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Glutamate-stimulated proliferation of rat retinal pigment epithelial cells

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Abstract

We investigated the effects of glutamate on cell proliferation and the expression of basic fibroblast growth factor (bFGF) and its receptor (FGF-R1) mRNA in cultured rat retinal pigment epithelial (RPE) cells. The number of primary RPE cells was significantly higher after treatment with 0.2 to 1.0 mM glutamate (maximum at 1.0 mM) for 7 days than in controls. Glutamate-stimulated cell proliferation was abolished by (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine (MK-801), but not by 6,7-dinitroquinoxaline-2,3-dione or L(+)-2-amino-3-phosphonopropionic acid. Proliferation was increased to a similar extent by *N*-methyl-D-aspartate (NMDA), but not by kainate, α-amino-3-hydroxy-3-methyl-4-isoxazolepropionic acid or *trans*-(±)-1-amino-1,3-cyclopentanedicarboxylic acid. NMDA-receptor-like immunoreactivity was detected in most cells cultured. Treatment of cells with glutamate increased the level of bFGF mRNA and, to a lesser extent, that of FGF-R1 mRNA, which peaked 2 and 4 days, respectively, after glutamate was added. The increase in bFGF mRNA induced by glutamate was inhibited by MK-801. These findings suggest that glutamate might stimulate proliferation of RPE cells through activation of NMDA receptors and expression of bFGF and further suggest that glutamate may be involved in the proliferative changes of RPE cells in retinal wound healing. © 1998 Elsevier Science B.V.

Keywords: Glutamate; NMDA receptor; Basic fibroblast growth factor; Retinal pigment epithelium; (Rat)

1. Introduction

Glutamate, an excitatory amino acid, is one of the most important neurotransmitters in the retina and the brain. As clearly demonstrated for brain ischemia (Mitani et al., 1990; Benveniste, 1991), glutamate is also reported to be transiently and excessively released from the ischemic retina (Louzada et al., 1992; Neal et al., 1994; Masai et al., 1995) and causes neuronal degeneration in the retina (El-Asrar et al., 1992; Mosinger et al., 1991; Lombardi et al., 1994). Furthermore, glutamate is believed to be released from retinal neurons in response to various types of stress, such as trauma, photocoagulation, retinal detachment and diabetes (Kalloniatis, 1995; Solberg et al., 1996). When these pathologic events occur, retinal pigment epithelial (RPE) cells proliferate and migrate into the retina (Okisaka et al., 1991; Toti et al., 1991; Matsumoto et al., 1994). Therefore, glutamate might be involved in proliferative changes of RPE cells after stress, such as during wound healing.

Glutamate receptors can be classified as ionotropic or metabotropic. Ionotropic receptors have been further divided pharmacologically into *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate (KA) receptors. Both NMDA and metabotropic receptors have been identified pharmacologically with binding assay in chick and human RPE cells (López-Colomé et al., 1993, 1994). However, their possible physiological or pathological roles in RPE cells remain unclear.

Basic fibroblast growth factor (bFGF) is a widely distributed polypeptide mitogen that may be involved in neuronal growth and survival within the central nervous system (Emoto et al., 1989; Wagner, 1991). Protective effects of bFGF against inherited retinal dystrophy (Faktorovich et al., 1990) and laser injury (Schuschereba et

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al., 1994) have also been demonstrated in retinal neurons. Both bFGF and FGF receptors are also reported to be present or synthesized in mammalian RPE cells (Schweigerer et al., 1987; Sternfeld et al., 1989; Bost and Hjelmeland, 1993; Malecaze et al., 1993; Rakoczy et al., 1993). Furthermore, bFGF was demonstrated to have a mitogenic effect on RPE cells (Sternfeld et al., 1989; Esser et al., 1992; Malecaze et al., 1993; Rakoczy et al., 1993). Because expression of bFGF by RPE cells of damaged regions increases after various types of stress (Zhang et al., 1993; Yamada et al., 1996; Yamamoto et al., 1996), RPE cells might self-proliferate by means of bFGF.

Therefore, after exposure to stress, RPE cells might proliferate in response to excessive extracellular glutamate through glutamate receptors and, subsequently, through expression of bFGF. To examine this hypothesis, we studied the effects of glutamate on cell proliferation and levels of bFGF and FGF receptor messenger RNA (mRNA) using reverse transcription-polymerase chain reaction (RT-PCR) in cultured rat RPE cells.

2. Materials and methods

2.1. Cell culture

Breeding pairs of Long–Evans rats were purchased from Charles River (Wilmington, MA) and maintained in our animal facilities. All animal use procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by our Animal Care and Use Committee. Isolation and primary culture of RPE cells were performed according to a previously published method (Chang et al., 1991). Briefly, eyes from 5- to 6-day-old rats anesthetized with pentobarbital sodium were enucleated and rinsed three times in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY), pH 7.4, supplemented with antibiotics (100 U/ml penicillin G, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B, Gibco). The intact eyes were incubated at 37°C for 18 to 20 min in a medium containing 2% dispase (grade II, Boehringer Mannheim, Mannheim, Germany). After incubation, the eyes were washed twice in fresh medium. Microdissection was performed in calcium- and magnesium-free Hanks' balanced salt solution (HBSS, Gibco). The eyes were opened by a vertical incision from the lamina cribrosa and the posterior segment, which consisted of the sclera, choroid, retina and RPE sheet, was cut from the ora serrata. Under a dissecting microscope, the sclera and choroid were gently peeled away from the retina and RPE sheet. The retinas with adherent RPE sheets were removed to fresh HBSS and incubated for 3 min at 37°C, causing the intact RPE sheets to separate from the retinas. Collected RPE sheets were incubated with 0.1% trypsin (trypsin–EDTA, Gibco) in the same buffer for 1 min and mechanically separated to a single-cell suspension by trituration. After enzyme quenching, the RPE cell suspension was centrifuged for 5 min at 1000 rpm and the supernatant was exchanged for the medium supplemented with 10% fetal bovine serum (Gibco). These cells were seeded onto 96-well plates (Falcon Primaria, Becton Dickinson, Lincoln Park, NE) for cell counting (1×10^3 cells/well) or onto 6×35 mm well plates (Falcon Primaria) for RNA isolation (cells from 3 to 4 eyes per well) and kept in a humidified environment of 95% air/5% CO_2 at 37°C. Throughout the experiments, primary cultured cells were grown in medium supplemented with 10% fetal bovine serum (growth medium).

2.2. Cell counting

24 h after cells were plated, growth medium was replaced with medium containing 0 (control) to 1.0 mM L-glutamic acid monosodium salt (glutamate, Sigma, St. Louis, MO); half of the growth medium with or without (control) glutamate was changed once daily. After treatment with glutamate for 1, 2, 4 or 7 days, RPE cells were rinsed twice with calcium- and magnesium-free phosphate-buffered saline (CMF-PBS) and suspended by trituration in CMF-PBS containing 0.15% trypsin. Then, cells that excluded Trypan blue (0.4%) were counted with phase optics under an inverted microscope (Diaphoto-TMD, Nikon, Tokyo).

To clarify which subtypes of glutamate receptors were involved, cells were treated with 1.0 mM glutamate and concomitantly with 10 or 100 μM (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine (MK-801, RBI, Natick, MA), an NMDA-receptor antagonist; 6,7-dinitroquinoxaline-2,3-dione (DNQX, Tocris, Bristol, UK), an antagonist of non-NMDA ionotropic receptors; or L(+)-2-amino-3-phosphonopropionic acid (AP-3, RBI), a metabotropic-glutamate-receptor antagonist. The effects of selective agonists of glutamate receptor subtypes were also investigated: NMDA (Sigma), kainic acid (kainate, Sigma), AMPA (Tocris) or *trans*-(±)-1-amino-1,3-cyclopentanedicarboxylic acid (*trans*-ACPD, RBI), a

Table 1
Specific oligonucleotide primers

	5′ Primer	3′ Primer	Length (bp)
bFGF	TCACTTCGCTTCCCGCACT	TGGAGTATTTCCGTGACCG	352
FGF-R1	GTCCAGACAACTTGCCGTAT	CTTGTAGATGATGACGGAGC	292
GAPDH	TGAAGGTCGGTGTCAACGGATTTGGC	CATGTAGGCCATGAGGTCCACCAC	983

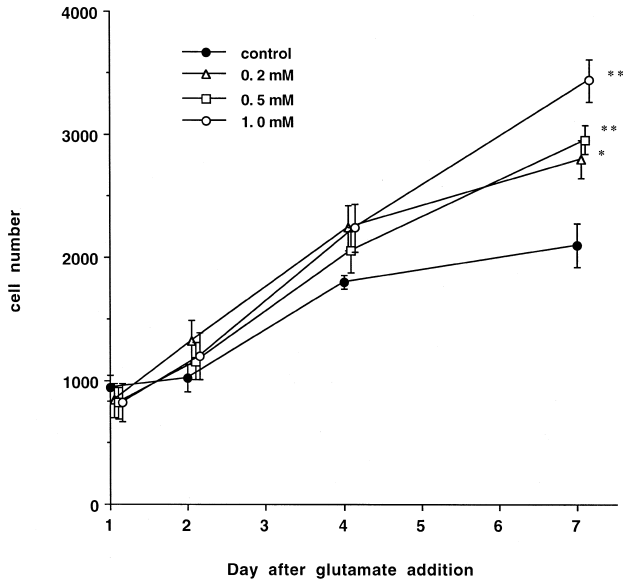

Fig. 1. Glutamate-stimulated RPE cell proliferation. Glutamate at concentrations from 0 (control) to 1.0 mM was added to the medium, and cells were cultured for 1, 2, 4 or 7 days. Data are expressed as the means ± S.E. ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the corresponding control.

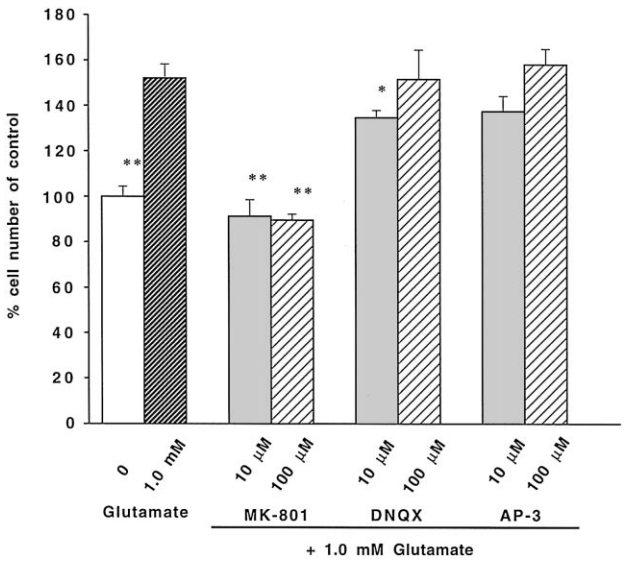

Fig. 2. Effect of selective antagonists of glutamate receptor subtypes on glutamate-stimulated RPE cell proliferation. RPE cells were cultured with 1.0 mM glutamate for 7 days in the presence or absence of the antagonists shown. Cell number in the control was considered 100%. Data are expressed as the means ± S.E. ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with 1.0 mM glutamate.

metabotropic glutamate receptor agonist. Cells were counted after being cultured for 7 days with drugs.

2.3. Immunohistochemistry

RPE cells were plated in eight-well chambered coverglasses (Nunc Lab-Tek, Naperville, IL) and cultured in growth medium. Seven days later, the cells were washed three times with 0.1 M PBS and fixed with 4% paraformaldehyde in a phosphate-buffered solution overnight. After being washed three times with PBS, the cells were incubated for 30 min in 10% normal goat serum and incubated overnight at 4°C with mouse monoclonal anti-human pancytokeratin antibody (1:400 dilution of clone C-11, Sigma) or with rabbit polyclonal anti-rat NMDA receptor (NMDA-R1) antiserum (0.5 μg/ml of AB1516, Chemicon International, Temecula, CA). The cells were washed in PBS three times and then incubated for 45 min with biotinylated goat anti-mouse and anti-rabbit IgG (1:200) against cytokeratin and NMDA receptor, respectively. After being rinsed with three changes of PBS, the cells were incubated for 30 min with avidin-biotinylated alkaline phosphatase complex (Vector Laboratories, Burlingame, CA). After being washed with PBS, the cells were incubated for 10 to 15 min with a substrate of alkaline phosphatase (Kit III, Vector Laboratories), which turned blue when stained positively.

2.4. RT-PCR

The RPE cells were cultured for 7 days with or without (control) treatment with 1.0 mM glutamate for 1, 2, 4 or 7 days. The cells were then washed with CMF-PBS and homogenized in 1.0 ml of Isogen (Nippon Gene, Tokyo). Total RNA was isolated according to the manufacturer's instructions. The purity of isolated RNA was assessed by measuring OD260/OD280 and its quantity was estimated from OD260. The specific primers for bFGF and FGF-R1, designed on the basis of the nucleotide sequences of rat bFGF cDNA (M22427, Shimasaki et al., 1988) and rat FGF-R1 cDNA (D12498, Yazaki et al., 1993) corre-

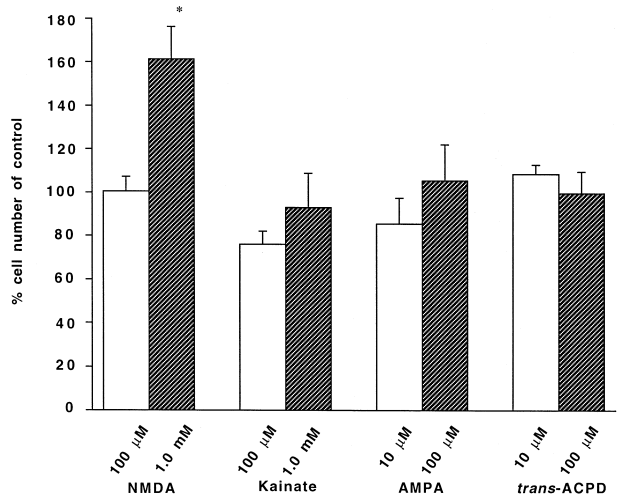

Fig. 3. Effects of selective agonists of glutamate receptor subtypes on RPE cell proliferation. RPE cells were cultured for 7 days with the agonists shown. Cell number in the control was considered 100%. Data are expressed as the means ± S.E. ($n = 4$). $^{*}P < 0.05$ compared with control.

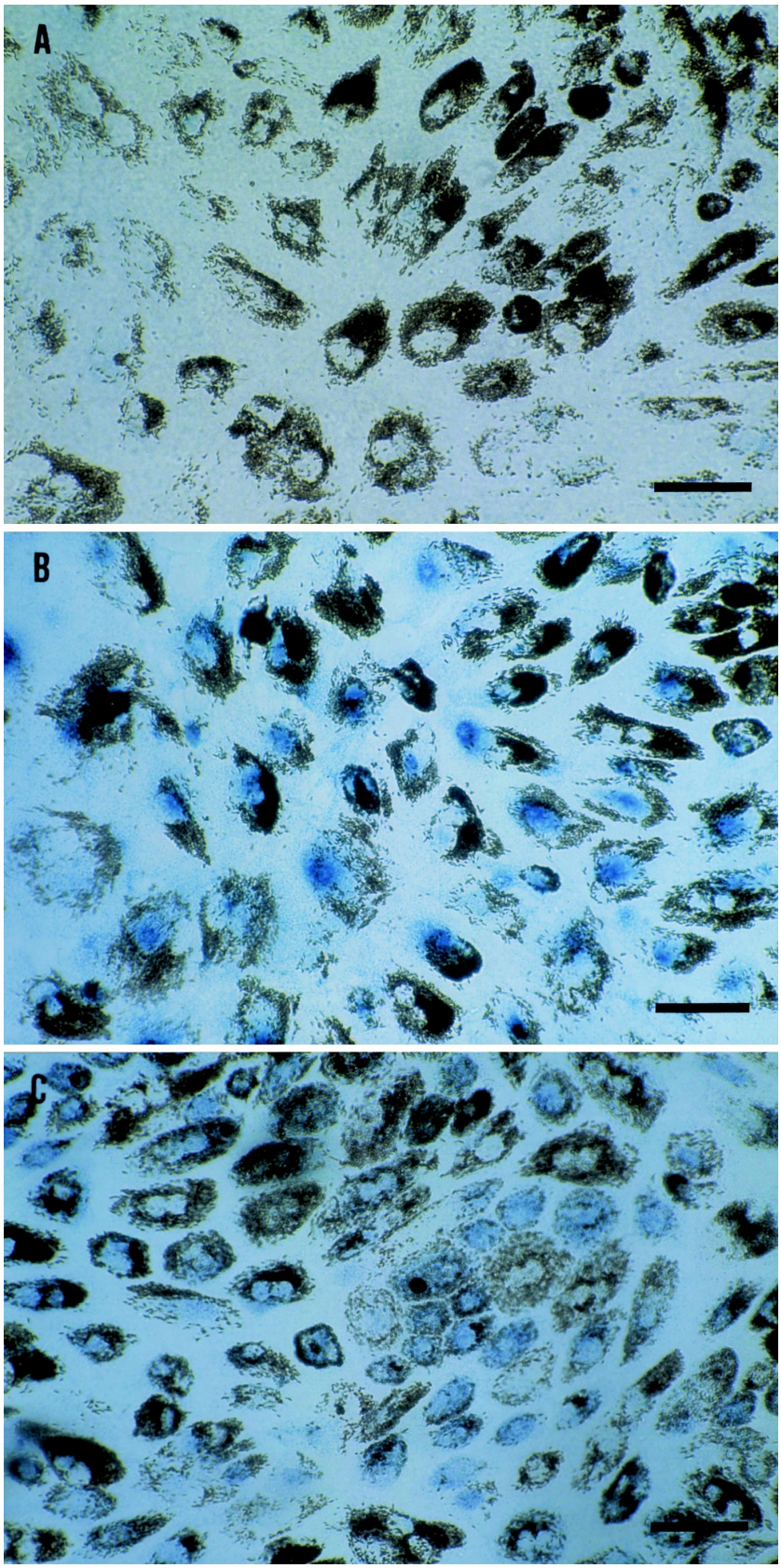

Fig. 4. Immunohistochemical staining of RPE cells for cytokeratin and NMDA receptor. RPE cells cultured for 7 days were immunostained with antibody against cytokeratin (B) and antiserum against NMDA receptor (NMDA-R1) (C). Control cells (A) were incubated with normal goat serum instead of specific antibody or antiserum. Specific cytokeratin- and NMDA-receptor-like immunoreactivity is observed as a blue stain in the cytoplasm of most RPE cells, more diffuse in the latter. Scale bar = 10 μm.

sponded to base pairs 542–560 and 978–996 for bFGF and 902–921 and 1175–1194 for FGF-R1 (Table 1). Primers for amplification of rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA, a house-keeping gene, were obtained from Clontech (Palo Alto, CA). The first strand of cDNA was synthesized from 1.0 μg of total RNA with reverse transcriptase and a 3′ primer and was amplified with a GeneAmp RNA PCR Core kit (Perkin-Elmer, Branchburg, NE). Amplification was performed as follows: 2 min at 95°C for initial denaturation, 26 cycles of amplification with a step program (95°C, 1 min; 62°C, 1 min; 72°C, 1 min), followed by a final extension at 72°C for 7 min. The PCR reaction without RT was performed as a negative control. The PCR products were detected with agarose gel electrophoresis and confirmed with sequencing according to the method of Sanger et al. (1977).

2.5. Statistical analysis

The results are expressed as means ± S.E. The Student's *t*-test was used to determine statistical significance of differences. A *P*-value of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Cell proliferation

The effect of glutamate on the proliferation of cultured RPE cells is shown in Fig. 1. Control RPE cells were allowed to proliferate in the growth medium; after 7 days of culture the number of cells had increased 2.2-fold. Four days after addition of glutamate, the number of cells appeared to be greater than that in control cultures, but the difference was not significant. However, by the end of the 7th day the number of cells was significantly greater than that in the control at all glutamate concentrations (0.2, 0.5 and 1.0 mM). The most effective concentration of glutamate was 1.0 mM, which increased the number of cells to 1.7 times that of the control.

The effects of glutamate receptor antagonists on glutamate-stimulated cell proliferation are shown in Fig. 2.

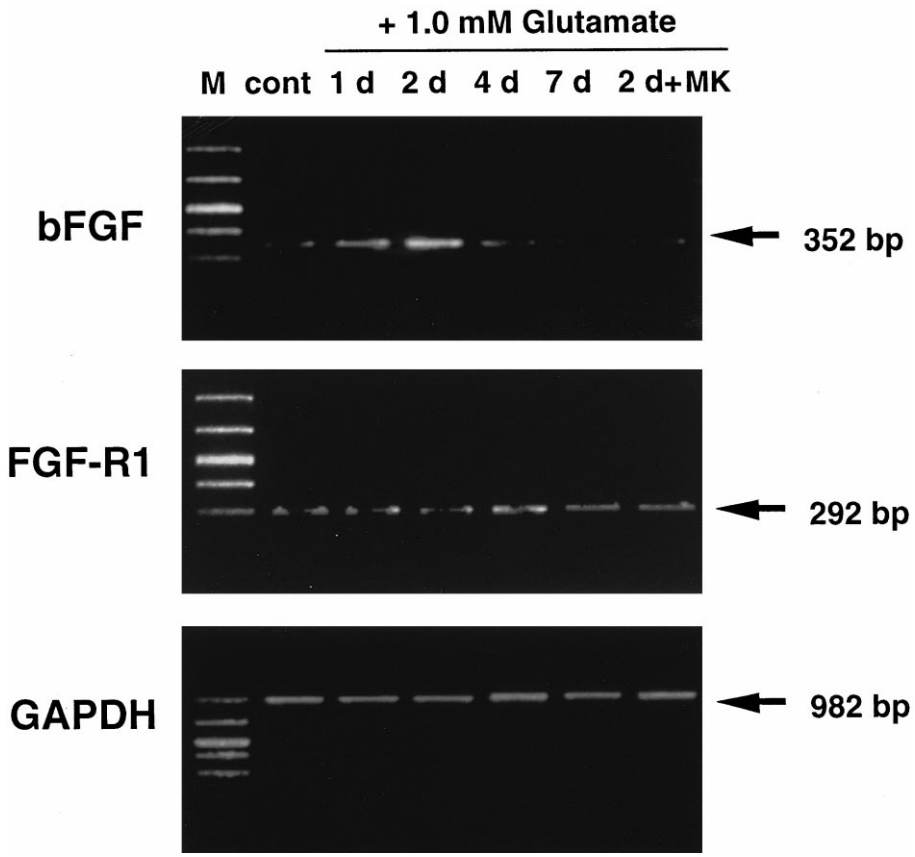

Fig. 5. RT-PCR analysis for bFGF, FGF-R1 and GAPDH mRNA levels in cultured RPE cells treated with glutamate. RPE cells were cultured for 7 days and total RNA was extracted and subjected to RT-PCR analysis. Glutamate (1.0 mM) was added to the medium for 1, 2, 4 or 7 days (d) before the extraction. Combined treatment with 100 μM MK-801 (MK) and 1.0 mM glutamate was performed for 2 days. In the control (cont), only growth medium was used. Panels show the ethidium bromide-stained PCR products in 3% agarose gel with a molecular weight marker (M: 300, 400, 500, 700 and 1000 bp). Arrows indicate the expected size of each PCR product.

Treatment with 10 and 100 μM MK-801 for 7 days suppressed the cell proliferation stimulated by 1.0 mM glutamate to near the control level. DNQX slightly but significantly inhibited glutamate-stimulated proliferation at 10 μM but not at 100 μM, whereas AP-3 did not inhibit proliferation at either concentration. On the other hand, none of the antagonists significantly affected cell proliferation when added alone without glutamate (data not shown). Of selective agonists of glutamate receptor subtypes, NMDA at 1.0 mM significantly stimulated proliferation compared with the control to an extent similar to that observed with 1.0 mM glutamate, while kainate, AMPA and *trans*-ACPD did not significantly affect cell proliferation (Fig. 3).

3.2. Immunohistochemistry

The results of immunohistochemical staining for cytokeratin and for NMDA receptors are shown in Fig. 4. Brown to black granular densities were most likely pigment granules, since they were also evident on control staining in the absence of specific antibodies (Fig. 4A). Cytokeratin-like immunoreactivity was detected in the cytoplasm of nearly all cells observed (Fig. 4B). NMDA-receptor-like immunoreactivity was also found in the cytoplasm of most cells (Fig. 4C) but was more diffuse than cytokeratin-like immunoreactivity (Fig. 4C).

3.3. Levels of bFGF and FGF-R1 mRNA

As shown in Fig. 5, PCR amplification with specific primers for bFGF and FGF-R1 resulted in single products of expected lengths (352 and 292 base pairs, respectively). Nucleotide sequence analysis of PCR products revealed that their sequences corresponded to the reported sequences of target genes (data not shown). When PCR amplification was performed without RT, no PCR products were detected, indicating that samples of extracted RNA had not been contaminated by genomic DNA. The number of PCR amplification cycles (26 cycles) was chosen on the basis of preliminary experiments using [^{32}P]dCTP as a substrate of PCR which established the linear range of the reaction (data not shown). Therefore, changes in the amounts of PCR products can be assumed to reflect changes in the levels of bFGF and FGF-R1 mRNA in RPE cells after culture with glutamate.

Levels of bFGF mRNA after treatment with glutamate for 1 and 2 days were higher than those in the control cultures in the absence of glutamate. The highest level of bFGF mRNA was observed after treatment with glutamate for 2 days, after which the levels gradually decreased to less than the control after 7 days of treatment. Although the FGF-R1 mRNA level was also increased after treatment with glutamate, the increase was less and slower than that of bFGF mRNA (the highest level was observed after 4 days of treatment). The increase in bFGF mRNA caused by glutamate was suppressed by MK-801 (100 μM) to near the control level, but the level of FGF-R1 mRNA was little affected by MK-801. On the other hand, the level of GAPDH mRNA was not affected by either glutamate or MK-801.

4. Discussion

The present study demonstrated that treatment with glutamate for more than 4 days stimulates the proliferation of cultured rat RPE cells in a concentration-dependent manner. Furthermore, only MK-801, a selective inhibitor of NMDA receptors, could completely abolish glutamate-stimulated proliferation of RPE cells, whereas inhibitors of non-NMDA ionotropic receptors (DNQX) and metabotropic receptors (AP-3) suppressed proliferation only slightly. In addition, NMDA, but not kainate, AMPA, or *trans*-ACPD, was shown to stimulate proliferation of cultured RPE cells with a potency comparable to that of glutamate. These findings suggest that glutamate may stimulate proliferation of RPE cells through activation of the NMDA receptor.

The possible existence of receptors with properties of both NMDA and metabotropic glutamate receptors is suggested by results of binding assays (López-Colomé et al., 1993, 1994). In the present study, positive immunostaining for NMDA receptors was observed in most cultured RPE cells. In a preliminary experiment, we also detected mRNA of NMDA-R1, 2A and 2D subunits of NMDA receptors using RT-PCR method in cultured RPE cells (data not shown). These observations support the possible involvement of NMDA receptors in glutamate-stimulated proliferation.

NMDA receptor function is suppressed by extracellular Mg^{2+} (Nowak et al., 1984). However, we did not remove Mg^{2+} from the culture medium (approximately 0.9 mM), since we intended to investigate the effect of glutamate on its receptors under physiological conditions. Our decision might be justified by the following observations. Recent studies demonstrated NMDA receptors which were resistant to Mg^{2+} block (Kutsuwada et al., 1992; Pearson et al., 1992). Furthermore, in a preliminary experiment with confocal laser-scanning microscopy, we confirmed that 1.0 mM glutamate induced a rapid $[Ca^{2+}]i$ rise in RPE cells in the presence of 1.0 mM Mg^{2+} (data not shown).

Both glutamate and NMDA induce the death of neurons derived from the brain (Choi et al., 1987, 1988; Kohmura et al., 1990). Furthermore, exposure of rat primary cultured retinal neurons to 1.0 mM glutamate or NMDA for only 10 min induces cell death within 1 h, but cell death is prevented by the addition of MK-801 (Kashii et al., 1994). Several authors have suggested the possible involvement of glutamate in neuronal degeneration through excessive stimulation of NMDA or non-NMDA receptors after retinal ischemia (Mosinger et al., 1991; El-Asrar et al., 1992; Lombardi et al., 1994). In fact, some in vitro studies have

demonstrated an excessive release of glutamate from retinal neurons after hypoxia (Neal et al., 1994; Rego et al., 1996). Recently, massive glutamate release (at levels 5- to 10-fold higher than the basal level) from rabbit (Louzada et al., 1992) and cat (Masai et al., 1995) retina during and after ocular ischemia has been demonstrated with in vivo microdialysis. Retinal neurons may also release large amounts of glutamate in response to other types of ocular stress, such as trauma, photocoagulation and retinal detachment. Solberg et al. (1996) have shown that treatment with MK-801 reduces rat retinal lesions 20 and 60 days after laser exposure. Thus, release of excessive glutamate in response to various types of ocular stress is likely to promote degeneration of retinal neurons (Kalloniatis, 1995).

Millimolar concentrations of glutamate have been reported to activate phagocytosis of the rod outer segment by *Xenopus* RPE cells in eye cups (Greenberger and Besharse, 1985). Such results, together with the results of the present study which show that glutamate stimulates proliferation of cultured rat RPE cells, suggest that glutamate at relatively high concentrations has opposite effects on retinal neurons and RPE cells. In a preliminary experiment, we determined that the basal glutamate concentration was approximately 7.0 μM in growth medium after 7 days of culture. This value is comparable with that reported for the extracellular space in the brain (Mitani et al., 1990; Benveniste, 1991). On the other hand, extracellular glutamate levels may increase to 200 μM or 1.0 mM during brain ischemia (Mitani et al., 1990; Benveniste, 1991). Thus, a direct effect of glutamate on RPE cells might be found in vivo: under conditions of ocular stress, when excessive amounts of glutamate accumulate in the extracellular space, RPE cells can proliferate and be activated through stimulation of NMDA receptors despite neuronal degeneration. Therefore, glutamate might reasonably be assumed to play an important role in the activation of RPE cells observed in wound healing after retinal damage (Okisaka et al., 1991; Toti et al., 1991).

Rat RPE cells have been reported to express both bFGF (Malecaze et al., 1993; Rakoczy et al., 1993) and FGF-R1 (Malecaze et al., 1993) mRNA. Immunohistochemical studies have also identified bFGF in the RPE of rats (Connolly et al., 1992) and primates (Hageman et al., 1991). In the present study, bFGF and FGF-R1 mRNAs were detected with RT-PCR in RPE cells and the PCR products obtained were identified on the basis of size and the results of direct sequencing. In RPE cells cultured with glutamate, levels of bFGF and FGF-R1 mRNAs increased before cell proliferation had increased significantly, a finding that suggests that glutamate may promote the expression of bFGF, a potent mitogenic cytokine and the bFGF receptor. The stimulation of bFGF mRNA expression by glutamate seems to be mediated by activation of NMDA receptors because stimulation was inhibited by MK-801.

Pechán et al. (1993) have reported that glutamate also stimulates the expression of bFGF and FGF-R1 mRNAs in rat cortical astrocytes. They have demonstrated that mRNA levels peak 4 h after the addition of 100 μM glutamate. However, in the present study with RPE cells, the highest levels of bFGF and FGF-R1 mRNA were observed at 2 days and 4 days, respectively, after glutamate was added, after which levels gradually decreased. The level of bFGF mRNA in cultured RPE cells has been reported to be dependent on cell density and to be markedly suppressed in proportion to increases in cell density (Bost and Hjelmeland, 1993). Thus, lower levels of bFGF mRNA after 7 days of treatment with glutamate might reflect cell density-dependent suppression of expression, since a significantly increased number of cells were present after 7 days of glutamate treatment compared with the control.

bFGF has been reported to have a mitogenic effect on cultured bovine RPE cells in vitro (Schweigerer et al., 1987) and on RPE cells in rabbit eyes in vivo (Lewis et al., 1992). Thus, glutamate-stimulated proliferation of RPE cells might be due to a mitogenic effect of bFGF produced by RPE cells themselves. However, since increases in the protein levels of bFGF and FGF receptors in RPE cells or in the growth medium were not observed in this study, definitive conclusions about the role of growth factors in the proliferation of RPE cells cannot yet be drawn.

bFGF is also known to protect retinal neurons, for example, in inherited retinal dystrophy (Faktorovich et al., 1990). Recently, the possible involvement of bFGF in retinal wound healing is suggested by reports of accelerated healing of photocoagulated rabbit retina treated with exogenous bFGF (Schuschereba et al., 1994) and reports of enhanced positive immunostaining for bFGF in RPE cells of photocoagulated rat retina (Zhang et al., 1993). Yamada et al. (1996) also report that bFGF mRNA and receptors in cultured human RPE cells increase after photocoagulation, after which intense immunoreactivity for bFGF appears in the cytoplasm and nuclei of cells surrounding the wound. Together with these findings, our results suggest that bFGF expression in RPE cells during wound healing may be stimulated by the release of excessive amounts of extracellular glutamate.

In addition to having a protective effect, glutamate might also be involved in the pathogenesis of proliferative vitreoretinopathy, which often results from ocular stress, such as retinal detachment. Several findings in vitreoretinopathy might be associated with the effects of glutamate on RPE cells observed in this study: proliferation and migration of RPE cells in surgically removed membranes (Jerdan et al., 1989; Vinores et al., 1990) or vitreous and subretinal fluid (Baudouin et al., 1991) of patients with vitreoretinopathy and increased expression of bFGF mRNA in a rabbit vitreoretinopathy model (Planck et al., 1992). However, further studies should be performed to test our hypothesis.

In summary, proliferation of RPE cells is stimulated by glutamate through activation of NMDA receptors. Levels of bFGF and FGF-R1 mRNA in RPE cells also increase

after glutamate treatment. Our results suggest the possible involvement of glutamate in retinal wound healing after various types of ocular stress.

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