α_2 -Adrenergic Agonists Induce Basic Fibroblast Growth Factor Expression in Photoreceptors *In Vivo* and Ameliorate Light Damage

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We observed an induction of basic fibroblast growth factor (bFGF) mRNA in the rat retina after systemic administration of the α_2 -adrenergic agonists xylazine and clonidine. A single injection of xylazine or clonidine transiently increased bFGF mRNA. Preinjection of yohimbine, an α_2 -adrenergic antagonist, completely inhibited this increase. Higher dosage of yohimbine inhibited the baseline expression of bFGF. Of particular interest is the finding that the induced bFGF expression occurred almost exclusively in the inner segment region of photoreceptors.

No increase in bFGF mRNA was found in the brain after either xylazine or clonidine injection. Xylazine or clonidine given systemically before and during constant light exposure also reduces photoreceptor degeneration in albino rats. These results indicate that regulation of bFGF expression in photoreceptors is unique in the CNS and suggest that endogenous bFGF promotes photoreceptor survival.

Key words: α_2 -adrenergic receptors; xylazine; clonidine; bFGF; retina; photoreceptors; degeneration; brain; rat

Basic fibroblast growth factor (bFGF) is one of the best characterized members of a family of at least nine structurally related heparin-binding growth factors (Baird, 1994). It stimulates the proliferation and modulates the differentiation of a variety of cells of mesodermal and ectodermal origin and plays a role in wound healing and angiogenesis (Gospodarowicz et al., 1987; Gospodarowicz, 1991). bFGF also exhibits neurotrophic activities (Wagner, 1991; Baird, 1994). It induces neurite outgrowth of PC12 cells (Togari et al., 1985; Rydel and Greene, 1987) and motor neurons (Gurney et al., 1992), synapse formation (Peng et al., 1991), and retinal regeneration *in vivo* (Park and Hollenberg, 1993). In addition, it has been shown that bFGF promotes the survival of neurons, including promoting neuronal survival in culture (Ishikawa et al., 1992; Kushima et al., 1992), protecting neurons from NMDA receptor-mediated cytotoxicity (Freese et al., 1992), and reducing axotomy-induced neuronal cell death (Sievers et al., 1987; Cummings et al., 1992). Injection of bFGF into the eye was found to rescue photoreceptors in two retinal degeneration models, the Royal College of Surgeons (RCS) rat with an inherited retinal degeneration, and constant light-induced photoreceptor degeneration in the albino rat (Faktorovich et al., 1990, 1992; LaVail et al., 1992).

In the brain, bFGF expression was elevated by various insults, including mechanical trauma, chemical injury, and ischemia (Wagner, 1991; Baird, 1994). In addition, activation of β -adrenergic receptors increased bFGF mRNA in rat hippocampus, cerebral cortex, and cerebellum (Follesa and Mocchetti,

1993). Recently, it has been shown in rat retina that bFGF mRNA was upregulated by mechanical injury (Wen et al., 1995).

The present work was stimulated by results of experiments in rat in which xylazine was used as an anesthetic. We now report that, in rat, systemic administration of the α_2 -adrenergic agonists xylazine and clonidine upregulates bFGF mRNA in retina, but not in brain, and that the α_2 -adrenergic antagonist yohimbine inhibits the upregulation. Of particular interest is the finding that this upregulation occurred mainly in the inner segment region of photoreceptors. Our results also show that systemic application of xylazine or clonidine before and during constant light exposure reduces photoreceptor degeneration in albino rats. These results indicate that regulation of bFGF expression in photoreceptors is unique and suggest that endogenous bFGF promotes photoreceptor survival.

MATERIALS AND METHODS

Animals. Male Sprague Dawley rats, 2–3 months of age, were used in all experiments. Animals were kept in a 12:12 hr light–dark cycle at an in-cage illuminance of < 25 foot-candles (1 ft-c = 10.76 lux) for 7 d before experiments. Ketamine (Fort Dodge Laboratories, Fort Dodge, IA), xylazine (either from Lloyd Laboratories, Shenandoah, IA, or Sigma, St. Louis, MO), or PBS was injected intramuscularly into the right hind leg. Clonidine (Sigma) or yohimbine (Sigma) was injected intraperitoneally.

RNA preparation and Northern blot analysis. Animals were killed by CO₂ overdose. Whole retinas were dissected, snap-frozen in liquid nitrogen, and stored at –80°C. Pooled retinas were homogenized in 5.5 M guanidinium thiocyanate solution (5.5 M guanidinium thiocyanate, 25 mM sodium citrate, and 0.5% sodium lauryl sarcosine, pH 7.0), and total RNA was isolated by a CsTFA (cesium trifluoroacetate, Pharmacia, Piscataway, NJ) gradient method (Farrell, 1993). Total RNA (20 μ g of each sample) was electrophoresed on 1% agarose formaldehyde gels and downward wick-transferred in 20 $\times$ SSC (1 $\times$ SSC = 0.15 M NaCl and 0.15 M sodium citrate, pH 7.0) to a nylon membrane (Hybond-N, Amersham, Arlington Heights, IL). Blots were UV-irradiated to immobilize RNA and then prehybridized for 4 hr in a hybridization solution containing 50% formamide, 5 $\times$ Denhardt's solution, 5 $\times$ SSPE (1 $\times$ SSPE = 0.15 M NaCl, 10 mM NaH₂PO₄, and 1 mM EDTA, pH 7.4), 200 μ g/ml denatured salmon sperm DNA, and 5% SDS at 50°C. Random primed ³²P-labeled cDNA

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Figure 1. Induction of bFGF mRNA expression in the retina by xylazine. Total RNA (20 μ g/lane) was electrophoresed on a 1% formaldehyde denaturing gel. Northern blot hybridization was performed with 32 P-labeled probes complementary to mRNA encoding bFGF. A major bFGF transcript of 7.0 kb was detected in all blots. Then each blot was stripped and rehybridized with probes for 18S rRNA, which served as a control for RNA loading. Migration of 28S and 18S rRNA was indicated for each blot. *A*, Xylazine-induced bFGF expression. Animals were injected with either the ketamine–xylazine mixture (*Ket+Xyl*; ketamine, 40 mg/kg; xylazine, 6 mg/kg, i.m.), ketamine alone (*Ket*; 40 mg/kg, i.m.), xylazine alone (*Xyl*; 6 mg/kg, i.m.), or *PBS* (0.5 ml, i.m.). Control animals

probes for rat bFGF (gift of Dr. A. D. Baird, Whittier Institute for Diabetes and Endocrinology, La Jolla, CA; Shimasaki et al., 1988), or rat 18S rRNA (gift of Dr. D. Schlessinger, Washington University, St. Louis, MO; Bowman et al., 1981) were added to the hybridization buffer (10^6 cpm/ml) and hybridized at 50°C overnight. Then blots were washed twice in $2\times$ SSC, 0.1% SDS at room temperature for 5 min, and twice in $0.1\times$ SSC, 0.1% SDS at 65°C for 10 min. After posthybridization wash, blots were exposed to a Storage Phosphor Screen (Molecular Dynamics, Sunnyvale, CA), and data were digitized by scanning the phosphor screen with a Phosphor Imager System (Molecular Dynamics). Blots were re-probed then with 18S rRNA probe, and data of 18S rRNA served as a control for RNA loading. Quantitative analysis was performed for the 7.0 kb transcript of bFGF mRNA, normalized with data of 18S rRNA by using Image Quant (Molecular Dynamics). Hard copies were obtained by exposing blots to Hyper Film (Amersham).

In situ hybridization. Animals were killed by CO_2 overdose and immediately perfused with PBS and then with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Eyes were enucleated, and the cornea and lens were removed. The rest of the eye was post-fixed in 4% paraformaldehyde overnight and then in 30% sucrose in PBS for 4 hr. Eyes were embedded in Tissue-Tek O.C.T. compound (Miles, Elkhart, IN), frozen in powdered dry ice, and stored at -80°C . Sections of 15–20 μm were cut through the entire retina, along the vertical meridian, on a Cryostat at -20°C and thaw-mounted onto Super Frost Plus glass slides (Fisher Scientific, Pittsburgh, PA). Sections on the glass slides were air-dried for 2 hr and fixed in 4% paraformaldehyde for 20 min before treatment with 10 $\mu\text{g}/\text{ml}$ proteinase K for 10 min at 37°C . Sections then were washed and treated with 0.25% acetic anhydride and 0.1 M triethanolamine, pH 8.0, for 10 min, 0.1 M Tris/glycine buffer, pH 7.0, for 30 min, dehydrated in graded alcohols, and air-dried. Then sections were hybridized with ^{35}S -labeled antisense RNA probe for rat bFGF (10^7 cpm/ml) at 50°C overnight. Some sections were hybridized with sense probe, which served as a control for nonspecific labeling. The hybridization buffer contained 40% formamide, $4\times$ SSC, 1 mg/ml yeast tRNA, 1 mg/ml denatured salmon sperm DNA, 10% dextran sulfate, 10 mM DTT, and $5\times$ Denhardt's solution. After hybridization, sections were washed twice in $2\times$ SSC for 10 min at room temperature, once in 50% formamide, $2\times$ SSC at 52°C for 10 min, and then treated with RNase A (10 mg/ml) in $2\times$ SSC for 20 min at 37°C . Sections were washed once again in 50% formamide, $2\times$ SSC for 10 min at 52°C and then $2\times$ SSC, 0.05% Triton X-100 for 1 hr at room temperature. Finally, sections were dehydrated in graded alcohols and cleaned in xylene. Slides were coated with NTB-3 photoemulsion (Eastman Kodak, Rochester NY), exposed at 4°C for 10–20 d, and then developed.

Histology and constant light exposure. Animals were injected systemically with either xylazine or clonidine according to a 10 d injection protocol, in which injection was given each day starting 4 d before constant light exposure and continuing throughout the constant light exposure. An additional 4 d injection protocol was used for xylazine injection, in which injection was given each day for 4 d immediately before constant light exposure, but no injection was made during the 7 d constant light exposure. For constant light exposure, animals were placed into constant fluorescent light at an illuminance of 115–130 ft-c for a period of 7 d. Then animals were killed by overdose of CO_2 , followed immediately by vascular perfusion of mixed aldehydes, and eyes were

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were uninjected. Retinas were collected 12 hr after injection. There was a 4.5-fold increase in bFGF signal in retinas from ketamine–xylazine-injected animals and also a 4.5-fold increase in retinas from xylazine-treated animals. No significant change occurred in bFGF mRNA in either ketamine- or PBS-treated retinas. *B*, Time course of xylazine-induced bFGF expression. Animals were injected with xylazine (6 mg/kg, i.m.), and retinas were collected 12, 24, and 48 hr after the injection. Expression of bFGF mRNA increased 5.7-fold at 12 hr after xylazine injection, declined to twofold, and returned to the control level by 48 hr. *C*, Inhibition of xylazine effect by yohimbine. Animals were injected with *PBS* (0.5 ml, i.m.), xylazine (*Xyl*; 6 mg/kg, i.m.), or yohimbine (5 mg/kg, i.p., 20 min before xylazine) plus xylazine (*Yoh+Xyl*) or yohimbine alone (*Yoh*; 5 mg/kg, i.p.). Retinas were collected 12 hr after injection. There was a significant increase in bFGF expression in xylazine-treated animals. Pretreatment with yohimbine completely blocked the effect of xylazine. No significant change in bFGF expression occurred in either PBS or yohimbine-treated animals.

embedded in an Epon/Araldite mixture for sectioning at 1 μ m thickness to provide sections of the entire retina along the vertical meridian of the eye (LaVail and Battelle, 1975).

RESULTS

Induction of bFGF mRNA expression in the retina by xylazine

Xylazine and ketamine are anesthetics commonly used in animal surgery. In our rat experiments, we routinely used a combination of ketamine and xylazine (ketamine, 40 mg/kg; xylazine, 6 mg/kg, i.m.). In early studies, we were surprised to find a transient increase in bFGF mRNA in the retinas of sham-operated eyes (data not shown). To determine whether the increase in bFGF mRNA was induced by ketamine or xylazine, we injected animals with either the ketamine–xylazine mixture, ketamine alone (40 mg/kg, i.m.), xylazine alone (6 mg/kg, i.m.), or PBS (0.5 ml, i.m.) and examined bFGF mRNA in the retina 12 hr after injection. Northern blot analysis, with radioactively labeled DNA probes complementary to mRNA encoding bFGF, detected a major bFGF transcript of 7.0 kb, along with several smaller transcripts (Fig. 1*A*). A significant increase in bFGF mRNA was observed in animals injected with the ketamine–xylazine mixture or xylazine. Quantitative analysis of this transcript indicated that there was a 4.5-fold increase in retinas of ketamine–xylazine-injected animals and also a 4.5-fold increase in retinas of xylazine-treated animals. No significant change was observed in either ketamine or PBS-treated animals. Thus, xylazine, an α_2 -adrenergic agonist, was responsible for the increase in bFGF mRNA we observed in sham-operated eyes.

To characterize further the effect of xylazine on bFGF mRNA expression in the retina, we injected animals with xylazine (6 mg/kg, i.m.) and estimated bFGF mRNA level in the retinas 12, 24, and 48 hr after the injection. As shown in Figure 1*B*, the xylazine-induced bFGF expression was transient. Expression of bFGF mRNA increased 5.7-fold 12 hr after xylazine injection. By 24 hr, it had declined to approximately twofold and had returned to the control level by 48 hr.

To confirm that the xylazine effect on bFGF mRNA expression was mediated via α_2 -adrenergic receptors, we used yohimbine, a specific α_2 -adrenergic antagonist. When injected (5 mg/kg, i.p.) 20 min before xylazine, yohimbine completely blocked the effect of xylazine (Fig. 1*C*). The complete inhibition of the xylazine-induced bFGF expression provides additional evidence that the effect of xylazine was mediated via α_2 -adrenergic receptors. Furthermore, injection of hydralazine (5 mg/kg, i.m.), which reduces mean arterial blood pressure substantially (Burney et al., 1995), produced only a small increase (40%) in bFGF expression in the retina (data not shown), indicating that reduction in blood pressure had only a small contribution to the increase in bFGF expression induced by xylazine.

Induction of bFGF mRNA expression in the retina by clonidine

We next used clonidine, another α_2 -adrenergic agonist, to confirm further that the activation of α_2 -adrenergic receptors was responsible for the induction of bFGF expression. Animals were injected with clonidine (0.5 mg/kg, i.p.), and retinas were collected 12, 24, or 48 hr after injection. The temporal pattern of bFGF mRNA expression after a single injection of clonidine was very similar to that of xylazine. There was a transient increase (3.2-fold) in bFGF mRNA 12 hr after injection, which declined to baseline level by 24 hr after injection (Fig. 2*A*). Pretreatment with yohimbine (15 mg/kg, i.p.) 20 min before clonidine injection completely inhibited

Figure 2. Induction of bFGF expression by clonidine. Northern blot hybridization was performed as described in Figure 1. *A*, Time course of clonidine-induced bFGF expression. Animals were injected with clonidine (0.5 mg/kg, i.p.), and retinas were collected 12, 24, and 48 hr after the injection. Expression of bFGF mRNA increased 3.2-fold at 12 hr after clonidine injection and returned to the control level by 24 hr. *B*, Inhibition of clonidine effect by yohimbine. Animals were injected with PBS (0.5 ml, i.m.), clonidine (*Cln*; 0.5 mg/kg, i.m.), or yohimbine (15 mg/kg, i.p., 20 min before clonidine) plus clonidine (*Cln+Yoh*), or yohimbine alone (*Yoh*; 15 mg/kg, i.p.). There was a marked increase in bFGF expression in clonidine-treated animals. Note that pretreatment with yohimbine at this dosage not only completely blocked the effect of clonidine but also inhibited the normal expression of bFGF by 40%. Injection of yohimbine alone at this dosage inhibited normal bFGF expression by the same amount.

the clonidine effect on bFGF expression (Fig. 2*B*). In addition, at this dosage, yohimbine also inhibited the normal expression of bFGF in the retina by 40% (Fig. 2*B*).

Expression of bFGF mRNA in different regions of the brain

Because the retina is part of the CNS, we wanted to learn whether xylazine or clonidine induced bFGF expression in the brain, as well. Animals were injected with either xylazine (6 mg/kg, i.m.) or clonidine (0.5 mg/kg, i.p.), and eight brain regions—septum, striatum, thalamus, hypothalamus, hippocampus, olfactory bulb, cerebellum, and cerebral cortex—were dissected 12 hr after injection. Northern blot analysis showed virtually no change in bFGF

Figure 3. Expression of bFGF in the brain. Northern blot hybridization was performed as described in Figure 1. Animals were injected with either (*A*) xylazine (Xyl; 6 mg/kg, i.m.) or (*B*) clonidine (Cln; 0.5 mg/kg, i.p.). Control animals were uninjected. Eight brain regions—septum (Sep), striatum (Str), hippocampus (Hip), thalamus (Th), hypothalamus (Hy), olfactory bulb (OB), cerebellum (Cb), and cerebral cortex (Cx)—were dissected 12 hr after injection. Although there were differences in bFGF expression among brain regions, there was virtually no change in each brain region after xylazine or clonidine treatment.

mRNA expression after either xylazine (Fig. 3*A*) or clonidine (Fig. 3*B*) injection in any of the brain regions. These findings indicate that both xylazine- and clonidine-dependent bFGF expression is selective for the retina.

Localization of bFGF mRNA in the retina

To localize the xylazine-induced bFGF mRNA expression, we performed *in situ* hybridization with radioactively labeled RNA probes complementary to mRNA encoding bFGF. Animals were injected with xylazine (6 mg/kg), and eyes were collected 12 hr after injection. As shown in Figure 4, *A* and *B*, in the normal retina bFGF mRNA was expressed at a low level in the retinal pigment epithelium (RPE), the inner segments of photoreceptors, the inner nuclear layer, and the ganglion cell layer. After xylazine injection, the increased bFGF mRNA was found almost exclusively in the inner segments of photoreceptors, whereas little change in expression was observed in the other regions of the retina (Fig. 4*C,D*). Retinas hybridized with sense probe showed nonspecific hybridization that did not form any specific pattern (Fig. 4*E,F*).

Protection of photoreceptors against light damage by xylazine or clonidine injection

Because exogenous bFGF rescues photoreceptors in RCS rats and also in albino rats exposed to constant light (Faktorovich et al., 1990, 1992; LaVail et al., 1992), we asked whether induction of bFGF expression by α_2 -adrenergic agonists in photoreceptors could protect them from cell death in a retinal degeneration. We used constant light-induced photoreceptor degeneration in albino rats as a model. Because the induction of bFGF expression by xylazine or clonidine injection was transient, we used a multiple injection protocol to produce a sustained upregulation of bFGF expression. In this protocol (10 d injection), systemic injection of either xylazine or clonidine was made each day, starting 4 d before constant light exposure and continuing throughout the constant light exposure. We also used an additional protocol (4 d injection) for xylazine injection to determine whether stimulation of bFGF

expression in the retina before constant light exposure would ameliorate light damage. In the 4 d injection protocol, systemic injection of xylazine was given each day for 4 d immediately before constant light exposure, but no injections were given during the 7 d constant light exposure.

Figure 5 shows representative sections of superior retinas from animals exposed to constant light with or without xylazine treatment. Severe photoreceptor degeneration was observed in uninjected animals after 7 d of constant light exposure. The outer nuclear layer (ONL), where photoreceptor nuclei reside, was reduced from 10–11 rows of nuclei in normal animals (Fig. 5*A*) to 3–4 rows (Fig. 5*B*). There was almost a complete absence of photoreceptor inner segments, and outer segments that remained formed large rounded or oblong profiles (Fig. 5*B*). In injected animals, however, the photoreceptor degeneration was much less severe. There were, on average, 6–8 rows of photoreceptor nuclei in the ONL. The inner segments, shorter than normal, were present. The outer segments were better preserved, although many also showed the rounded and oblong profiles (Fig. 5*C*).

To assess the degree of photoreceptor preservation after constant light exposure, we used a scoring system that took into account the well known nonuniform distribution of light damage across the retina and, in each retinal region, the number of surviving photoreceptor nuclei as well as the condition of the inner and outer segments. A five point scale was used, with the score for normal retina being five and the score being one for retina with the most severe loss of photoreceptors. Each tissue section was assessed in a “double-blind” manner by four scientists equally familiar with the scoring criteria; a score was given by unanimous decision. In uninjected animals, the degree of photoreceptor preservation after 7 d of constant light exposure was 1.83 ± 0.60 (mean $\pm$ SD, $n = 23$), whereas in all three groups of animals receiving xylazine or clonidine injection the degrees of photoreceptor preservation was significantly higher. The score for animals receiving the 10 d injection of xylazine was 3.54 ± 0.63

Figure 4. Localization of xylazine-induced bFGF expression. *In situ* hybridization was performed with a bFGF antisense RNA probe. Photomicrographs of Nomarski are shown in *A*, *C*, and *E*, and dark-field images of the same sections are shown in *B*, *D*, and *F*, respectively. *A*, *B*, *A* section of the superior retina from a normal control eye. *C*, *D*, *A* section of the superior retina from an animal 12 hr after injection of xylazine (6 mg/kg, i.m.). In the normal retina (*A*, *B*), there was a low level of bFGF expression in the retinal pigment epithelium (*pe*), the inner segments of photoreceptors (*is*), the inner nuclear layer (*in*), and the ganglion cell layer (*gc*). After xylazine injection, the induced bFGF expression was found almost exclusively in the inner segments, with no significant change in bFGF mRNA in other regions of the retina. *E*, *F*, *A* section of superior retina from an animal 12 hr after injection of xylazine (6 mg/kg, i.m.). This section of retina had been hybridized with sense probe to serve as a nonspecific hybridization control. *on*, Outer nuclear layer. Scale bar, 100 μ m.

($n = 34$), 3.08 ± 0.39 ($n = 18$) for 4 d injection of xylazine, and 2.97 ± 0.55 ($n = 15$) for the 10 d injection of clonidine (Fig. 6).

DISCUSSION

We have shown that a single injection of the α_2 -adrenergic agonist xylazine or clonidine transiently increased bFGF mRNA mainly in the photoreceptors of rat retina, but not in the brain. Our results provide evidence that this increase in bFGF expression was mediated via activation of α_2 -adrenergic receptors. First, the increase was induced by two α_2 -adrenergic agonists in a very similar manner. Second, the effects of both xylazine and clonidine were inhibited by the specific α_2 -adrenergic antagonist yohimbine.

α_2 -adrenergic receptors have been identified in the retina. Binding studies with bovine retinal membranes showed that the major α -adrenergic receptor in the retina was the α_2 subtype (Bittiger et al., 1980; Osborne, 1982). Using [H^3]paralaminoclonidine and autoradiography, Zarbin et al. (1986) mapped α_2 -adrenergic receptors in the rat retina. They found that the major binding sites were localized to the inner plexiform layer, with a lower density of binding sites in the ganglion cell layer. Although these authors did not mention binding sites in the inner segments of photoreceptors, these could be identified clearly in their published microphotographs [Zarbin et al. (1986), their Fig. 4*A,B*]. This raises the possibility that the increase in bFGF mRNA expression resulted from direct stimulation of α_2 -adrenergic receptors in photoreceptors by xylazine and clonidine. In cultured chromaffin cells from bovine adrenal medulla, it has been shown that direct stimulation of nicotinic acetylcholine receptors or angiotensin II receptors increased bFGF protein expression via cAMP or protein kinase C pathways, respectively (Stachowiak et al., 1994). Because α_2 -adrenergic receptors are believed to be coupled negatively to adenylate cyclase (Jakobs, 1979; Bylund, 1992) and it has been shown that direct stimulation of α_2 -adrenergic receptors resulted in inhibition of cAMP production in the rabbit retina (Osborne, 1991), our results may point to a new regulatory mechanism for bFGF expression.

The physiological functions of α_2 -adrenergic receptors in the retina are not understood. Our finding that a high dosage of yohimbine (15 mg/kg) inhibited expression of bFGF in normal retina (Fig. 2*B*) indicates that α_2 -adrenergic receptors are of

physiological importance in regulating bFGF expression in the retina.

A most striking finding in the present work is that the induction of bFGF mRNA by α_2 -adrenergic stimulation was found in the inner segments of photoreceptors. Increased bFGF immunoreactivity in the photoreceptors has been reported in both mouse and rat after optic nerve crush (Kostyk et al., 1994), whereas expression of bFGF mRNA was found to be elevated in mouse and rat after constant light exposure (Gao and Hollyfield, 1996). On the other hand, we recently found that mechanical injury to the rat retina induced a marked increase in bFGF expression, and the greatest increase was found in the inner nuclear layer (Wen et al., 1995).

Although xylazine and clonidine induced bFGF mRNA in the retina, it was surprising that the expression of bFGF in the brain was not changed. Similar results were reported by Follesa and Mocchetti (1993), in which injection of clonidine (0.5 mg/kg, i.p.) did not affect bFGF mRNA in the rat brain. Thus, there seem to be differences in the regulation of bFGF expression between retina and brain.

The neuroprotective activities of bFGF have been well studied (Wagner, 1991; Baird, 1994), and evidence is accumulating that bFGF promotes photoreceptor survival. In RCS and light-damaged rats, bFGF slows or prevents photoreceptor degeneration (Faktorovich et al., 1990, 1992; LaVail et al., 1992). Optic nerve crush upregulates bFGF expression in photoreceptors (Kostyk et al., 1994), which is believed to result in photoreceptor protection against light damage in rats after optic nerve section (Bush and Williams, 1991). Gao and Hollyfield (1995, 1996) found that bFGF in photoreceptors was elevated in light-stressed mice and rats and also in inherited mouse retinal degeneration models. They suggest that bFGF upregulation may function to enhance photoreceptor survival. In addition, upregulation of bFGF in retina by mechanical injury is believed to be responsible for the injury-induced photoreceptor rescue in RCS and light-damaged rats (Wen et al., 1995). It is very likely that the protection of photoreceptor by α_2 -adrenergic agonists against light damage was mediated by upregulated bFGF in photoreceptors, especially considering that even the 4 d injection of xylazine *before* constant light exposure resulted in significant photoreceptor preservation. How bFGF expression is regulated in the photoreceptor is certainly an

Figure 5. Protection of photoreceptors by xylazine treatment. *A*, Normal retina of rat reared in cyclic light. The photoreceptor outer segments (*OS*) are apposed to the retinal pigment epithelium (*RPE*), distinct photoreceptor inner segments (*IS*) are seen, and the outer nuclear layer (*ONL*) consists of 10–11 rows of photoreceptor cell nuclei. *B*, Retina of rat exposed to 7 d of constant light without injection (*superior* region). The *ONL* is reduced to 3–4 rows of nuclei, the inner segments are missing or are reduced to very short stumps, and the few remaining outer segments are in the form of large rounded or oblong profiles. *C*, Retina of rat that received 7 d of constant light exposure and a 10 d injection of xylazine (*superior* region). The *ONL* shows 6–8 rows of nuclei; inner segments are present, albeit shorter than normal; the outer segments are better preserved but disorganized, many showing the rounded and oblong profiles. *OPL*, Outer plexiform layer; *INL*, inner nuclear layer. Toluidine blue stain was used. Scale bar, 20 μ m.

Figure 6. Degree of photoreceptor preservation in retinas of rats injected with xylazine or clonidine and exposed to constant light. Preservation of photoreceptors was determined by weighing the overall thickness of the ONL and the integrity and organization of the inner and outer segments in a retina. The degree of the photoreceptor preservation for uninjected animals (*Uninj*) after 7 d of constant light exposure was estimated at 1.83 ± 0.60 (mean $\pm$ SD, $n = 23$) on a five point scale; the degree for animals receiving 10 d injections of xylazine (*10-d xyl*) was 3.54 ± 0.63 ($n = 23$), 3.08 ± 0.39 ($n = 18$) for 4 d injection of xylazine (*4-d xyl*), and 2.97 ± 0.55 ($n = 15$) for 10 d injections of clonidine (*10-d cln*). *** $p < 0.001$; Student's t test.

important topic for future studies. On the practical side, this means that photoreceptors can be targeted specifically, systemically, to increase their bFGF production, which may promote photoreceptor survival. Finally, the therapeutic potential of α_2 -adrenergic agonists for photoreceptor degenerative diseases should not be overlooked.

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