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THYROTROPIN-LIKE IMMUNOREACTIVITY IN HUMAN RETINA: IMMUNOREACTIVE CO-LOCALIZATION IN GANGLION CELLS AND PERIVASCULAR FIBERS

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Abstract—The present immunocytochemical study has demonstrated immunoreactive thyrotropin-like ganglion cell populations as well as perivascular fibers in the human retina by using specific antiserum. Thyrotropin is a pituitary glycopeptide involved in the synthesis and release of thyroid hormones. The existence and functions of peptides in vertebrate retinas are still not well known. Many authors have reported neuropeptide immunoreactivity in the human retina which have had their functions established in the neuroregulatory processes of vision. Moreover, some authors have reported the possibility that the fiber terminal of peptidergic neurons may also be a blood vessel. The appearance of immunoreactive-cells in human retina, e.g. existence of retinal ganglion cells with thyrotropin-like immunoreactivity, indicates the existence of specific mechanisms that would be mediated by these peptides which are located near immunoreactive ganglion cells. We hypothesize that there is an intrinsic mechanism for blood flow control, mediated by retinal ganglion cells which may regulate vessel diameter according to its luminous stimuli. No-one has demonstrated the presence or the functional existence of thyrotropin-like immunoreactive structures in the vertebrate retina, or on the side of the pituitary–thyroid axis. To the best of our knowledge this is the first time that thyrotropin has been immunocytochemically demonstrated in the human retina. Thus, we suggest that thyrotropin acts as a neuromodulator in the human retina, which is implicated in blood flow control. Copyright © 1996 Elsevier Science Ltd.

Retinal vessels have a well known distribution although they exhibit a great diversity in location and number. From the central retinal artery large and middle-size vessels arise, which are located at the inner plexiform layer, detached from the internal limiting membrane by sparse glial cells. Only capillaries located in the inner nuclear layer reach outer retinal layers. Arteries are flanked by corresponding veins (Ernest, 1989).

The retina is a high oxygen consuming tissue, so its blood flow must be maintained even in the case of important systemic blood pressure changes. However, retinal vasculature control still remains controversial. Sympathetic fibers innervate the ophthalmic artery to the central retinal artery, which is left without sympathetic innervation. Thus, the blood flow mechanism at the retina differs from elsewhere in the body, where the autonomic nervous system exercises control, even in the most critical situations. Other intrinsic mechanisms must exist in the retina, regulated by the non-nervous system, which adjust blood flow according to metabolic necessity (Bill and Nilsson, 1985).

Some hypotheses have been reported to explain this intrinsic mechanism. Several authors have postulated the possibility that "the effector organ of autoregulation is the smooth muscle, the tone of which controls vessel diameter, (. . .) the vessels dilate in response to the accumulation of metabolic by-products such as carbon dioxide and decreased pH" (Blahser, 1984).

More recently, by using immunohistochemistry and cytochemistry techniques, many authors have reported peptidergic immunoreactivity in different retinal neurons. However, only a few of these explain the active but opposing influence of several substances over the control of retinal vasculature. These include angiotensin II, substance P, VIP, NPY, CGRP, prostaglandin I_2 and the endothelins (1, 2 and 3). Angiotensin II, one of the most potent vasoconstrictors, is a heptapeptide derived from lung alveoli synthesized angiotensinogen. Its existence has been reported in a high concentration in retinal arterioles as well as in

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pericytes, although in the case of pericytes it is without effect (Dodge *et al.*, 1991; Ferrari Dileo *et al.*, 1991; Nyborg *et al.*, 1990; Sato *et al.*, 1993).

The relaxation property of prostaglandin PgL_2 has been studied in pericyte binding-sites (Dodge *et al.*, 1991). Other peptides such as Substance P, CGRP or VIP have been reported as vasodilator agents. Substance P and CGRP have effects on retinal small arteries and VIP on choroidal vessels (Fitzgerald *et al.*, 1992; Kitamura *et al.*, 1993; Prieto *et al.*, 1991; Ye *et al.*, 1990). In this way, substances which are locally synthesized, like PgI_2 or thromboxane A_2, could affect the blood flow across local control mechanisms. Thromboxane A_2 is a vasoactive substance, as shown by its pericyte contraction. In recent years, a group of substances called endothelins (1, 2 and 3) has been reported. They are a family of high vasoconstrictors in which endothelin 1 is the most potent vasoconstrictor known. Endothelins are synthesized by lung endothelial cells and neuroretinal cells. Endothelins provoke the constriction of microvasculature pericytes (McCumber *et al.*, 1991; Ramachandran *et al.*, 1993; Takei *et al.*, 1993).

Conversely, thyrotropin (TSH) is a 28.3 kDa glycoprotein, synthesized by the anterior pituitary gland. It is released by a tripeptide hypothalamic factor, the thyrotropin releasing hormone (TRH), which in recent years has also been reported as a neurotransmiter (Jackson and Lechan, 1987). The secretion of TSH is regulated both by the decrease of thyroid hormones and by TRH. The important role of TRH is implied not only in its releasing function but in TSH conformational structure and glycosylation. TSH has a double peptide sequence called α (96 residues) and β (114 residues). Moreover, it has four glycosidic chains; two chains in each of the α and β sequences. TSH has a common structure for a hormone group which includes prolactin, follicle stimulating hormone (FSH) and luteinizing hormone (LH). Within the group, the α sequence is identical to those within one species, but the β sequence is only similar. Both peptidergic chains must be bound for hormonal function, thus glycosylation is necessary for α–β interaction. Glycosidic chains are rich in manose residues (Granner, 1990).

Ganglion cells are named the first neurons in the optic nerve and are involved in vision processing information and selection functions of on–off pathways. We hypothesize that ganglion cells exercise a possible intrinsic control mechanism over blood flow. These ganglion cells would control microvasculature contraction according to their received luminous stimuli.

EXPERIMENTAL PROCEDURES

Histological specimens

Four Bouin's liquid-fixed normal human eyeglobes from donor patients were selected from the University Hospital "Puerta del Mar" at Cadiz. The case histories were revised to ascertain that cytostatic drug or radiotherapy treatment had not been administered. The eyeglobes were fixed in Bouin's liquid for 4 h, dehydrated through graded alcohols and benzol and embedded in medium paraffin by routine procedures. Serial sections were cut at 20 μm and placed on albumin-coated glass slides. They were counterstained with modified hematoxilin-VOF according to Gutierrez (Gutierrez *et al.*, 1963).

Immunocytochemical techniques

The sections were deparaffinized in xylol and rehydrated in degraded alcohol series and distilled water. Later the endogenous peroxidase and pseudo-peroxidase activities were removed with treatment for 30 min in 3% H_2O_2 solution. Sections were washed twice in phosphate buffered saline (0.1 M PBS, pH = 7.4; Sigma P-4417) for 5 min, then were incubated with primary antibody for 72 h at 4°C. Sections were then washed twice in PBS. The avidin–biotinylated peroxidase complex (Biogenex Supersensitive Multilink HRP) was used as follows: incubation for 30 min in secondary biotinylated antibody at a dilution of 1/50 (room temperature), twice in PBS for 5 min, 30 min with 1/50 diluted avidin–peroxidase complex (always at room temperature), and finally twice in PBS for 5 min.

Subsequent colour development was for 10 min using a solution of 10 mg/15 ml diaminobenzidine (3,3′diaminobenzidine, Sigma D-5905) in Tris buffer saline (Sigma T-5030) with 20 μl/10 ml H_2O_2. Afterwards, sections were dehydrated and coverslips mounted with Entellan (Merck 7961).

We used a rabbit polyclonal antibody against human TSH (Histogen HI33-5P) at a dilution of 1/500 and followed the staining control by incubation of some sections without primary antibody.

The immunoreactive tissues were viewed using a Leitz LABORLUX light microscope with photomicrography unit.

RESULTS

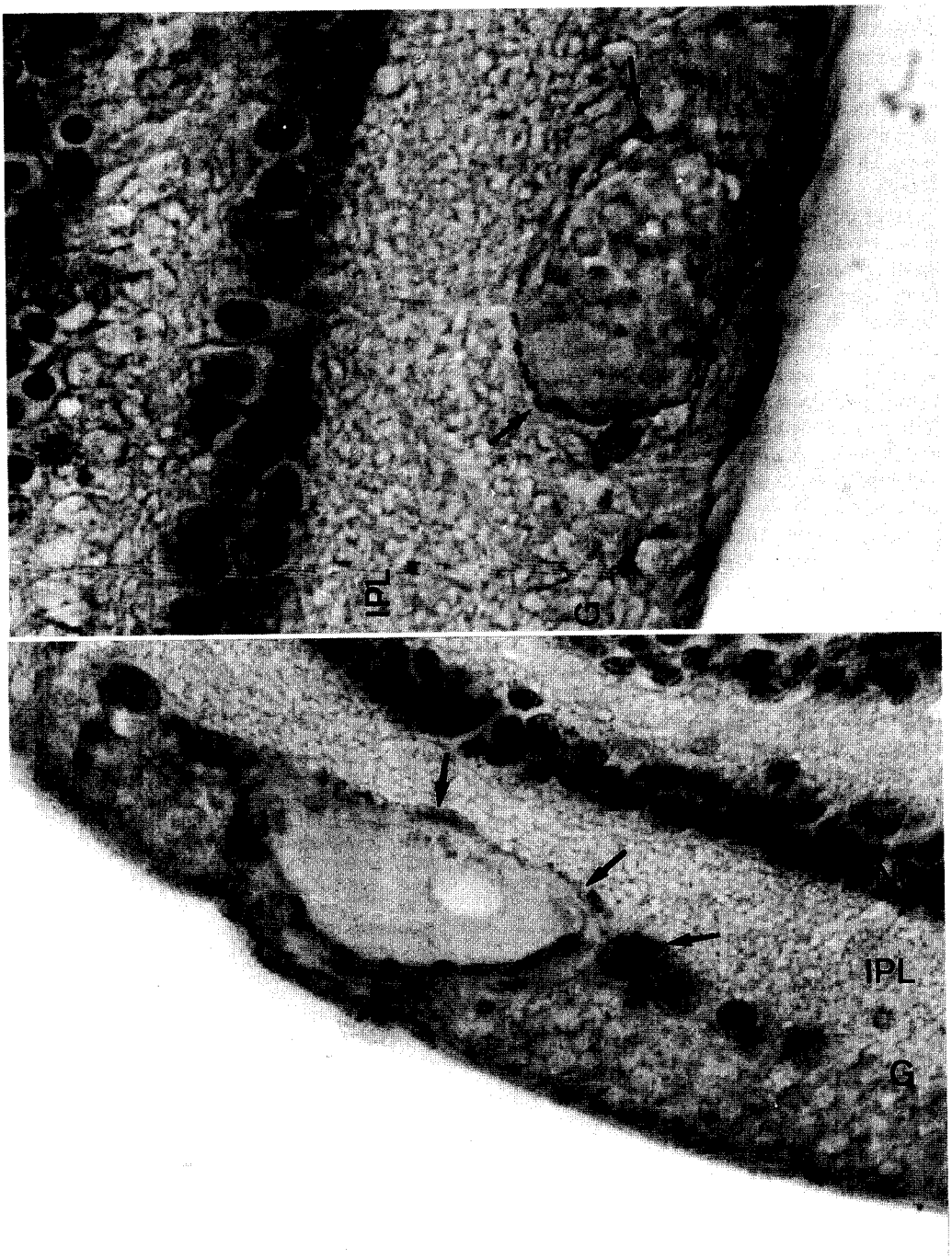
Modified hematoxilin–VOF by Gutierrez staining of the available human retina showed a normal histological state. All retinal layers were present in a good state of preservation.

TSH-like immunoreactive nerve fibers were observed around the middle-sized and small blood vessels in the inner plexiform layer. They were situated at the blood vessels, smooth muscle layer and had a typical varicose form with a characteristic thickness. We also observed nerve fibers smaller than these, not associated with blood vessels.

It was possible to differentiate clear and strong TSH-like immunoreactive ganglion cells associated with the blood vessels. Immunoreactivity was located in the whole neuron, and showed a diffuse cytoplasmic staining. Some of these ganglion cells were in close

proximity to the thin fibers which surround the retinal vessels.

The TSH-like immunoreactivity did not appear in all the retinal vessels. Therefore, it is important to note that immunoreactivity was not present in all the ganglion cells (Figs 1 and 2).



Figs 1 and 2. Perivascular fiber in human retina stained with polyclonal anti-TSH, using DAB chromogen ×400. G: ganglion cells; IPL: inner plexiform layer; INL: inner nuclear layer. Arrows, immunoreactive reaction.

DISCUSSION

Structures containing a particular neurotransmitter are usually demonstrated by immunocytochemical methods. However, these methods have yet to be used to demonstrate the localization of a high-affinity peptidergic system.

Thus, we have employed TSH specific polyclonal antiserum. Its presence in both ganglion cells and perivascular fibers in retinal vessels suggests that TSH-like immunoreactivity could act as a neuroregulator at this level.

A number of neuroregulators have been identified in retinas of various species. Most of them have been located in amacrine cells, ganglion cells or the inner plexiform layer. Nevertheless we do not know their specific behavior, although they have been postulated to act as neuronal function regulators. Blahser (1984) had reported the possibility that the fiber terminal of peptidergic neurons may also be a blood vessel. The localization of TSH-like immunoreactivity in ganglion cells and in perivascular retinal vessels suggests a role in blood flow control.

Speculation as to the detailed role of TSH as a ganglion cell neuroregulator is clearly premature, particularly as electrophysiological and pharmacological research as well as previous histological studies have not been carried out.

In conclusion there are solid arguments backing the role of TSH as a neuroregulator and this hormone could act in controlling the diameter of retinal blood vessels. Nevertheless further investigations are necessary in order to study, in depth, the distribution, localization and structural implications of TSH at a retinal level.

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