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Coexpression of opsin- and VIP-like-immunoreactivity in CSF-contacting neurons of the avian brain

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Summary. Cerebrospinal fluid-contacting (CSF) cells in both the septal and the tuberal areas in the brain of the ring dove are labeled by RET-P1, a monoclonal antibody to opsin that reacts with inner and outer segment membranes of rod photoreceptors in a variety of vertebrates. Immunoblot analysis of proteins from diverse brain regions, however, revealed bands of anti-RET-P1 immunoreactivity that did not correspond to opsin. Binding of RET-P1 to opsin-containing membranes, was not inhibited by membranes rich in muscarinic and β-adrenergic receptor proteins (red blood cells, heart, lung) taken from doves. RET-P1-immunoreactive CSF-contacting cells emit a dendritic process that penetrates the ependyma and ends in a knob-like terminal suspended in the ventricle. These cells also possess other processes that penetrate more or less deeply into the neuropil. Additionally, a band of labeled fibers occurs in the external layer of the median eminence. A double-label technique demonstrated that RET-P1-positive cells coexpress VIP-like immunoreactivity. VIP-positive cells in other brain areas are not RET-P1-positive.

Key words: Cerebrospinal fluid-contacting neurons – Monoclonal antibodies – Opsin – VIP – Extraocular photoreceptors – Ring dove – *Coturnix coturnix* – *Anas platyrhynchos*

Solar radiation can penetrate the brain in vertebrates (Hartwig and van Veen 1979). Furthermore, it has been demonstrated repeatedly that nonmammalian vertebrates possess encephalic photoreceptors that lie outside of the retina and the pineal gland (Oliver and Baylé 1982). In several avian species, encephalic photoreceptors control photoperiodic induction of gonadal growth and regression (for review, see Yokoyama et al. 1978; Groos 1982). The spectral sensitivity function of these responses suggests the involvement of a rhodopsin-like pigment (Foster et al. 1985; Foster and Follett 1985; McMillan et al. 1975).

A starting point in the study of encephalic photoreceptors is to establish their location. Direct stimulation of the brain in several avian species indicates that these photoreceptors are concentrated in the ventral hypothalamic region, although there is some evidence of photosensitivity in extrahypothalamic loci as well (Yokoyama et al. 1978; Homma et al. 1979).

Opsin-like-immunoreactivity has been reported previously in outer segments of pineal photoreceptors in birds and other species. In contrast, there is neither structural nor immunochemical evidence for the presence of photoreceptive elements in the hypothalamus (Foster et al. 1987; Korf et al. 1985; Vigh and Vigh-Teichmann 1981; Vigh et al. 1982; 1983; Vigh-Teichmann et al. 1980, 1982, 1983). In the present study, we attempted to localize encephalic photoreceptors by means of immunocytochemistry using two monoclonal antibodies raised against opsin.

We used both indirect immunofluorescence and immunoperoxidase labelling of neural tissue at the light-microscopic level to determine the distribution and specificity of immunoreactive material. Labelled cells were seen in two distinct brain regions, the lateral septum and the infundibular zone of the hypothalamus. Immunoreactive fibers were found in the external layer of the median eminence. Finally, cells that stained positively with an anti-opsin antibody also showed VIP-like immunoreactivity.

Materials and methods

Animals. Brain tissue for the study was from 8 adult male ring doves, 2 adult male Japanese quail (*Coturnix coturnix*), and 1 juvenile domestic Cayuga duck (*Anas platyrhynchos*) kept in laboratory cages at 22–23° C, exposed to alternating periods of light and darkness (on 0600, off 2000 h), and fed ad libitum. Animals were sacrificed between 0900–1100 h.

Fixation solutions. One of the following 2 fixatives was used: 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer pH 7.3–4, or Zamboni's fixative; 1.8% paraformaldehyde and 7.5% picric acid in 0.1 M phosphate buffer pH 7.3. In addition, 2% sucrose was added to the fixative solution when the tissue was to be sectioned frozen. Postfixation solutions were: (1) Zamboni's fixative if perfused with Zamboni's, or (2) 4% paraformaldehyde in 0.1 M sodium bicarbonate buffer pH 10.4 if perfused with the mixed aldehydes (Eldred et al. 1983). Four percent sucrose was added to the postfixative solution for tissues to be frozen for sectioning.

Tissue preparation. Animals were anesthetized with 0.4 cc Chloropent IM (Fort Dodge, Fort Dodge, Iowa) followed

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by 0.3 cc ammonium heparin IV. The animals then were anesthetized deeply with an additional 1.0 cc Chloropent IM and perfused via the carotid artery using a peristaltic pump. The initial perfusion was with 60–75 ml of 0.1 M phosphate buffer pH 7.3–4 at 37° C containing 4% dextran and 2 mg/100 ml sodium heparin salt administered at a rate of 21 ml/min. Thereafter the fixative was administered at a rate of 8.4 ml/min for 10 min. The brain was left in situ for 1–2 h, then removed and immersed in cold (4° C) post-fixative solution for 2–24 h. Tissue destined for frozen sectioning was cryoprotected at 4° C with 20% sucrose in 0.1 M phosphate buffer pH 7.3–4. Brains were sectioned frozen at 50 μm on a Lipshaw sliding microtome equipped with a freezing stage. For the double-labelling study (see below), sections of fixed unfrozen tissue were cut at 60 μm on a vibratome. All tissue was collected in chilled 0.01 M phosphate-buffered 0.9% saline (PBS).

Immunocytochemical processing. Two monoclonal antibodies to opsin, RET-P1 and Rho-ID4, were tested. RET-P1 was raised in mice against the membrane fraction of rat retina; it reacts with rod photoreceptors from a wide range of vertebrates including goldfish, salamander, turtle, mouse, rat and rabbit (Barnstable 1980). RHO-1D4 was raised in mice against bleached membranes of outer segment disks of bovine rod (Molday and MacKenzie 1983). Both antibodies have been shown to react with opsin by inhibition with purified opsin or synthetic opsin corresponding to regions of opsin (Fekete and Barnstable 1983; Hargrave et al. 1986; Molday and MacKenzie 1983). The RET-P1 epitope is located on amino acid residues 4–10; the RHO-1D4 epitope is at the C-terminus. Both RET-P1 and RHO-1D4 reacted positively with dove retinal photoreceptors. A polyclonal antiserum against vasoactive intestinal polypeptide (VIP), produced in rabbit, was obtained from Immunonuclear Corp. (Stillwater, Minnesota).

Tissue was washed in 3 changes of PBS for 1 h to remove excess aldehydes, then exposed for 10 min to either 0.1% hydrogen peroxide or 0.1% sodium borohydride to reduce endogenous peroxidase activity. After preincubation in the appropriate normal serum (for RET-P1 and Rho-ID4 – 10% normal horse serum; for anti-VIP serum – 1% normal goat serum), sections were incubated overnight at 4° C at the following dilutions: RET-P1 1:1000, Rho-ID4 1:10, and anti-VIP 1:3000, with 0.3% Triton X-100 in PBS. In control sections either the primary antibody was omitted, or the sections were treated with diaminobenzidine (DAB) only, or the appropriate normal serum only.

For immunoperoxidase detection of antigenicity, sections then were processed by an avidin-biotin-HRP procedure (Vectastain, Vector Labs, Burlington, California). HRP label was demonstrated using DAB as the chromogen, and in the case of avidin-biotin-HRP procedure, the hydrogen peroxide substrate was generated by a glucose oxidase reaction (Itoh et al. 1979), or by adding H_2O_2. Sections were mounted from buffer, dehydrated, cleared, coverslipped, and viewed under both bright- and dark-field illumination.

For analysis of co-localization of RET-P1 and VIP-like immunoreactivity, a double-label immunofluorescent procedure was used. Sections first exposed for 48 h to RET-P1 were incubated for 60 min in goat anti-mouse IgG conjugated to fluorescein (FITC; Dako Corp, Santa Barbara, California). After washing in buffer, sections were incubated with VIP antiserum (rabbit) for 48 h, followed by sheep-anti-rabbit IgG conjugated to rhodamine (TRITC; Dako Corp.) for 60 min. The order of exposure to opsin and VIP antisera, and their respective secondary antisera, was reversed for alternate sections. Control sections involved omission of the second primary antibody from the above protocol. The procedure established that no RET-P1 FITC fluorescence was visible with the VIP rhodamine filter system and vice versa. Sections were viewed on a Leitz Dialux II microscope equipped for fluorescence with either a H2 (FITC) or N2 (TRITC) cube.

Immunoblots. Brain regions were dissected and frozen on dry ice until use. Frozen tissue samples of approximately equal volumes were homogenized rapidly in SDS sample buffer to lessen proteolytic degradation. Samples were then boiled for a further 2 min and loaded on 10% polyacrylamide gels. Gels were run and nitrocellulose blots prepared and incubated as previously described (Fekete and Barnstable 1983; Drager et al. 1984). Bound peroxidase-conjugated secondary antibody was visualized with 3,3-diaminobenzidine.

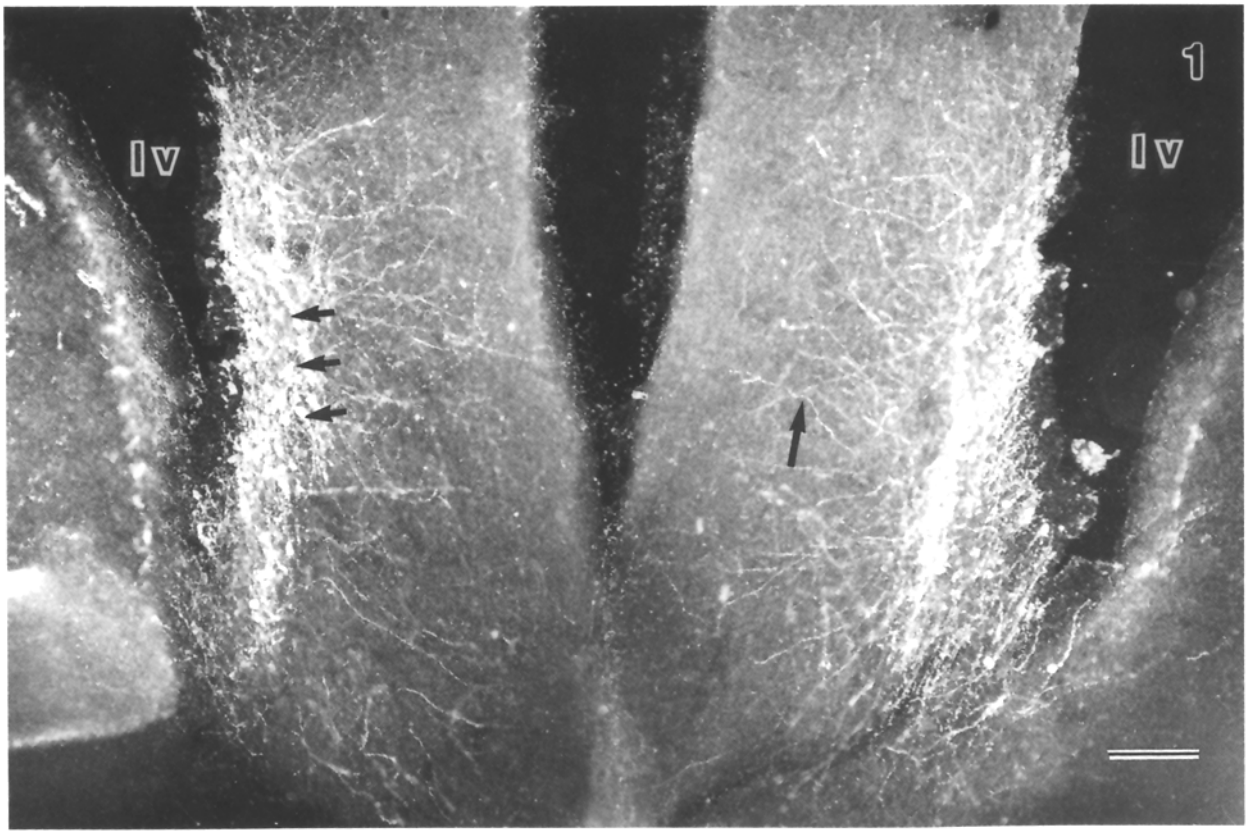
Inhibition assays. Crude membrane fractions were prepared as described previously (Barnstable 1982), and used to inhibit RET-P1 binding measured in a solid-phase indirect radioactive binding assay (Gozes and Barnstable 1982). Briefly, PVC microtiter plates were coated with opsin-containing membranes and then washed with PBS-1% BSA. Varying concentrations of inhibitor and a dilution of RET-P1 chosen to be limiting in the assay were added to the wells and incubated for 1 h at room temperature. After washing with PBS-0.1% BSA, antibody bound to the target membranes was measured using an ^{125}I-labelled $F(ab)_2$ fragment of an affinity-purified rabbit-anti-mouse IgG serum. Radioactive antibody binding was carried out for 1 h at room temperature. After washing with PBS-0.1% BSA the wells were cut from the plate and bound radioactivity measured in a gamma counter. Results were expressed for a given amount of inhibitor protein as the percentage of counts relative to controls with no inhibitor present.

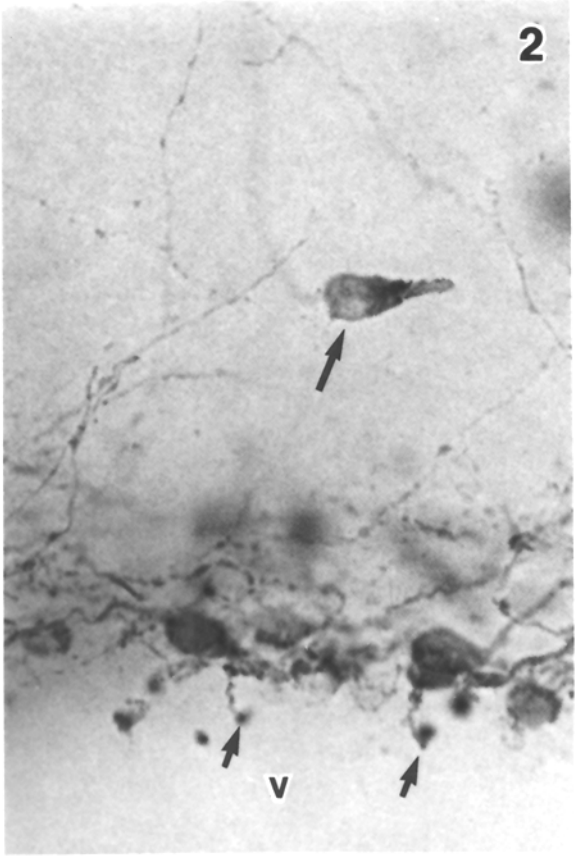
Results

In attempting to locate opsin-containing neurons in the brain, we used two antibodies to opsin, namely RET-P1 and RHO-1D4. The results described below are based on RET-P1 anti-opsin immunoreactivity only, as no RHO-1D4 staining was detected in our material.

In dove brain, RET-P1 immunoreactive cell bodies were seen in subependymal neurons in the lateral region of the septum (Figs. 1, 2) and in the infundibular region of the

Fig. 1. Cross section through forebrain of ring dove at the level of the septum, reacted with RET-P1 antibody and seen in dark field. A collection of stained cell bodies (three *arrows*) is located symmetrically adjacent to the lateral ventricle (*lv*). Stained fibers emerging from the group of cell bodies course into deeper layers of the septum (*single arrow*). Marker bar = 100 μm. × 129





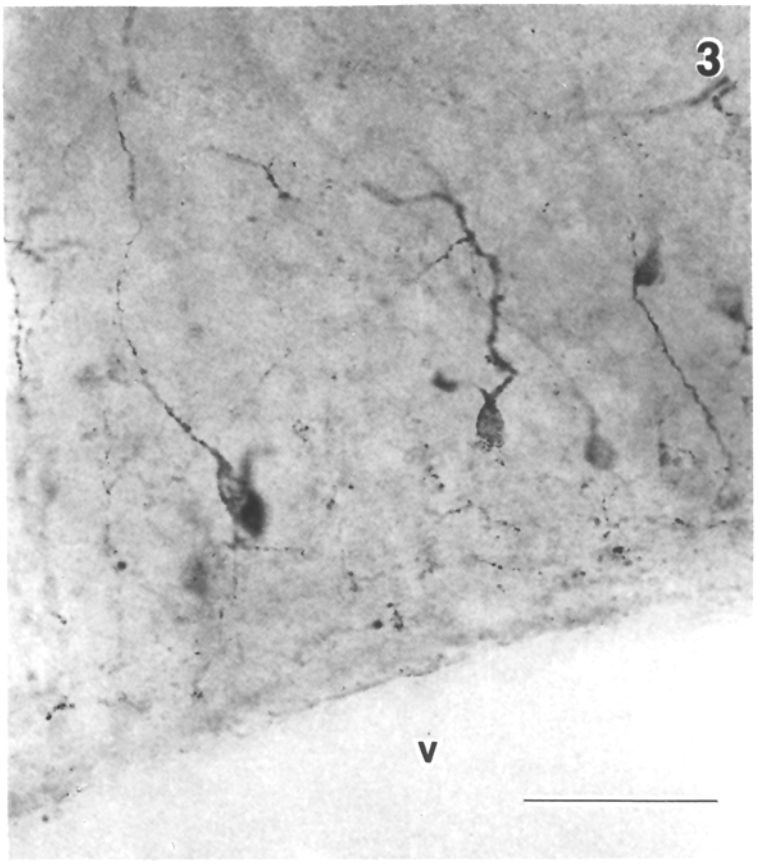


Fig. 2. RET-P1-stained cells (perikaryal diameter 8–12 μm in the septal region of forebrain of ring dove. Most reactive cell bodies lie adjacent to the ventricle, but an occasional cell is displaced to deeper layers (*large arrow*). Many cells emit a process that ends at the ventricular surface (*v*) in a small knob (diameter 2–3 μm, *small arrows*). Other reactive processes are oriented parallel to the ventricular border; still others are directed away from the ventricle into the brain tissue. × 515

Fig. 3. RET-P1-stained cells in the hypothalamic region of brain of ring dove. Stained cell bodies are 5–8 μm in diameter and emit one or more processes. Marker bar = 50 μm for Figs. 2 and 3. × 515

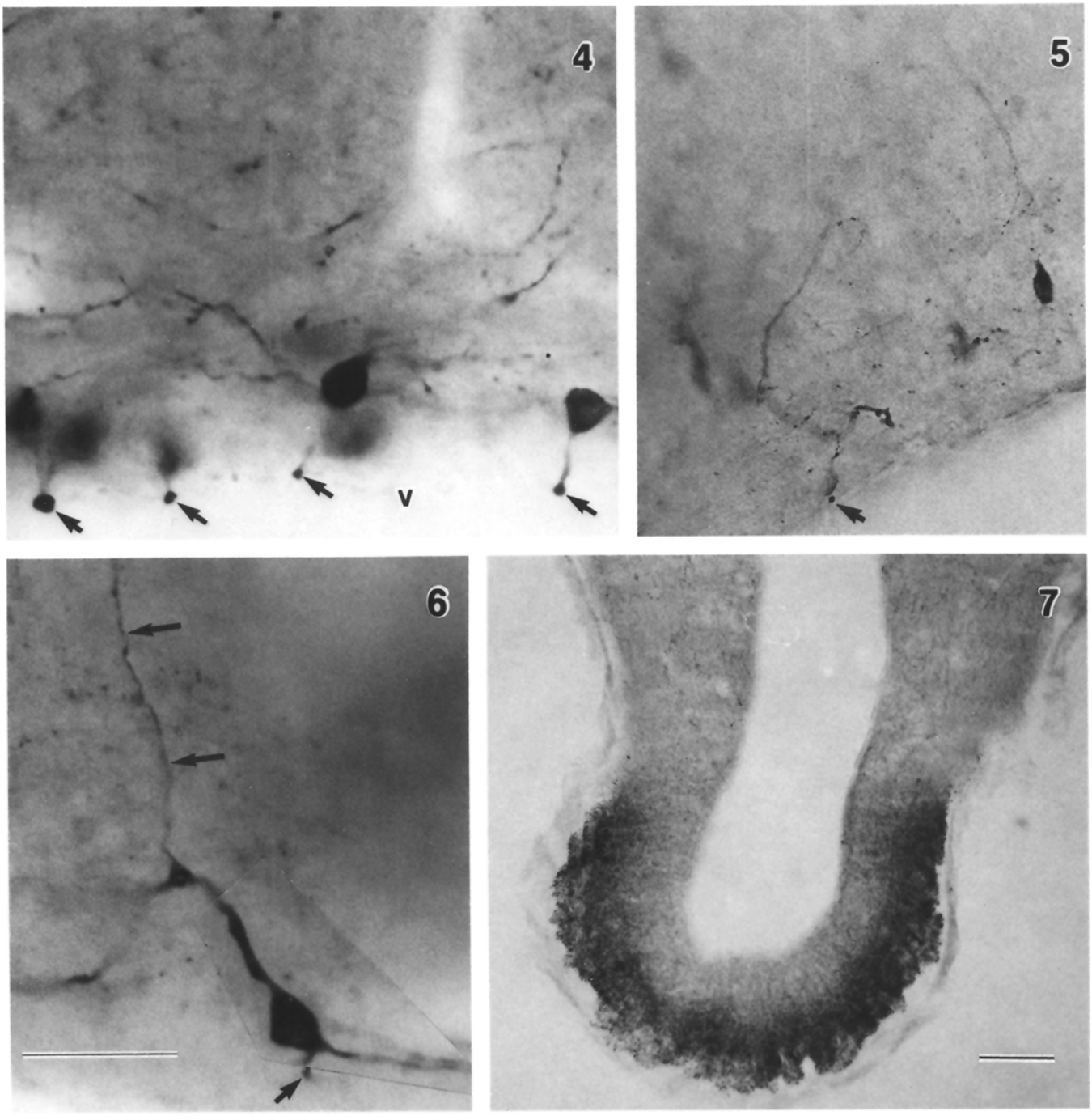


Fig. 4. RET-P1-stained cell in the septal region of brain of ring dove. Most reactive cell bodies situated adjacent to the ventricle (*v*) emit a short process with a terminal knob that contacts the ventricular fluid. × 515

Fig. 5. RET-P1-stained cells in the hypothalamic region of the dove brain. Only occasional processes terminating at the ventricular surface are noted (*arrow*). × 515

Fig. 6. A montage of a RET-P1-stained cell in the septal region of the dove forebrain. This cell is seen to give rise to multiple processes, one of which is directed towards the ventricle (*small arrow*). Another process, presumably an axon, is oriented orthogonally to the ventricular surface and penetrates deeply into the brain tissue (*large arrows*). Marker bar = 50 μm for Figs. 4, 5 and 6. × 515

Fig. 7. A dense band of RET-P1-stained fibers in the median eminence of the dove brain. Marker bar = 100 μm. × 129

hypothalamus (Fig. 3). Immunoreactive cells in the septal region of the quail and duck brain were similar to those of the dove and are not described separately. No label was seen in control tissue prepared without primary antiserum, with normal mouse serum only, or with DAB only.

In the septal region (Fig. 1), the labelled neurons are located in a highly circumscribed region of the lateral ventricular wall of the lateral septum (nucleus lateralis septalis of van Tienhoven and Juhasz 1962), corresponding to about A-9.75 in the atlas of the pigeon brain of Karten and Hodos

(1967). The rostrocaudal extent of the labelled cells is about 350 μm, whereas the dorsoventral extent from the tip of the ventricle is about 600 μm, measured in the perfused material. The labelled neurons of the infundibular region are more confined, with a rostrocaudal extent of about 150 μm, and a dorsoventral extent of about 500 μm along the ventricle. There are many fewer labelled neurons in the hypothalamus compared to the septal area.

Morphological characteristics of the immunoreactive cells. Because stained neurons in the septum and the hypothalamic regions were similar, we provide a single description. As can be seen in Figs. 2 and 3, immunoreactive cell bodies in the septal region are densely clustered, whereas those in the hypothalamic region are more sparse. The labelled perikarya are 5–12 μm in diameter with round or pyriform cell bodies. Many, though not all, of these subependymal neurons are oriented perpendicularly to the ventricular surface and contact the cerebrospinal fluid via a process that penetrates the ependymal layer and terminates in a single knob-like swelling measuring 1–3 μm in diameter (Figs. 4, 5). Some cells have two such processes. The ependymal cells around which the reactive cell bodies and processes lie never themselves display positive immunoreactivity.

In addition to the cluster of cell bodies and their ventricular processes, the immunoreactive cells give rise to a dense plexus of processes, a part of which arborizes in the area of stained cell bodies. Other processes penetrate deeply into the adjacent neuropil (Fig. 6) and give rise to the axonal projection described below. Both infundibular and septal cells clearly give rise to beaded axons. In the infundibular region, some of these could be traced into surrounding brain tissue from which they turned ventrally toward the median eminence. A discrete band of fibers occurs in the external layer of the median eminence (Fig. 7). In the septal region, axons have diverse orientations in the region of the immunoreactive cells. The location and distribution of RET-P1 immunoreactive cells in the septal and hypothalamic regions are shown in Fig. 8.

VIP. With regard to their morphology, the RET-P1 immunoreactive perikarya strongly resembled VIP-positive cells described in the infundibular and septal regions of quail and duck (Yamada et al. 1982; Korf and Fahrenkrug 1984). Our initial survey of VIP-like immunoreactivity in dove brain, using PAP-labelled VIP antibody, indicated that VIP- and RET-P1 labelled cells lay in the same regions. However, VIP immunoreactivity also was found in brain regions devoid of RET-P1 staining, e.g., in the deeper layers of the septum, scattered in the preoptic area, and in the paraventricular region. To establish whether opsin-like and VIP-like immunoreactivity were coexpressed in the same cell, we first treated tissues stained with anti-RET-P1 antibody by the immunoperoxidase method, with an anti-VIP antibody followed by goat-anti-rabbit FITC. These sections revealed that both RET-P1 and VIP-like immunoreactivity are co-localized in some but not all cells of the septal and infundibular regions. However, the DAB marker for RET-P1 tended to quench the fluorescent label. We therefore repeated this part of the study using two fluorescent labels. We detected immunofluorescent RET-P1 (labelled with FITC) and VIP (labelled with rhodamine) either in alternate sections or in the same section. Fig. 9a–d shows two examples of double fluorescent labelling in cells of the septal

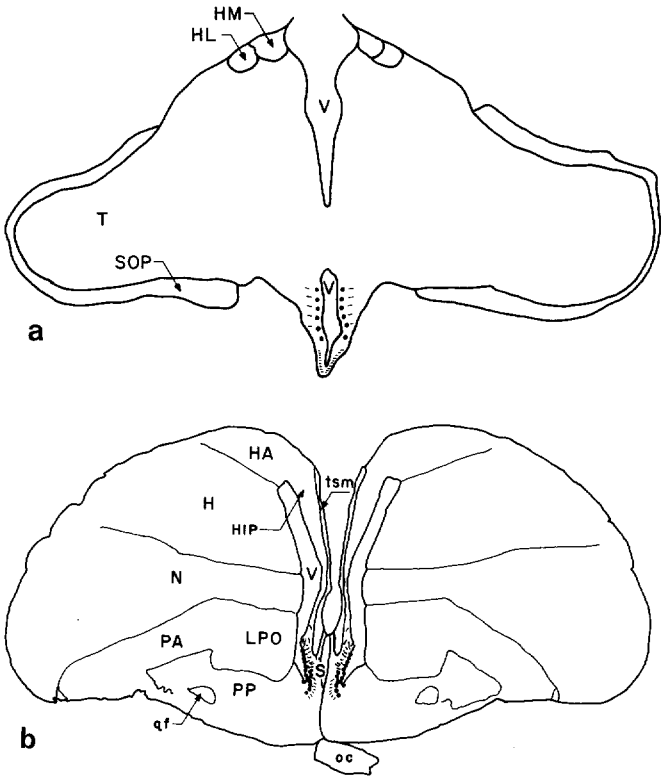


Fig. 8a, b. The distribution of RET-P1-immunoreactive perikarya (*solid dots*) and fibers (*lines*) in drawings of coronal sections of the dove hypothalamus (8a) and forebrain (8b). Abbreviations (after Karten and Hodos 1967) are as follows: *H* hyperstriatum; *HA* hyperstriatum accessorium; *HL* nucleus habenularis lateralis; *HM* nucleus habenularis medialis; *HP* hippocampus; *LPO* lobus parolfactorius; *N* neostriatum; *OM* tractus occipitomesencephalicus; *PA* paleostriatum augmentatum; *PP* paleostriatum primitivum; *qf* tractus quintofrontalis; *S* nucleus septalis; *SOP* stratum opticum; *T* tectum opticum; *tsm* tractus septomesencephalicus; *V* ventricle

region, indicating that RET-P1- and VIP-like immunoreactivity is contained in the same cell. All RET-P1 positive cells co-localize with VIP, but the converse is not invariably the case. Other VIP cells in septal and diencephalic regions do not stain for RET-P1. Control sections in which the second primary antibody was omitted from the protocol showed immunofluorescence corresponding to the first primary antibody alone.

Inhibition assays. The ability of material from erythrocytes, heart and lung to inhibit RET-P1 binding was measured to determine whether the antibody cross reacted with adrenergic receptors. As shown in Fig. 10a, 50% inhibition on RET-P1 binding was achieved by approximately 25 μg of a crude fraction from bovine retina. No significant inhibition was found even with much greater amounts of erythrocytes (Fig. 10b), heart (Fig. 10c) or lung (Fig. 10d) membranes.

Immunoblot labelling by antibody RET-P1. Approximately equal amounts of tissue from relevant regions were run on different lanes of the same gel. The whole gel was transferred to nitrocellulose and, after blocking with 5% normal goat serum in PBS, incubated sequentially with RET-P1 antibody and a peroxidase-conjugated goat anti-mouse IgG

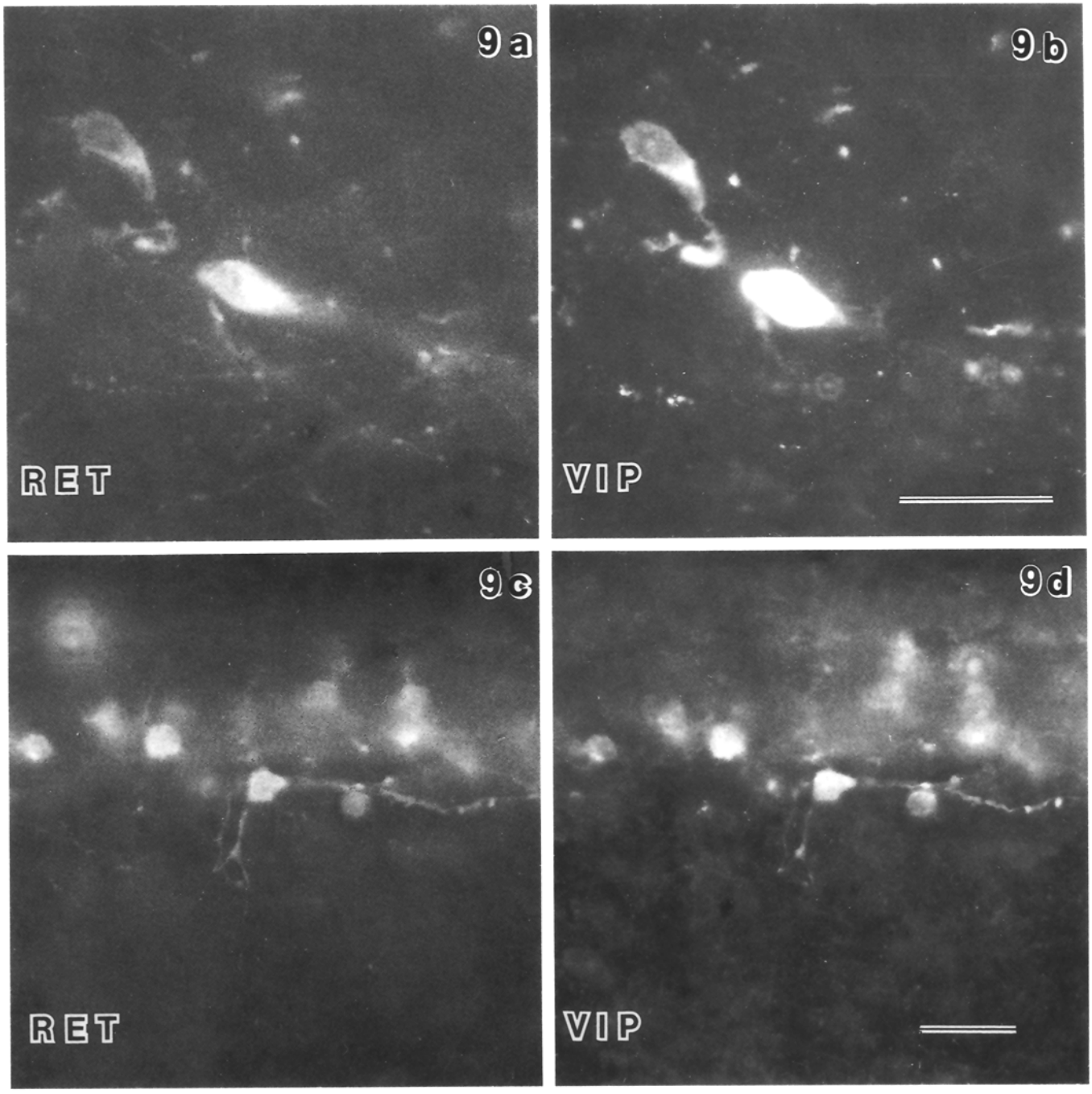


Fig. 9a–d. Co-localization of opsin-like and VIP-like immunoreactivity in neurons from the septal region of forebrain of ring dove. Sections **a** and **c** were reacted with RET-P1 antibody and visualized with an FITC fluorescent label; **b** and **d** were reacted with an anti-VIP antibody and visualized with a rhodamine label. It is apparent that the same cell bodies and processes are labelled by the two antisera. Marker bar = 50 μm for Fig. 9a–d; a–b × 515; c–d × 322

antibody. Fig. 11 shows the results of such an analysis. In lane 1 is shown a retinal control. Because of the homogenization and subsequent boiling in SDS, the opsin has formed a ladder of different sized aggregates. Bands from monomers to decamers are shown on the figure. A similar series of bands is found with the pineal sample (lane 4). Samples from pituitary (lane 2) and hypothalamus (lane 3) showed a series of bands, some of which corresponded with bands in the retinal and pineal lanes. The major bands of the pituitary sample were at 251 kD and 288 kD and those of the hypothalamus were 331 kD and 269 kD. No clear bands were found in the tissue sample from the septal area (lane 5).

Discussion

Characterization of CSF-contacting neurons in the vertebrate brain. The neurons that express opsin- and VIP-like immu-

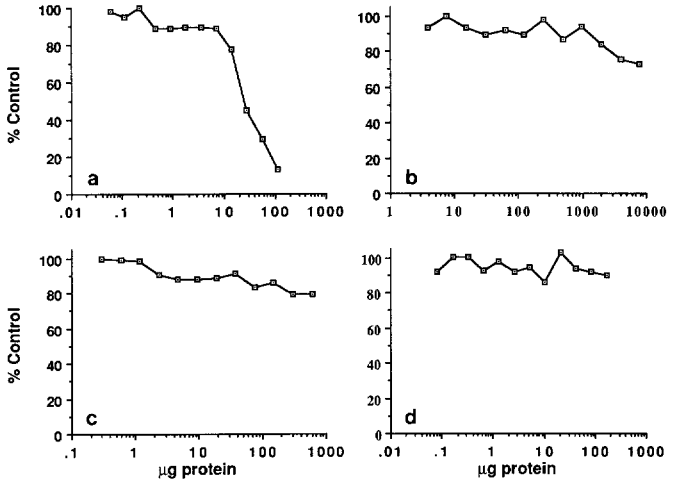


Fig. 10a–d. Inhibition of RET-P1 binding by membrane fractions. (**a**) Crude bovine retinal outer segment preparation used as an opsin-containing positive control, (**b**) erythrocytes, (**c**) heart and (**d**) lung membranes. Each point represents the mean of three determinations. 50% inhibition is found in **a** at about 25 µg of inhibitor protein. None of the other three samples gave significant inhibition

noreactivity in the septal and the infundibular region in the dove brain are morphologically similar to CSF-contacting neurons found in hypothalamic and medullospinal regions of other vertebrate brains (Vigh and Vigh-Teichmann 1973). Such CSF-contacting cells are typically bipolar; one of their processes enters the CSF while the other enters the overlying neuropil. A globular or club-shaped CSF-contacting terminal protrudes into the ventricular lumen. Cells with opsin- and VIP-like immunoreactivity described in the present report share these features. In addition, they emit numerous processes that form a plexus in and around the area of the stained cell bodies. Based on light-microscopic evidence it seems clear that our immunoreactive cells are the same as the VIP-like immunoreactive cells described by Yamada et al. (1982) and Korf and Fahrenkrug (1984) in Japanese quail and mallard, respectively.

Specificity of the RET-P1 antibody employed. Does opsin-like immunoreactivity in septal and infundibular cells indicate that they bear authentic opsin and serve as the deep encephalic photoreceptors? Binding by a monoclonal antibody does not prove the presence of a specific antigenic determinant (see Landis 1985). However, Barnstable (1982) and Fekete and Barnstable (1983) found that the RET-P1 antibody recognizes only a single cell type, photoreceptors, in the rat retina, and no cells in the central nervous sytem. More detailed analysis has shown that RET-P1 reacts with a determinant that consists primarily of amino acids between positions 1 and 12 at the N-terminus of opsin (Hargrave et al. 1986).

One possible source of cross reactivity of the antibody is with other members of the G-protein-linked receptor family. This is not likely since the N-terminal region of opsin that RET-P1 reacts with is not well conserved between these genes. Indeed muscarinic acetylcholine receptors do not possess a region corresponding to the RET-P1 binding site. However, considerable amino acid homology has been shown between rhodopsin and the β-adrenergic receptor (Dixon et al. 1987). To examine possible cross reactivity with adrenergic receptors, the ability of erythrocytes, heart and lung to inhibit RET-P1 binding was measured. No significant inhibition was given by any of these tissues (Fig. 10). On the other hand, we failed to observe immunoreactivity in response to RHO-1D4 monoclonal antibody, which is specific for the C-terminal region of opsin (Molday and Mackenzie 1983), even though this antibody did react with retinal photoreceptors. Together these results suggest that the molecule labelled in our experiments is not identical to retinal opsin. The immunoblots tend to support this conclusion although, in view of the small amounts of tissue available, the strength of the bands labelled is not sufficient to draw absolute conclusions. Opsin was clearly detectable on immunoblots of retina and pineal body.

Although a number of bands could be seen in pituitary and hypothalamus, none of these could be clearly identified as opsin. At present it is impossible to state whether these bands represent non-specific binding of antibody or whether they correspond to the immunoreactivity seen histologically. Though a series of bands corresponding to opsin and its aggregated forms could be found in blots of the retina and pineal body, only faint staining was observed in the septum. We have no ready explanation for the fact that we found vigorous staining with RET-P1 in the septal region but were unable to identify a corresponding band of stained protein in the immunoblot. In pituitary gland no bands corresponding to opsin were observed. Instead, two bands of higher molecular weights (approximately 251 kD and 288 kD) were seen. Samples from the hypothalamus revealed a more complicated picture. In addition to higher molecular weight bands of approximately 331 kD and 269 kD several other lower weight bands were observed. Some, but not all, of the bands were at the positions of the opsin bands observed in the retinal and pineal samples. Because of the complexity of the pattern no firm conclusions about the identity of the reactive molecules can be drawn from this experiment alone.

Encephalic receptors. A substantial literature suggests that the deep brain photoreceptors lie in the basal hypothalamus, and there is somewhat weaker evidence for extrahypothalamic sites (see Groos 1982 for review). Vigh and Vigh-Teichmann (1973) and their collaborators have long argued that CSF-contacting cells exhibit a morphological similarity to sensory cells. They suggest that the ciliated dendrites of CSF-contacting neurons resemble inner and outer segments of pinealocytes in higher vertebrates and of developing retina, and that these CSF-contacting cells may correspond to the encephalic photoreceptors (Vigh-Teichmann et al. 1980). Ekström et al. (1987) showed that neurons of the habenular nucleus, pineal and parapineal organs reacted with antibodies against photoreceptor outer segment proteins. On the other hand, the CSF-contacting cells of hypothalamus failed to react with a variety of anti-opsin antibodies (Foster et al. 1987; Korf et al. 1985; Vigh and Vigh-Teichmann 1981; Vigh et al. 1983; Vigh-Teichmann et al. 1980, 1982). These authors did not however, utilize the RET-P1 antibody employed in the present study. An additional argument against equating CSF-contacting cells with encephalic photoreceptors is the cell shape of the former group. In both septal and hypothalamic regions, the CSF-contacting neurons that we studied had long, branching dendritic and axonal processes not associated with typical photoreceptors, but rather resembling CSF-

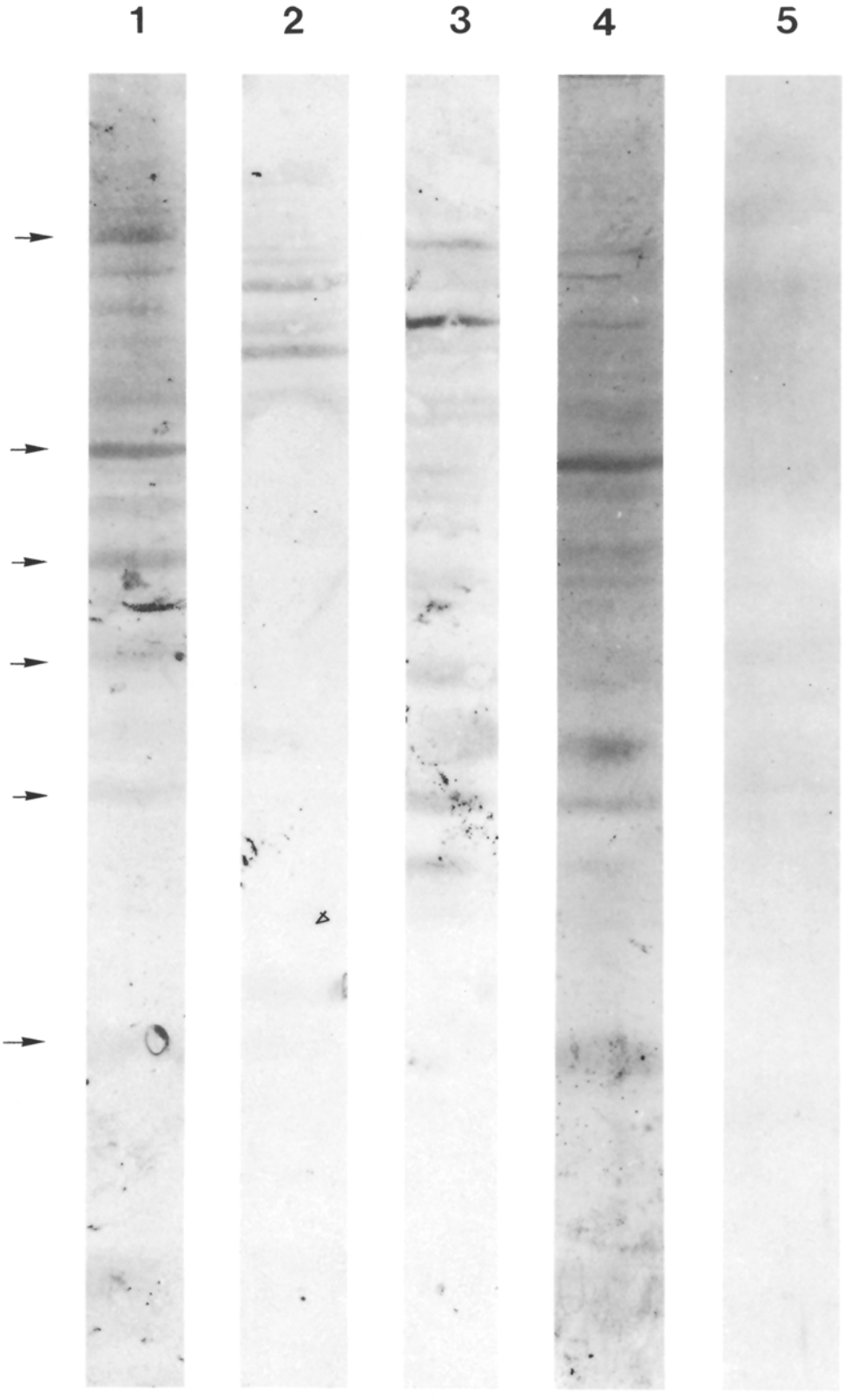


Fig. 11. Immunoblot analysis of RET-P1 immunoreactivity. Samples were homogenized and boiled in SDS sample buffer and electrophoresed on 10% polyacrylamide gels and transferred to nitrocellulose. Immunoreactive bands were revealed using a peroxidase-conjugated secondary antibody. Samples analyzed were retina (lane 1), pituitary (lane 2), hypothalamus (lane 3), pineal (lane 4), and septum (lane 5). *Arrows* point to migration positions of opsin monomer (39 kD) and its dimer, trimer, tetramer, pentamer and decamer (origin at bottom of figure)

contacting neurons in the pineal organ (Ekström 1987). Moreover, photoreceptors are not known to contain VIP (Brecha 1983).

Whether or not the cells studied in the present report have a specialized outer segment region, with the high concentration of visual pigment characteristic of photoreceptors, remains to be determined. In this regard, a structure of interest is the knob-like intraventricular end bulb (Figs. 2, 3). Preliminary observation of its ultrastructure does not indicate an outer segment-like arrangement of membranes (work in progress). On the other hand, several authors have suggested that deep encephalic photoreceptors

are unlikely to have specialized receptor endings (as these have never been found despite many attempts to locate them) and that the rhodopsin-like sensitivity of the encephalic photoreceptor may be mediated by a non-specialized membrane-bound protein or photosensitive enzymes (Foster and Follett 1985; Hartwig 1982).

Role for VIP. Present evidence suggests that VIP can serve as a neurotransmitter and as a neuromodulator, affecting neural communication, energy metabolism, cerebral blood flow, and hypothalamic-pituitary function (Abrams et al. 1985). In the present study, RET-P1 labelled a subset of VIP-immunoreactive cells that contact the CSF. If these cells are photosensitive, it may be significant for seasonal reproduction that VIP has been found in high concentrations in the median eminence (Samson et al. 1981) and in the hypophyseal portal blood (Said and Porter 1979; Shimatsu et al. 1981). Furthermore, VIP has been implicated in the release of prolactin, LH, and GH in rats (Kato et al. 1978; Vijayan et al. 1979), and prolactin in hens (Macnamee et al. 1986) suggesting that VIP plays a role in the control of hypophyseal hormone secretion.

Conclusion. The cells that we describe in this paper contact the CSF in both the lateral and the third ventricles. The opsin-like immunoreactivity of the CSF-contacting cells may be fortuitous in the sense that these cells may not be photoreceptive. Nevertheless the RET-P1 label allowed us to visualize a subset of VIP-immunoreactive cells and their axonal projections. Thus, whatever role VIP plays in regulating brain and pituitary function, it appears to depend on information from multiple ventricular sites.

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