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Insulin Receptors in Human Ocular Tissues

Immunohistochemical demonstration in normal and diabetic eyes

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ABSTRACT

The α- and β-subunits of the insulin receptor have been localised in human eyes by immunohistochemistry. In the normal eye staining for both receptor subunits was distinct at the same sites of the anterior part of the eye, i.e. cornea, smooth muscle and epithelium of the ciliary body and the lens capsule. In the retina, the receptor was clearly demonstrated in the nerve fibre layer, the ganglion cells and Müller cells, the outer nuclear layer, inner segments of rods and cones, the outer limiting membrane and in the pigment epithelium. In eyes with diabetic retinopathy, the receptor did not stain in the inner segments of the rods and cones and the staining of the other layers was weak.

Endothelial cells stained positively in normal and diabetic eyes, but pericytes of normal and new vessels did not stain. The receptor staining did not change in cornea, iris, ciliary body and lens.

All together, the study shows that α- and β-subunits of the insulin receptor are present in the retina, and that the staining reaction for the receptor is reduced in diabetes. To what extent these findings are of importance for the development of diabetic retinopathy, remains to be clarified.

INTRODUCTION

Diabetes mellitus is a disease in which insulin is either totally lacking (Type 1), or is produced in insufficient quantities (Type 2). The risk for the patients to develop changes of the retina ultimately leading to a deranged function, is significant in both types of diabetes. Diabetic retinopathy is at present the dominant cause of blindness in Western countries. Binding of insulin to tissues has a major influence on their metabolism (7,11). The blood-retinal barrier, which consists of the pigment epithelium and the endothelial cells of the retinal capillaries, allows only molecules much smaller than insulin to pass (8,12). It may be anticipated that a small amount of insulin normally reaches the retinal cells. Insulin from the blood binds to retinal endothelial cells (6), and insulin receptors in retinal endothelial cells may internalise insulin from their apical side by receptor mediated endocytosis (15). The sensitivity of the insulin receptors in the retina may be changed in rats with experimental diabetes (18). The object of the present investigation was to study the

distribution of the α- and β-subunits of the insulin receptor in the retina and other parts of the normal human eye. Furthermore, possible changes of the receptor pattern in diabetic eyes were studied.

MATERIAL AND METHODS

Chemicals: All chemicals in the study were purchased from Bie & Berntsen, Rödovre, Denmark, unless otherwise stated.

Antibodies: Monoclonal antibodies against the subunits of the insulin receptor (Insulin R α (N-20) and Insulin R β (C-19), Santa Cruz Biotechnology) were obtained from SDS, Falkenberg, Sweden. Swine serum (Dako X 901), SWAR (swine-anti-rabbit, Dako Z 196, diluted 1:20 in TBS(tris buffered saline; 0.05 M Tris (2-amino-2(hydroxymethyl)propane -1,3-diol)) and PAP complexes (Dako Z 196, diluted 1:40 in TBS) were from DAKO A/S, Glostrup, Denmark.

Eyes and biopsy material: Three paraffin embedded normal eyes, three eyes with proliferative diabetic retinopathy without retinal detachment, and two eyes with severe retinopathy with retinal detachment were used for the investigation. Striated muscle and liver tissue served for testing the antibody dilution. All tissues used in the present investigation were procured from the files of the Eye Pathology Institute, Copenhagen.

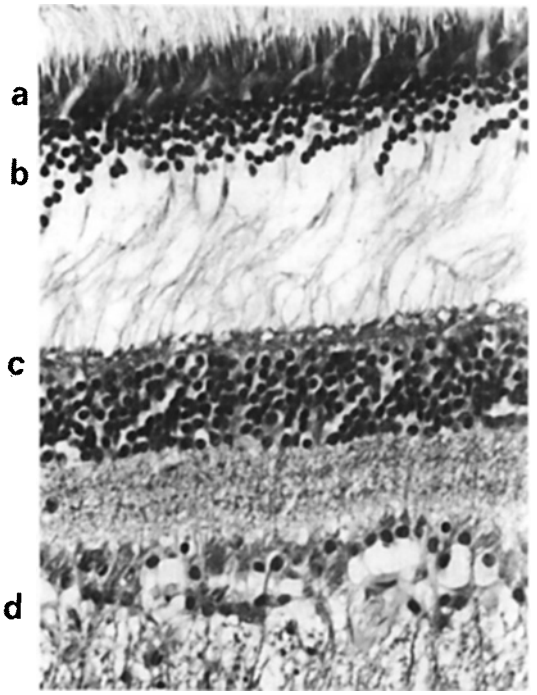

Fig 1. Normal retina showing insulin β-receptor staining in the inner segments of rods and cones (a), cells of the outer granular layer (b), outer limiting membrane (c), and ganglion cells (d). x 40, Mayer's acid haemalum counterstaining.

Immunostaining: The immunohistochemical stainings were performed with PAP standard methods(14). The paraffin embedded material was cut in 5 µm thick sections and placed on object slides (Super Frost / Plus, Menzel Gläser, Germany) before being deparaffinized. Sections were treated with pronase (0.1% w/v) for 5 min, and endogenous peroxidase activity was blocked by incubation in 1 % H_2O_2 – methanol for 15 min. Both insulin receptor antibodies were first diluted in a 1:10 mixture of swine serum and TBS, 0.145 M NaCl, pH 7.6). These solutions were then further diluted to a final dilution of the antibody from 1:10 to 1:10000. Incubations were performed for 18-20 h at room temperature. After washing in TBS, incubation with the secondary antibody was performed, followed by incubation with the PAP complexes for 15 min. Bound enzyme was visualised with AEC (3-amino-9-ethylcarbazole). Sections were counterstained with Mayer's acid haemalum.

RESULTS

Antibodies against the α- and β-subunits diluted to 1:50 and 1:100 demonstrated reliable and reproducible staining in skeletal muscle and liver. There was no difference in the distribution of α- and β-subunits, and they are collectively referred to as the insulin receptor below.

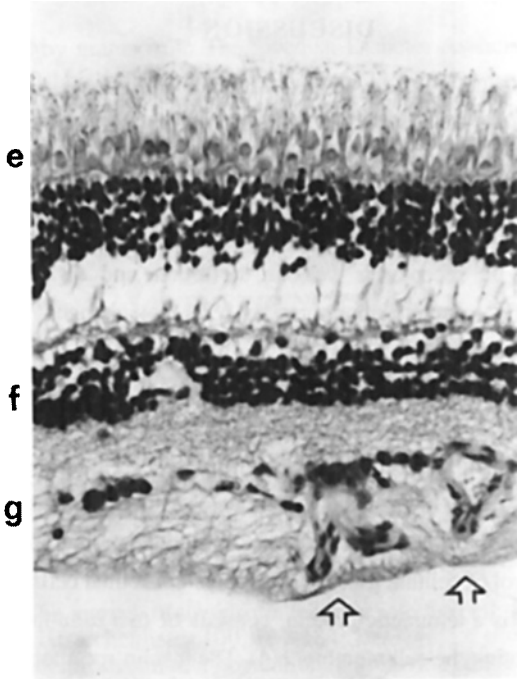

Fig 2. In the diabetic retina β-receptor staining is negative in the inner segments of rods and cones (e), and there is a weak reaction in the inner nuclear layer (f) and the ganglion cells (g). There endothelial cells of a neovascular tuft displays insulin receptor staining (arrows). x 40, Mayer's acid haemalum counterstaining

Normal eyes. Cornea displayed a marked staining for the receptor in both the epithelium, the endothelium and the stromal cells. Smooth muscle fibres of the pupillary sphincter and dilator, and of the ciliary body also reacted. The pigmented and non-pigmented epithelial cells of the ciliary body were receptor negative. Lens capsular epithelium showed a marked staining for the receptor.

In the retina, the receptor was clearly demonstrated in the nerve fibre layer, the ganglion cells and Müller cells, the outer nuclear layer, inner segments of rods and cones, the outer limiting membrane and in the pigment epithelium. The retinal vessels showed a positive reaction in the endothelial cells, but not in the pericytes. The endothelial cells of the choroid were also positive.

The striated extraocular muscles showed distinct receptor staining in the perimysium.

Diabetic eyes. Most tissues had an unchanged expression of the insulin receptor. The receptor in the cornea was clearly seen, and this was also the case in the iris and the ciliary body. In the lens, however, the staining of the capsular epithelium was lost when there was significant cataract development. In the retina, receptor staining could not be demonstrated in the rods and cones, and only a weak reaction was found in the inner nuclear layer and in the ganglion cells (Fig 2). The endothelial cells of the vessels stained positively both in original retinal vessels, and in microaneurysms and new vessels. The staining of the extraocular muscles was unchanged

DISCUSSION

The present study shows that binding of antibodies against the α- and β-subunits of the insulin receptor may be demonstrated in routinely formalin-fixed and paraffin embedded eyes, and that the receptor is present in the cornea, the iris, the ciliary body, the lens, and the retina. The receptor is also present in the endothelial cells of arterioles, venules, and capillaries of the retina and the choroid. The extraocular striated eye muscles also have receptors for insulin. In eyes from diabetic patients, the expression of the receptor is weak in the retina, and disappears in the lens when cataract has developed. The receptor immunoreactivity is not changed in smooth and striated muscles and vascular endothelial cells.

The demonstration of the α- and β-subunits of the insulin receptor within the eye is of great interest, since this indicates an active transportation of insulin across the blood -retina barrier and binding of insulin to retinal cells. The insulin receptor and insulin synthesis have been demonstrated in the developing chick retina (16). They have also been shown in the neuroretina and the retinal pigment epithelium of rats (5). Thus, there is evidence that insulin under normal circumstances may play a role for the membrane control of cellular glucose metabolism of retinal cells(3).

The insulin receptor which is a tetrameric protein, consists of two α-subunits on the cell surface, and two β-subunits transversing the cell membrane(4). The insulin receptor and transport of insulin have been demonstrated in the central nervous system (1). Transport of insulin into the central nervous system is a saturable process which may be facilitated by an insulin-receptor mediated transport process (2). A similar mechanism may act in the eye. In bovine retina there are receptors with high affinity for insulin. The receptor stimulates a tyrosine-specific activity that is capable of phosphorylating both the β-subunits of the receptor and exogenous substrates (17).

The observed decreased immunoreactivity for the insulin receptor in the diabetic retina is difficult to understand. An explanation may be that the insulin receptor, like the glucose transport system (GLUT 1), is downregulated (9), or blocked due to glycation (10). Leakage of blood plasma containing small amounts of insulin into the retina may induce the neovascularisation directly by the growth factor function of insulin, but also by other hormones (13).

In the lens a decreased immunoreactivity for insulin receptor first develops when cataract is seen . This is probably only a part of the general degeneration of the diseased lens. The osmotic changes preceding cataract formation probably induce a cell degeneration within the lens epithelium. Also in this case, the findings may indicate that the insulin receptor is vulnerable to the high glucose concentrations occurring within the eye after the blood-eye barrier has broken down.

In the periocular tissues such as eye muscles and vessels the binding of insulin is not changed in diabetes. However, this was expected and mirrors differences in glucose metabolism of intra- and extraocular tissues.

In conclusion the present investigation indicates that the immunoreactivity of the insulin receptor in the human eye is changed during diabetes. The significance of the findings remains to be clarified.

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