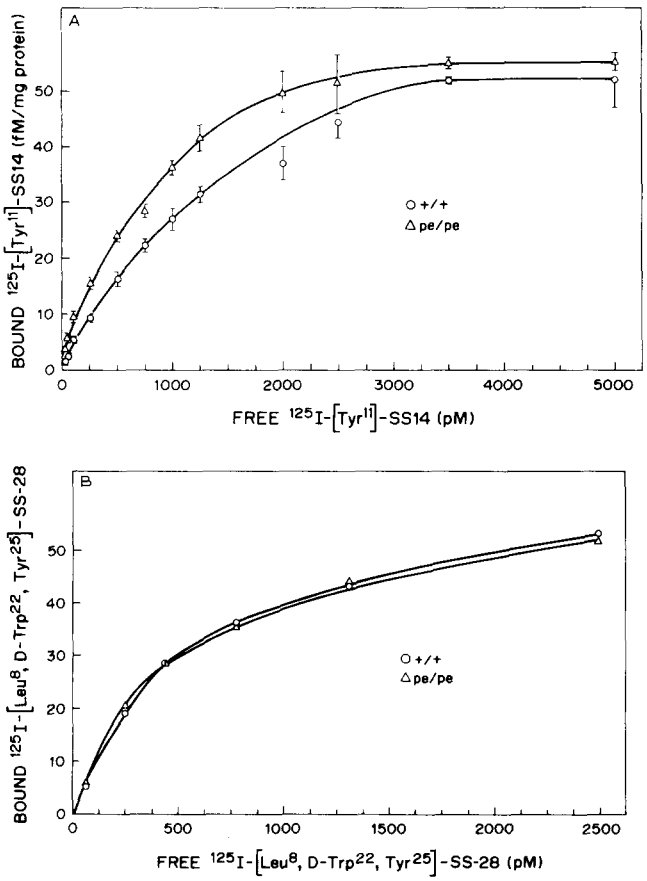


Fig. 2. Specific (total non-specific) binding of $^{125}$I-[Tyr$^{11}$]- somatostatin-14 (A) and $^{125}$I-[Leu$^8$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28 (B) to the inner plexiform layer of wild-type (○) and pearl mutant (△) retinas. Note the greater binding to pearl mutant retinas for the lower concentrations of only $^{125}$I-[Tyr$^{11}$]-somatostatin-14. Average data from 7 experiments in (A) and two experiments in (B).

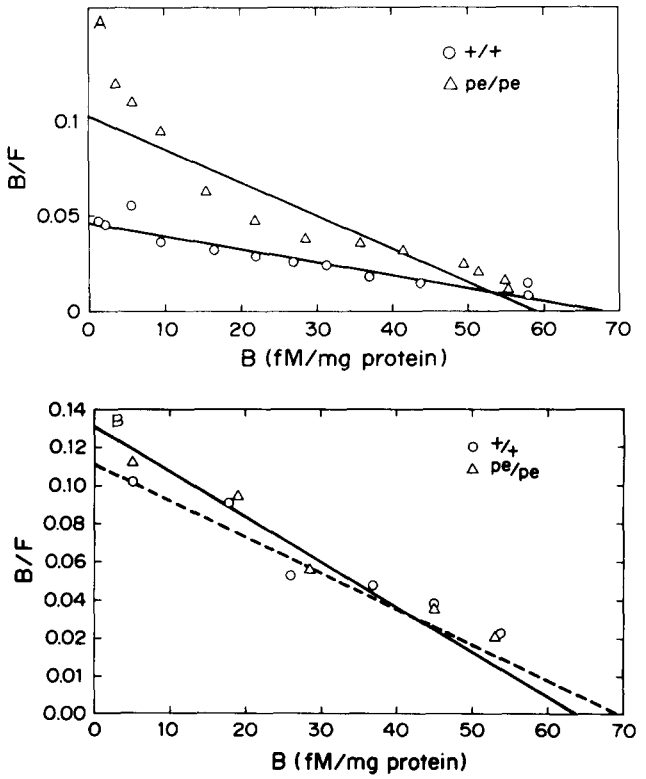


Fig. 3. Scatchard plots of specific binding to the inner plexiform layer of wild-type (○) and pearl mutant (△) retinas for $^{125}$I-[Tyr$^{11}$]-somatostatin-14 (A, taken from Fig. 2A) and $^{125}$I-[Leu$^8$, D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28 (B, taken from Fig. 2B). The interrupted line in B is fitted to the data from wild-type and the solid line to the data from pearl mutants.

this near equality in both of two experiments with $^{125}$I-[Leu$^8$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28 (see Fig. 5B for averaged data). In each of 4 experiments with $^{125}$I-[Tyr$^{11}$]-somatostatin-14 ligand, unlabeled somatostatin-28 was a slightly more potent competitor in wild-type retinas than in pearl mutant retinas (Fig. 5A) and thus had the reverse order of potency, seen in the two genotypes with somatostatin-14. For pearl mutant retinas $IC_{50}$ for somatostatin-28 was 510 pM against $^{125}$I-[Tyr$^{11}$]-somatostatin-14 and was 1.28 nM against $^{125}$I-[Leu$^8$,D-Trp$^{22}$, Tyr$^{25}$]-somatostatin-28. The corresponding values for $IC_{50}$ in wild-type retinas are 420 pM and 1.20 nM, respectively[7].

Two-way analysis of variance of the specific binding of each of the two ligands in the presence of all concentrations of each of the two competitors was performed. For every concentration of somatostatin-28 competitor, the specific binding of $^{125}$I-[Tyr$^{11}$]-somatostatin-14 (Fig. 4A) was significantly less in pearl mutant retinas than in wild-type retinas (ANOVA: $F = 100.2$; $P < 0.001$). The dependence of specific binding of $^{125}$I-[Leu$^8$,D-Trp$^{22}$, Tyr$^{25}$]-somatostatin-28 upon the concentration of somatostatin-14 competitor was significantly different for pearl mutant retinas than for wild-type retinas (Fig. 4B; ANOVA: $F = 14.6$; $P < 0.001$); for competitor concentrations of 100 pM and higher the specific binding was lower in the mutant retinas. The specific binding of $^{125}$I-[Tyr$^{11}$]-somatostatin-14 was slightly less in wild-type retinas than in pearl mutant retinas for every concentration of somatostatin-28 competitor (see Fig. 5A; ANOVA: $F = 5.0$; $P = 0.03$), but the specific binding of $^{125}$I-[Leu$^8$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28 was not different in the two genotypes for every concentration of somatostatin-28 competitor (Fig. 5B; ANOVA: $F = 0.13$; $P = 0.72$).

Somatostatin-(28) (1–14) and bombesin did not measurably compete with the binding of $^{125}$I-[Tyr$^{11}$]-

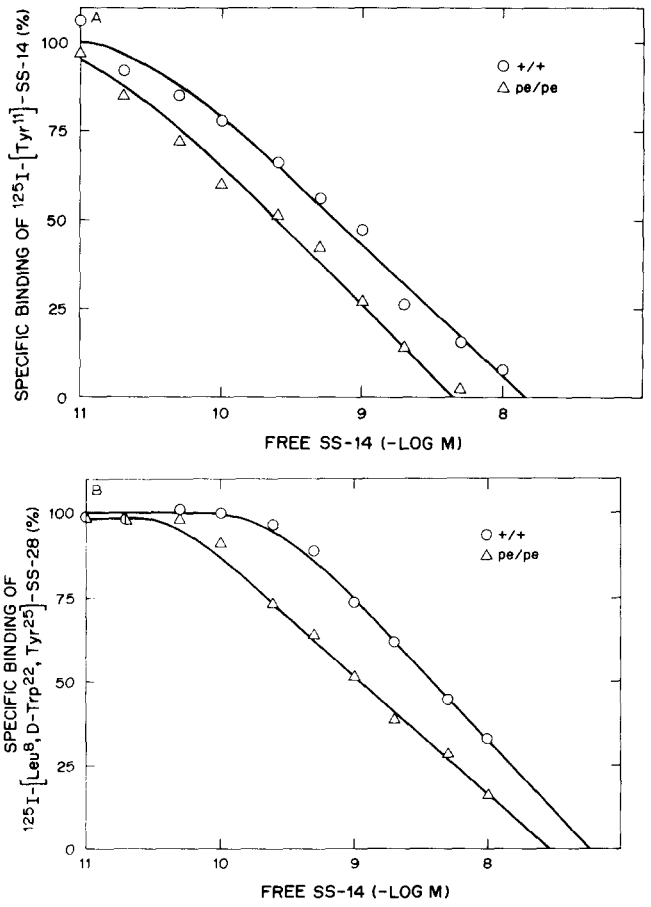


Fig. 4. Competition by somatostatin-14 for the specific binding of $^{125}$I-[Tyr$^{11}$]-somatostatin-14 (A) and $^{125}$I-[Leu$^8$,D-Trp$^{22}$,Tyr$^{25}$]- somatostatin-28 (B) in wild-type (○) and pearl mutant (△) retinas. Average data from 5 experiments in A and two experiments in B. The inhibitory binding constants ($K_i$), calculated for pearl mutant retinas using the formula of Cheng and Prussoff[3] was 240 pM against $^{125}$I-[Tyr$^{11}$]-somatostatin-14 and was 1.2 nM against $^{125}$I-[Leu$^8$, D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28. The corresponding values for $K_i$ in wild-type retinas are 900 pM and 4.59 nM, respectively[7].

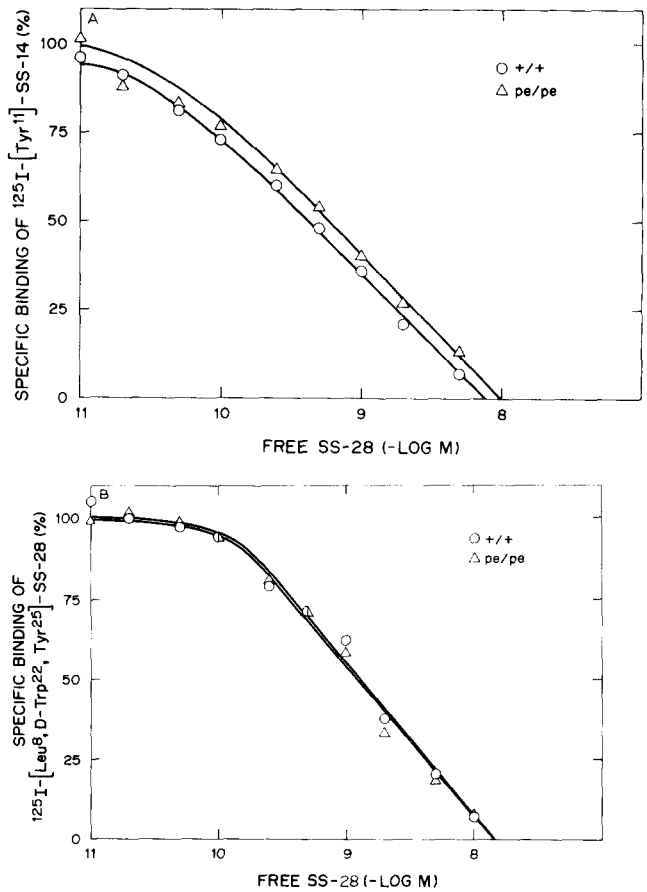


Fig. 5. Competition by somatostatin-28 for the specific binding of $^{125}$I-[Tyr$^{11}$]-somatostatin-14 (A) and $^{125}$I-[Leu$^8$,D-Trp$^{22}$,Tyr$^{25}$]- somatostatin-28 (B) in wild-type (○) and pearl mutant (△) retinas. Average data from 4 experiments in A and two experiments in B. For pearl mutant retinas $K_i$ for somatostatin-28 was 430 pM against $^{125}$I-[Tyr$^{11}$]-somatostatin-14 and was 690 pM against $^{125}$I-[Leu$^8$, D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28. The corresponding values for $K_i$ in wild-type retinas are 350 and 710 pM, respectively.

somatostatin-14 or $^{125}$I-[Leu$^{8}$,D-Trp$^{22}$,Tyr$^{25}$]- somatostatin-28 to pearl mutant retinas or wild-type retinas[7]. Bombesin, in concentrations as high as 1 mM, did not significantly reduce the binding of $^{125}$I-[Tyr$^{11}$]- somatostatin-14.

## DISCUSSION

Pearl mutant mice have served as a useful model for studying congenital night blindness. Although we do not know the basis for this phenotype, we have observed previously that the mutants have a specific electrophysiologically observable abnormality[14]. The light response of on-center retinal ganglion cells is increased in amplitude by somatostatin-28 and not by somatostatin-14 in wild-type animals. However, the responses of mutant animals are affected by both molecular forms. We reasoned that there are at least two explanations for these observations. One possibility is that the structure of somatostatin-14 in wild-type is different from that of the commercially available synthetic compound, based on the structure of somatostatin in other mammalian species. The corollary to this hypothesis is that the pearl mutants synthesize somatostatin-14 that is more similar to that found in the other mammalian species. We examined this possibility in previous studies and found that somatostatin of both wild-type and pearl mutant is identical to the commercially available somatostatin-14 (Aldrich et al., in preparation). An alternative explanation for our electrophysiological finding is that the pearl mutation results in an alteration of the affinity of retinal binding sites for somatostatin-14 and -28. Our current studies support this latter hypothesis.

Saturation binding and binding competition results, obtained with an experimental protocol that employed identically treated pairs of mutant and wild-type retinas, indicate that pearl mutant retinas bind somatostatin-14 more avidly than wild-type retinas. In saturation binding studies the amount of $^{125}$I-[Tyr$^{11}$]-somatostatin-14 ligand bound with concentrations of ligand of 1250 pM and lower was greater in pearl mutant retinas. This increase was reflected in a lower dissociation constant for pearl mutant retinas (600 pM) than for wild-type retinas (1.48 nM). Binding competition results showed that the inhibitory binding constants ($K_i$) for somatostatin-14 were lower in pearl mutant retinas against both $^{125}$I-[Tyr$^{11}$]-somatostatin-14 (240 pM) and $^{125}$I-[Leu$^{8}$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28 (430 pM) than in wild-type retinas (900 pM and 4.58 nM, respectively). However, no differences were found between mutant and wild-type retinas in saturation binding studies using $^{125}$I-[Leu$^{8}$,D-Trp$^{22}$,Tyr$^{25}$]-somatostatin-28 ligand or in the competition of somatostatin-28 against this ligand. We found that somatostatin-28 was slightly more effective in competing against $^{125}$I-[Tyr$^{11}$]-somatostatin-14 in wild-type retinas than in pearl mutant retinas.

The present data do not permit us to say to what degree, if at all, the altered binding of somatostatin is responsible for the night-blind phenotype seen in pearl mutants. Perhaps recordings from retinal interneurons of the mouse that are post-synaptic to the somatostatin-containing amacrine cells will help clarify somatostatin's role in the appearance of the night-blind phenotype in pearl mutants.

The availability of a mutation with altered somatostatin binding may be useful for understanding the role and mechanism of action on this peptide in the retina. Immunohistochemical studies have found somatostatin in the processes of amacrine cells in the inner plexiform layer and in the processes of unidentified cells in the outer plexiform layer[6,13]. The processes of these cells in the inner plexiform layer are presynaptic to amacrine, ganglion and bipolar cells[9,10]. Recently it has become possible to record membrane currents of solitary neurons of these cell types from mammalian retinas (ganglion cells, see ref. 8; bipolar cells, see ref. 5). It would not be surprising if various membrane currents of these cells were modified by somatostatin, as has been found in other neurons[2,4]. The pearl mutation affects the somatostatin receptor molecule and therefore may be useful in studying the mechanism for somatostatin-induced modification of membrane currents.

## REFERENCES


1 Balkema, G.W, Mangini, N.J. and Pinto, L.H., Discrete visual defects in pearl mutant mice, *Science,* 219 (1983) 1085–1087.

2 Bean, B.P., Neurotransmitter inhibition of neuronal calcium current by changes in channel voltage dependence, *Nature (Lond.),* 340 (1989) 153–155.

3 Cheng, Y.C. and Prusoff, W.H., Relationship between the inhibition constant ($K_I$) and the concentration of inhibitor which causes 50% inhibition (*IC*50) of an enzymatic reaction, *Biochem. Pharmacol.,* 22 (1973) 3033–3168.

4 Inoue, M., Nakajima, S. and Nakajima, Y., Somatostatin induces an inward rectification in rate of locus coeruleus neurones through a pertussis toxin-sensitive mechanism, *J. Physiol. (Lond.),* 407 (1988) 177–198.

5 Kaneko, A., Pinto, L.H. and Tachibana, M., Transient calcium current of retinal bipolar cells of the mouse, *J. Physiol. (Lond.),* 410 (1989) 613–629.

6 Kirsch, B. and Leonhardt, H., Demonstration of a somatostatin-like activity in retinal cells of the rat, *Cell Tissue Res.,* 204 (1979) 127–140.

7 Kossut, M., Yamada, T., Aldrich, L.B. and Pinto, L.H., Localization and characterization of somatostatin binding sites in the mouse retina, *Brain Research,* 476 (1989) 780–784.

8 Lipton, S.A. and Tauck, D.L., Voltage-dependent conductances of solitary ganglion cells dissociated from the rat retina, *J.*

*Physiol. (Lond.)*, 385 (1987) 361–391.
9 Marshak, D. and Yamada, T., Characterization of somatostatin-like immunoreactivity in vertebrate retina, *Invest. Ophthalmol. Vis. Sci.*, Suppl., 25 (1984) 11–115.
10 Marshak, D., Synapses of peptide immunoreactive neurons in macaque retina, *Invest. Ophthalmol. Vis.*, Suppl., 27 (1986) 331.
11 Maurer, R. and Reubi, J.C., Brain somatostatin receptor subpopulation visualized by autoradiography, *Brain Research*, 333 (1985) 178–181.
12 Park, J., Chiba, T. and Yamada, T., *J. Biol. Chem.*, 262 (1987) 14190–14196.
13 Spira, A.W., Shimizu, Y. and Rorstad, O.P., Location, chromatographic characterization, and development of somatostatin-like immunoreactivity in the guinea pig retina, *J. Neurosci.*, 4 (1984) 3069–3079.
14 Suzuki, H. and Pinto, L.H., Effects of somatostatin-14 and -28 upon the light responses of retinal ganglion cells and horizontal cells in the isolated retinas of wild-type (C57 Bl/6J), pearl mutant, and pearl revertant mice, *Biophys. J.*, 49 (1986) 367a.
15 Unnerstall, J., Niehoff, D., Kuhar, M. and Palacios, J., Quantitative receptor autoradiography using [$^3$H]ultrafilm: application to multiple benzodiazepine receptors, *J. Neurosci. Methods*, 6 (1982) 59–73.
16 Williams, M.A., Pinto, L.H. and Gherson, J., The retinal pigment epithelium of wild type (C57BL/6J +/+) and pearl mutant (C57BL/6J *pe/pe*) mice, *Invest. Ophthalmol. Vis. Sci.*, 26 (1985) 658–669.
17 Williams, M.A., Gherson, J., Fisher, L.J. and Pinto, L.H., Synaptic lamellae of the photoreceptors of pearl and wild-type mice, *Invest. Ophthalmol. Vis. Sci.*, 26 (1985) 992–1001.
18 Yamada, T., Marshak, D., Basinger, S., Walsh, J., Morley, J. and Stell, W., Somatostatin-like immunoreactivity in the retina, *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 1681–1695.