c-Fos Protein in Photoreceptor Cell Death after Photic Injury in Rats

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PURPOSE. To examine the involvement of c-Fos protein in light-induced photoreceptor cell death in rats.

METHODS. Thirty-two Lewis albino rats were exposed to green fluorescent light (480–520 nm) of 300 to 320 foot-candles (3228–3443.2 lux) for 3 hours, allowed to recover in the dark, and euthanatized at 0, 1, 3, 6, 12, 24, or 96 hours after light exposure. c-Fos was detected immunohistochemically and nicked DNA by in situ TdT-dUTP terminal nick-end labeling (TUNEL). Double labeling of c-Fos and DNA nicks was also performed.

RESULTS. There was a time-dependent change in the number of c-Fos–positive photoreceptor nuclei after light injury, which paralleled the change in the number of TUNEL-positive nuclei. The temporal and spatial appearance of these nuclei also matched the appearance of pyknotic nuclei of the outer nuclear layer. Double-labeling study revealed that some c-Fos–positive nuclei were also TUNEL-positive nuclei.

CONCLUSIONS. There was an acute accumulation of c-Fos protein in photoreceptors associated with cell death. This study further supports other studies showing that c-Fos is linked to apoptotic photoreceptor cell death. (Invest Ophthalmol Vis Sci. 2000;41:2755–2758)

Apoptosis in light-induced photoreceptor cell death has been demonstrated by several groups since 1996.1 However, the underlying mechanism of photoreceptor cell death remains poorly understood.

c-Fos, an immediate early gene,2 is one of the genes activated by external stimuli such as growth or differentiation factors and physical stresses like injury and heat and electric shocks. As a transcription factor alone or interacting with other transcription factors, it regulates the expression of many genes such as the c-jun family members that are associated with c-Fos within the AP-1 transcription complex.3

Accumulation of c-Fos was observed in the cytoplasm of fibroblasts undergoing apoptosis.4 Hafezi and his colleagues5 demonstrated that c-fos mRNA is necessary for light-induced apoptotic cell death of photoreceptors in mice, whereas photoreceptor degeneration in rd mice, which is not light-injury related, is c-Fos–independent.6 Hence, it is possible that mouse photoreceptors undergo c-Fos–dependent or –independent apoptotic pathways depending on the stimuli. It is not clear whether c-Fos is involved in apoptosis of photoreceptors in photic injury of rat retinas. In the present study, c-Fos after light damage was detected immunohistochemically, whereas cell death was indicated by TdT-dUTP terminal nick-end labeling (TUNEL). Double labeling of c-Fos by immunohistochemical method and DNA nicks by in situ TUNEL were also performed.

METHODS

Animals

Thirty-two 35-day-old Lewis albino rats were equally divided into eight groups. They were reared in cyclic light and darkness for 14 days before the experiment. All animal handling and experimentation adhered to the guidelines established in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by Institutional Animal Use and Ethics Committee.

Light Exposure

Seven groups of rats (four in each group) were dark-adapted for 24 hours before exposure to green light (480–520 nm) at 300 to 320 foot-candles (ft-cd) for 3 hours. After that, they were allowed to recover in the dark and euthanatized (in groups of four animals) after 0, 1, 3, 6, 12, 24, or 96 hours. One group of rats was kept in cyclic light and darkness for 14 days and then dark-adapted for 24 hours before euthanasia as control. All exposed rats were light-exposed at the same time in the morning and euthanatized in a darkroom equipped with a red light.

Tissue Preparation

All enucleated left eyes were fixed in Davidson’s solution and paraffin-embedded, whereas the right eyes were sampled into four strips of the retina from superior, inferior, nasal, and temporal quadrants. The strips were glutaraldehyde-fixed and epoxy-embedded.
Histopathology
One-micron-thick epoxy sections of the strips from the superior retinas were cut, stained in 1% toluidine blue, and examined under light microscopy.

In Situ TUNEL
Apop-Taq in situ Apoptosis Detection Kits from Oncor (Gaithersburg, MD) were applied to detect nicked DNA on 4-μm-thick paraffin sections containing the whole retina including the optic nerve head. Diaminobenzidine (DAB; Sigma Chemical, St. Louis, MO) was used as chromogen.

Immunohistochemistry of c-Fos
Four-micron-thick paraffin sections similar to those used for TUNEL were deparaffinized and incubated overnight at 4°C in rabbit antiserum to c-Fos [c-Fos(Ab-2); Calbiochem, La Jolla, CA]. Antibody binding was localized by the avidin-biotin-peroxidase method (Vector, Burlingame, VA) using DAB as chromogen.

Colocalization of c-Fos and DNA Nicks
Retinas at twenty-four hours after photic injury were selected for double labeling because it was the peak incidence of in situ TUNEL in this study. After in situ TUNEL using DAB as chromogen, immunohistochemistry for c-Fos was performed on the same section using fluorescein (Trevigen, Gaithersburg, MD) as chromogen. The section was excited at 495 nm, then visualized and photographed with a Leica microscope (Leica, Germany). The length of the outer nuclear layer (ONL) was recorded by an image analyzing system (Leica model Q500MC; Cambridge, UK). The number of positive cells per unit length of the retina was obtained. A pairwise multiple comparison test was applied to detect significant differences among different groups. P < 0.05 was considered statistically significant.

RESULTS
In this series of experiments we used a light exposure schedule (i.e., 300–320 ft-cd for 3 hours) to generate a mild injurious insult to rat retinas. Because the tissue responses of the retina varied according to the region, the results we present here were restricted to the most sensitive area (i.e., the equatorial region of the superior quadrant of the retina).7

Histopathology
Immediately after photic injury (Fig. 1), mild edema and isolated pyknotic nuclei were noted in the inner part of the ONL (Figs. 1A through 1E). At 12 hours (Fig. 1F through 1I), more pyknotic nuclei were noted and most of them were in the inner part of the ONL. At 24 hours (Fig. 1J through 1L), the ONL was focally thinned with 8 to 10 nuclei per column compared with 10 to 12 of the normal and showed more pyknotic nuclei, which were scattered in the ONL. After 96 hours (Fig. 1M through 1P), the ONL showed marked loss of photoreceptor nuclei and few pyknotic nuclei.

In Situ TUNEL
There was a gradual increase in the number of TUNEL-positive photoreceptor nuclei from 0 to 24 hours after light exposure, starting in the inner part of the ONL (Figs. 1G through 1J) and gradually spreading to the outer part of the ONL at 24 hours.
DISCUSSION

The present study showed temporal and spatial appearance of histologically degenerating cells that paralleled the appearance of DNA nicks and c-Fos accumulation after photic injury in rat retinas. Double-labeling showed intense c-Fos IM without DNA nicks, coexistence of weak c-Fos IM and extensive DNA nicks, and no c-Fos IM but extensive DNA nicks in scattered photoreceptor nuclei. Morphometry of c-Fos–positive nuclei and TUNEL-positive nuclei showed parallel trends. These observations are consistent with an active role for c-Fos in light-induced apoptosis of photoreceptor cells preceding DNA nicks in rats.

The three categories of double-labeling observed can be explained by an early appearance of c-Fos in the nuclei preceding the action of endonuclease in generating DNA nicks. As the chromosomal DNA continues to be cleaved in apoptosis, synthesis of c-Fos decreases or degradation of c-Fos increases until the cell ceases to have c-Fos but nicked DNA.

There are other possible explanations to our observation. Photoreceptors may die through both c-Fos–dependent or –independent pathways after light injury. However, the number of TUNEL-positive nuclei would have been higher than that of c-Fos–positive ones if the c-Fos–independent pathway played a significant role. Our morphometry study does not support this possibility. It is also possible that there is no causal relationship of c-Fos and photoreceptor cell death but rather a coincident induction of c-fos. For example, Hafezi et al. using c-fos−/− rd mice showed that there was extensive photoreceptor cell death without c-fos expression even though Rich and her colleagues observed aberrant expression of c-fos accompanying photoreceptor cell death in the rd mouse. Although this is possible, there is extensive documentation of a link between the expression of c-fos and impending cell death in a variety of neural and nonneural tissues during development or under pathologic conditions. For example, Estus and his colleagues revealed that c-fos induction was restricted to neurons undergoing chromatin condensation, a hallmark of apoptosis, leading to the hypothesis that c-fos is indeed involved in the early changes of gene expression in the apoptotic pathway. A recent study by Hafezi and his colleagues in which c-fos−/− transgenic mice were used, also demonstrated the requirement of c-fos in photoreceptor cell death after photic injury. Our observation supports a causal role for c-fos in light-induced photoreceptor cell death as suggested by Hafezi et al. It is also possible that c-fos expression may be synchronized, whereas cell death by apoptosis as shown by...
TUNEL may not be synchronized, giving rise to our observed different labeling patterns.

In summary, our study suggests that there may be a narrow window between the accumulation of c-Fos and the presence of DNA nicks in photoreceptors after photic injury. Inhibition of c-fos expression may ameliorate light-induced retinal apoptosis. Further studies on c-jun and AP-1 may also help to delineate the pathways of c-fos-related photoreceptor cell death.

References