Eur Arch Otorhinolaryngol (2002) 259:166–169

MISCELLANEOUS

Hideo Nakamura · Y. Higuchi · H. Kondoh · M. Obata
S. Takahashi

The effect of basic fibroblast growth factor on the regeneration of guinea pig olfactory epithelium

Received: 3 October 2001 / Accepted: 10 October 2001

Abstract Olfactory receptor cells are widely thought to regenerate after degeneration and also thought to show turnover in normal circumstances in animal olfactory epithelium. The identity of the factor that controls proliferation and differentiation of olfactory receptor cells is a very important problem that has yet to be resolved. In this study, the mitogenic effects of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) on olfactory receptor cells in guinea pig olfactory epithelium was examined. The intraperitoneal injection of 1,000 ng bFGF/day for 14 days increased the cells in proliferation detected by immunostaining with proliferating cell nuclear antigen (PCNA), while neither EGF nor low-dose bFGF had any effect. These results support the idea that an adequate dose of bFGF plays an important role in the neurogenesis in the olfactory epithelium. Further study is needed to clarify the efficacy of bFGF in the damaged olfactory epithelium, but bFGF may provide a therapeutic option for olfactory disturbances caused by complete or partial loss of olfactory receptor cells.

Keywords Olfactory epithelium · Olfactory receptor cells · Regeneration · Fibroblast growth factor

Introduction

Partial or complete loss of olfactory receptor cells from various causes can induce significant olfactory disturbance. Currently, there are no effective treatment methods that can reverse these serious olfactory disturbances. If we could establish a method of recovering olfactory receptor cells it would be possible to treat these patients.

H. Nakamura (✉) · Y. Higuchi · H. Kondoh · M. Obata
S. Takahashi
Department of Otolaryngology,
Niigata University School of Medicine,
Asahi-machi 1, Niigata 951-8510, Japan
e-mail: hident@med.niigata-u.ac.jp
Tel.: +81-25-2272305, Fax: +81-25-2270786

Olfactory receptor cells are widely thought to regenerate after degeneration caused by olfactory nerve axotomy or after chemical destruction, and also thought to show turnover in normal circumstances in the animal olfactory epithelium [1, 4, 12]. The stem cells for olfactory receptor cells are progenitor cells that divide just above the basal cells [13]. The regeneration of olfactory receptor cells depends on the activity of these progenitors, and it is very important to investigate the factor that affects the progenitors. Recently, epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) were reported to have different effects on neuronal progenitors in the rat brain; in particular, FGF-2 had a stimulatory effect on the generation of olfactory bulb neurons [11]. In addition, dissociated cells from the olfactory epithelium required basic fibroblast growth factor (bFGF) for proliferation and long-term survival in vitro [7]. It seems likely that growth factors also influence neural progenitors of the olfactory epithelium in vivo.

The aim of the present study was to explore the effects of EGF and bFGF on the proliferation of neural progenitor cells in the olfactory epithelium of guinea pigs.

Materials and methods

Four-month-old healthy male guinea pigs were used in this study. To study the effect of growth factors on the proliferation of adult olfactory progenitor cells in vivo, we used a program of chronic administration: injection of EGF, or bFGF intraperitoneally, each day for 14 days. Human recombinant bFGF (Promega, Madison, WI) or EGF (Promega, Madison, Wis.) was dissolved in 10 mM phosphate-buffered saline (PBS), pH 7.6. The animals received 1,000 ng of bFGF/day (group A, $n = 5$), 250 ng of bFGF/day (group B, $n = 5$), 1,000 ng of EGF /day (group C, $n = 5$), or PBS only (control, $n = 5$) by intraperitoneal injection each day for 14 days. One day after the final injection, animals were anesthetized with intraperitoneal injections of sodium-pentobarbital (100 mg/kg), and intracardial perfusion with Bouin's solution was performed. The olfactory epithelium was excised from the posterior-superior portion of the nasal septal mucosa. Specimens were embedded in paraffin and 5-μm-thick sections prepared. The sections were dewaxed in a graded ethanol series and treated with methanol containing 0.3% peroxidase for 30 min at room temperature, to inhibit any nonspecific reaction. The sections were then

treated with proliferating cell nuclear antigen (PCNA) antiserum with horseradish peroxidase (EPOS-HRP, Dako Japan, Kyoto, Japan) for 2 h at 37 °C. The immunopositive cells were stained with diaminobenzidine (DAB). To determine the number of immunoreactive cells in the olfactory epithelium, five microscopic fields (× 200) were chosen at random. The mean of five fields was represented as the number of immunopositive cells in the olfactory epithelium. Classic hematoxylin-eosin (HE) staining was also performed, and olfactory receptor cells in a 100-μm area of olfactory epithelium were counted. Analysis of variance (ANOVA) was used, with subsequent application of the Bonferroni/Dunn test, to compare the cell numbers of the groups. Differences were considered significant when $P < 0.05$.

Results

In the control group, cells immunoreactive to PCNA in the basal area appeared to form zonal arrangements (Fig. 1). More cells were immunoreactive to PCNA antiserum in the basal layer in group A, and the distribution of these cells appeared to be homogeneous (Fig. 2). In groups B and C, PCNA-immunoreactive cells were also recognized in the same location, but the number of positive cells was apparently lower than that in group A (Fig. 3). The thickness of olfactory epithelium and the number of olfactory receptor cells did not differ among groups A, B, and C, and controls. The average numbers of immunoreactive cells in the guinea pigs in groups A, B, C and control are summarized in Fig. 4. In each group, almost equal counts were obtained from five different microscopic fields. There were significant differences in the number of dividing cells between group A and control, but no difference was recognized between B or C and control.

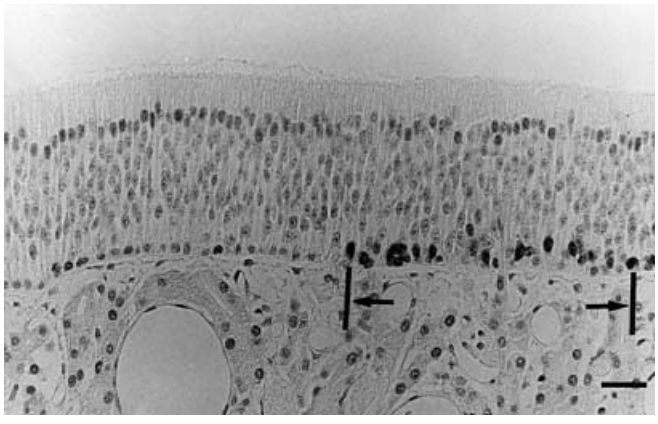

Fig. 1 Olfactory epithelium of a guinea pig at 4 months old after injection of PBS (control). Immunoreactive cells to anti-PCNA antibody can be found in the basal area. These cells have produced cellular zones *(arrows)*. *Bar* indicates 20 μm

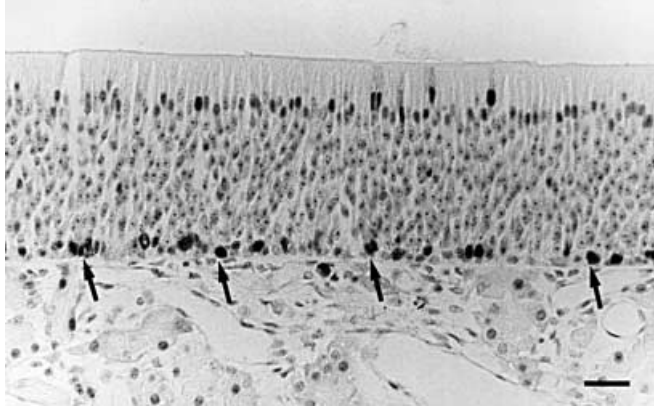

Fig. 2 Olfactory epithelium of a guinea pig at 4 months old after injection of 1000 ng/day of bFGF (group A). More immunoreactive cells *(arrows)* are present diffusely in the basal area. *Bar* indicates 20 μm

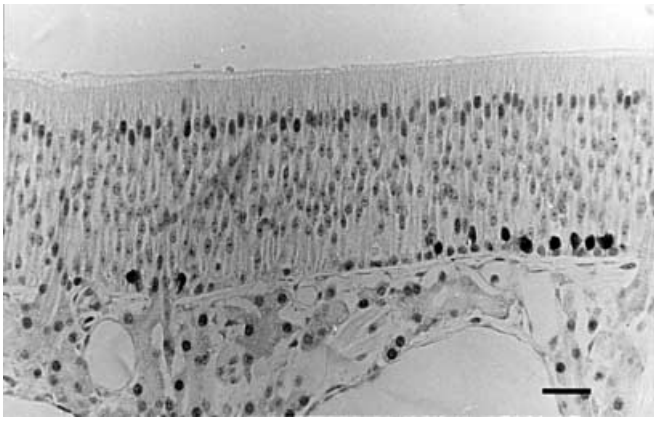

Fig. 3 Olfactory epithelium of a guinea pig at 4 months old after injection of 1,000 ng of EGF /day (group C). Immunoreactive cells can be found in the basal area. These cells have produced cellular zones. Almost same finding as in control group was recognized. *Bar* indicates 20 μm

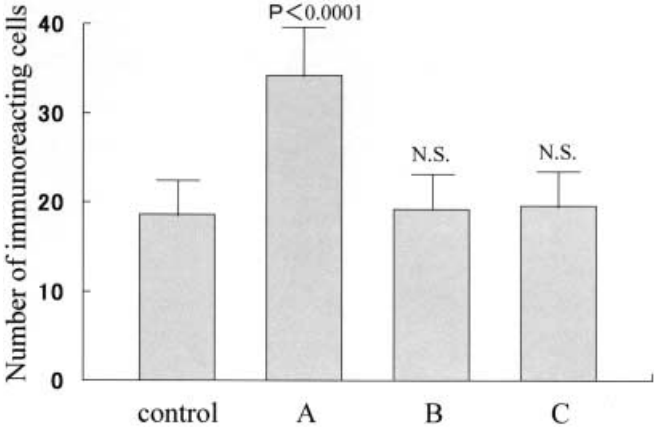

Fig. 4 The number of immunoreactive cells in each group was shown; *columns* and *bars* are mean ± SD. Only group A differed significantly from control ($P < 0.0001$) (*NS* not significant)

Discussion

Regeneration of olfactory epithelium may be affected by numerous factors. Therefore, it is very important to identify the factors that control proliferation and differentiation of olfactory receptor cells, and this could lead to

progress in the ability stimulate regeneration of the neural system. Recently, it has been confirmed in various fields that growth factors play a part in the degenerating and regenerating phases of tissues and organs [3, 9, 17]. To investigate the factors that affect olfactory generation, we used the EGF and bFGF in this study. Because, EGF and bFGF were reported to contribute to the regeneration of the neural cells in the central nervous system (CNS) [11], and bFGF receptor was confirmed to exist in the olfactory epithelium, so it is easy to speculate that bFGF is involved in the regeneration of olfactory receptor cells in the olfactory epithelium [7]. We have found earlier that a neutralizing antibody against bFGF inhibits the proliferation and regeneration of olfactory receptor cells in vitro (unpublished data).

In this study, we examined the olfactory epithelium of healthy guinea pigs and administered EGF, bFGF to register the influence of EGF and bFGF on the proliferation and regeneration of the olfactory receptor cells.

This study investigated the effects of EGF and bFGF on olfactory regeneration. bFGF increased the number of progenitor cells, but EGF did not affect the olfactory epithelium. This result is unexpected in view of results obtained in the central nervous system, where EGF does have a role in neural regeneration [11]. Ohta and Ichimura described the localization of EGF receptors in the olfactory epithelium [16]. However, we cannot base our conclusions on the effect of EGF on olfactory regeneration on this study alone. Further studies involving high-dosage EGF and other administration methods are warranted. There is some controversy as to the optimal administration route for EGF and bFGF in the central nervous system. Some authors maintain that they should be administered intracranially because of the blood–brain barrier (BBB) [10], while others are of the opinion that intravenous injection is satisfactory [5]. However, there is no barrier similar to the BBB in the olfactory epithelium. Thus, we administered EGF and bFGF intraperitoneally. The dosages of these agents were based on a previous report dealing with the central nervous system [11]. Further investigations into the dosage of bFGF in the tissue are needed, with reference to normal concentrations, which were not analyzed in this study.

Low-dose bFGF did not affect cell regeneration, but an adequate dosage of bFGF increased the number of PCNA-expressing cells. As yet, we cannot explain the mechanism by which progenitors were increased, but FGFs are mitogens in vitro for most of the mesoderm- and neuroectoderm-derived cell lines [8]. During development, growth factors may provide important extracellular signals regulating the proliferation and fate determination of stem and progenitor cells in the olfactory epithelium as in the CNS [2].

A system responsible for maintaining the overall size of the neuronal population (the number of olfactory cells) exists in normal animals [6]. In healthy young guinea pigs there are many more dividing cells in the olfactory epithelium than there are in older guinea pigs, but there is no difference in the number of olfactory receptor cells between young and old animals [15]. So it is doubtful whether the increase in dividing cells will be directly followed by an increase in the number of receptor cells in normal conditions. Further studies as to whether and how these additional dividing cells differentiate are necessary. However, we have confirmed that bFGF is an important factor for increasing the numbers of progenitor cells in healthy young animals at least and possessing the possibility to promote the regeneration of olfactory receptor cells when olfactory receptor cells have degenerated due to various causes.

FGFs have a broad spectrum of biological activities. Experimental studies of these factors that may be directly linked with clinical applications have been actively pursued, such as studies on wound healing, neuroprotection of ischemically damaged brain and heart [3, 5, 9, 17]. Currently, there are no effective treatments for olfactory disturbances originating from post-upper respiratory infection, traumatic axonal degeneration, drug-induced injuries [14] and so on. Further studies are needed to clarify the efficacy of bFGF on the olfactory epithelium of animals with olfactory deficits and older animals, but bFGF may provide a therapeutic option for these serious olfactory disturbances.

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