Immunoeytochemical Localization Of Epidermal Growth Factor, Its Receptor And Related Oncogenes On Rat Retina

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Introduction

Numerous studies have pointed out that neural retina or its extract promote cell division and fiber differentiation in lens epithelium and is the probable source of intraocular epidermal growth factor (EGF) and others (1,2). However, the distribution of EGF, its receptor (EGF-R) and related oncogenes (erb-B) on rat retina in situ is obscure and we have been using immunoperoxidase histochemical method in order to explore their effects on the retina.

Materials and Methods

Both neonatal and adult Sprague-Dawley rats of both sexes were used for this study. The animals were anaesthetized with intraperitoneal injections of sodium pentobarbital (30 mg/kg). The eye balls were removed surgically from the animals. After the cornea had been removed, eyes were fixed in Periodate-lysine Paraformaldehyde (PLP) solution according to the method described by Mclean and Nakane (3). Serial frozen sections 6-8 μm thick were cut with a cryostat microtome at -20 °C. Rabbit immunoglobulin G (IgG) against human EGF, EGF-R and erb-B served as the primary antibodies. They respectively were obtained from Oncogene Science, Inc. U.S.A. The streptavidin-biotin system (Zymed laboratories Inc. U.S.A.) was used for peroxidase staining. The secondary antibody was goat anti-rabbit antibody. Staining protocol was carried out according to the manufacturer’s suggested procedure. The following controls for staining and antibody specificities were performed: (1) the primary antibodies were substituted with normal rabbit serum. (2) 100 μl of diluted (1:100) rabbit EGF IgG, EGF-R IgG and erb-B IgG were absorbed respectively with 40 μg of lyophilized human EGF, EGF-R (peptide-1) and erb-B (peptide-1) obtained from Oncogene Science Inc. U.S.A. (3) goat anti-rabbit IgG was substituted with rabbit anti-mouse IgG. (4) the tissue sections were incubated in phosphate buffered saline pH 7.2 alone without the primary antibody and then treated with 0.02% of 3,3-diaminobenzidine and 0.005% hydrogen peroxide. Some of the
sections were counterstained with haematoxylin. All sections were dehydrated with alcohol, cleared in xylene, and mounted in permount for examination. They were viewed in a Zeiss microscope and photographed.

Results

Positive EGF, EGF-R or erb-B immunoperoxidase staining exhibited the characteristic brownish peroxidase staining, indicative of the presence of EGF, EGF-R or erb-B. On closer examination of EGF, EGF-R and erb-B which were present in the retina, they were distributed respectively over the ganglion cell layer (GCL), inner nuclear (INL), outer nuclear layer (ONL) and retinal epithelium cell (RPE). EGF immunostaining was not strong, but it was specific and distributed uniformly in GCL, INL, ONL and RPE. Weak immunostaining was prominent in the cells of GCL and INL, which could be either ganglion cells or amacrine cells (including displaced amacrine cells) (Fig. 2). EGF-R was distributed throughout both the cytoplasm and nuclei of these cells, and the nuclei appeared relatively prominent (Fig. 3). For the neonatal rat retina, ganglion cells or amacrine cells appeared to have relatively higher EGF-R than those for the adult. The erb-B immunostaining was distributed similarly as the pattern of EGF. However, heavy erb-B immunostaining band was seen in the ONL, which probably belong to the photoreceptor. The immunostaining was present mainly in the cytoplasm of photoreceptor (Fig. 4). The controls for staining and antibody specificities (see methods) were all negative.

Discussion

Using immunohistochemical method our present studies document for the first time direct evidence of the existence and location of EGF, EGF-R and erb-B in the rat retina. It is well known that EGF elicits mitogenic and maturation responses in a variety of ectoderm- and mesoderm-derived tissues (4). The binding of EGF to its specific membrane receptor (EGF-R) on target cell initiates a complex array of cellular responses (5). The erb-B oncogene was found to be a truncated EGF receptor gene (6). However, little is known about the effects of EGF, EGF-R and erb-B on the retina. The density of EGF-R had been found to be relatively higher in astrocytes, intermediate in oligodendrocytes, and low in neurons in rat brain (7). Several glial cell lines, derived from human brain had been demonstrated to bind and respond mitogenically to EGF (8). The retina is a part of the central nervous system. The reasons for
these different patterns of EGF, EGF-R and erb-B in retina remain to be determined.

Our finding also suggests that EGF, EGF-R and erb-B may play an important role in the retina development and undergoes various structural and physiologic changes with aging.

References


Figure Legends

Fig. 1 The section was cut through the wall of the eye, and incubated with rabbit anti-EGF antibody. Positive staining was distributed the layers of the retina, namely in the ganglion cell layer (GCL), inner nuclear layer (INL), outer nuclear (ONL) and retinal pigment epithelium (RPE). The nucleus of the retinal cell (see arrows) appeared to have less staining than the cytoplasm. x66.

Fig. 2 The section was similarly prepared and cut as Figure 1, incubated with rabbit anti-EGF-R antibody. Many positive staining cells were observed in the GCL and INL. x66.

Fig. 3 The figure was a portion of Figure 2, showing the cells in GCL and INL. Immunostaining for EGF-R was distributed throughout the cytoplasm and nuclei of cells. The nuclei stained relatively heavier than the cytoplasm. x120.

Fig. 4 The section was incubated with rabbit anti-erb-B antibody. Immunostaining was distributed similarly as the pattern of EGF. In ONL, however, immunostaining appeared relatively heavier than the rest of other layers. The nucleus of cell appeared to have less staining than the cytoplasm (see arrows). x66.