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Decreased Expression of PTEN in Olfactory Bulb of Rat Pub after Naris Closure

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PTEN (phosphatase and tensin homolog) is a dual specific phosphatase antagonizing phosphoinositide 3-kinase activity, and has first been cloned as a tumor suppressor for glioma. Although the role of PTEN as a tumor suppressor has been well studied, little is known about signaling mechanisms regulating expression and/or activity of PTEN in the central nervous system. In this study, we investigated whether PTEN expression is regulated by sensory deprivation. P5 rat pups were unilaterally naris-closed, and olfactory bulbs were immunohistochemically analyzed with PTEN antibody at the 7th day after naris closure. PTEN immunoreactivity was found to be down-regulated in both glomerular, external plexiform and subependymal cell layers, suggesting that odor deprivation signals down-regulate expression of PTEN in the olfactory bulb. To the best of our knowledge, this is the first report to suggest that PTEN expression is regulated by sensory deprivation signals in neonatal rats.

Key Words: PTEN (phosphatase and tensin homolog), Olfactory bulb, Naris closure

INTRODUCTION

The olfactory system is the area most susceptible to environmental influences during the development of the central nerve system. Moreover, this system exhibits life-long turnover and addition of receptor cells from the stem cells in olfactory epithelium and subventricular zone (Lois & Alvarez-Buylla, 1994; Weiler & Farbman, 1997; Weiler & Farbman, 1998). The olfactory bulb glomerulus is a discrete area where axons of olfactory receptor cells synapse with dendrites of mitral, tufted, and periglomerular neurons. The information received by olfactory receptor in olfactory epithelium is relayed to glomerular cells of an ipsilateral main olfactory bulb, and mitral cells receiving information from glomerular cells send signals to higher order cortex through the lateral olfactory tract. Odor deprivation by naris closure, specially in neonates, leads to cell death of periglomerular interneurons and synaptic reorganizations in ipsilateral side of olfactory bulb (Brunjes et al, 1985; Johnson et al, 1996).

PTEN was first discovered as a tumor suppressor, and is known to play major roles not only in apoptosis, but also in embryonic development and cell migration (Besson et al, 1999; Maehama & Dixon, 1999; Di Cristofano & Pandolfi, 2000). PTEN functions primarily as a lipid phosphatase to regulate crucial signal transduction pathway; key target is phosphatidylinositol 3,4,5-triphosphate (Eng, 2002). In addition, it displays weak tyrosin phosphatase activity, which may modulate signaling pathways that involve focal adhesion kinase (FAK) or Shc (Gu et al, 1999). Even the low level of PTEN protein present in early embryos is needed for successful embryonic development. Gene-targeting studies demonstrate that it has a crucial role in normal development including growth, adhesion, migration, invasion, and apoptosis (Li et al, 2003).

As PTEN appears to play important roles in regulating anoikis (apoptosis of cells after loss of contact with extracellular matrix) and cell migration (Yamada & Araki, 2001), a study of PTEN expression and activity may provide new insight into the roles of PTEN in sensory process and olfactory bulb development as well as neuronal cell death caused by stimulus deprivation. In the present study, we investigated expression of PTEN in the postnatal rat olfactory bulbs after odor deprivation by naris closure.

METHODS

Animals

Timed pregnant female rats (Sprague-Dawley - MJC, Inc., Seoul, Korea) were housed singly under constant temperature (22°C) on a 12/12 hour light/dark cycle with food and water *ad libitum*. Cages were checked daily to determine the date of birth, which was designated postnatal day 5.

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ABBREVIATIONS: PTEN, phosphatase and tensin homolog; FAK, focal adhesion kinase; TH, tyrosine hydroxylase; GFAP, glial fibrillary acidic protein; SVZ, subventricular zone.

Unilateral odor deprivation

Under anesthesia with Nembutal which was diluted 1:3 with saline, the left naris was closed using a bipolar coagulator. Following closure, all animals were allowed to recover from the anesthesia on a heating pad and then returned to mothers. Animals were checked every day after operation. There were no postoperative sick or dead animals.

Immunohistochemistry

Prior to sacrifice, all animals were examined under a microscope, and animals having a completely closed naris were selected for analysis and the ones with a partial opening of the naris were discarded. Animals were anesthetized with sodium pentobarbital (Nembutal) and perfused transcardially with saline containing 0.5% $NaNO_2$ and heparin (10 unit/ml) followed by 4% paraformaldehyde containing fixative. Olfactory bulbs were carefully removed to avoid any damage, postfixed for 1 h in the same fixative, and cryoprotected overnight with 30% sucrose. The bulbs were then sectioned coronally (45 μm) on a freezing microtome and collected in cryoprotectant for storage at $-20^{\circ}C$ until processed. Floating sections of olfactory bulb were processed according to the published methods (Baker & Farbman, 1993). Sections were rinsed three times with phosphate-buffered saline (PBS) to remove cryoprotectant, and pre-incubated for 30 min in 0.1 M PBS containing 1% bovine serum albumin and 0.2% Triton X-100 and incubated overnight with the following primary antisera: anti-rabbit polyclonal TH (1 : 2500, Protosbiotech, USA), PTEN-specific mouse monoclonal antibody (1 : 1000, Cell Signalling Technology, USA) and anti-goat GFAP (1 : 1000, Santacruz). On the following day, sections were incubated for 1 h in biotinylated secondary antibody obtained from Vector laboratories. After incubation with the Vector Elite ABC kit, antigens were detected with 3,3-diaminobenzidine tetrahydrochloride (DAB) as the chromogen. Sections were mounted, air-dried, dehydrated through graded ethanols, cleared in histoclear, and coverslipped using Permount (Fisher).

RESULTS

In olfactory system, unilateral naris closure performed on neonatal rats produces various morphological, histochemical, and metabolic changes in the main olfactory bulb ipsilateral to the occluded naris, including a reduction in its total bulb size (Brunjes, 1985; Brunjes et al, 1985; Brunjes & Frazier, 1986; Frazier & Brunjes, 1988) and decrease in the expression of tyrosine hydroxylase (TH), the first and rate-limiting enzyme in the synthesis of catecholamine neurotransmitters (Nagatsu et al, 1964). As seen in Fig. 1A, TH expression in glomerular neurons was down-regulated in ipsilateral olfactory bulb by naris closure as previously reported. To examine the effects of unilateral neonatal olfactory deprivation on the expression of PTEN, PTEN was immunohistochemically detected in pairs of olfactory bulb and found to be down-regulated in glomerular, external plexiform, mitral cell and subependymal cell layers of ipsilateral olfactory bulb (Fig. 1B). PTEN is known to be expressed mostly in neuronal cell body but not in neuronal process or matured astrocytes. However, PTEN immunostaining was detected in a process-like structure. Since we recently reported that PTEN is expressed in the early stage of reactive glia in mouse hippocampus after excitotoxic injury with kainic acid (Cho et al, 2002), we investigated whether odor deprivation induced reactive gliosis. As shown in Fig. 1C, GFAP immunostaining between ipsilateral and contrallateral sides did not appear to be much different. However, immunostaining pattern between PTEN and GFAP was different, suggesting that PTEN was not stained in astrocytes but neuronal processes. Higher magnification showed that PTEN expressing cells were located around cell bodies of glomerular cells (Fig. 2). Furthermore, PTEN was expressed in subependymal cell layers and its expression was down-regulated by odor deprivation (Figs. 3A, D, E). Down-regulation of TH and PTEN occurred without prominent cell death at this time point (Figs. 3B and C).

DISCUSSION

Signals regulating expression of PTEN are of great interest, however, little is known about signals which regulate normal PTEN expression in nervous system. There are reports that transfected PTEN is regulated by NGF in PC 12 cells (Lachyankar et al, 2000), and that PTEN is transactivated by p53 in primary cell and tumor cell lines, analyzed by deletion and mutation of p53 binding element

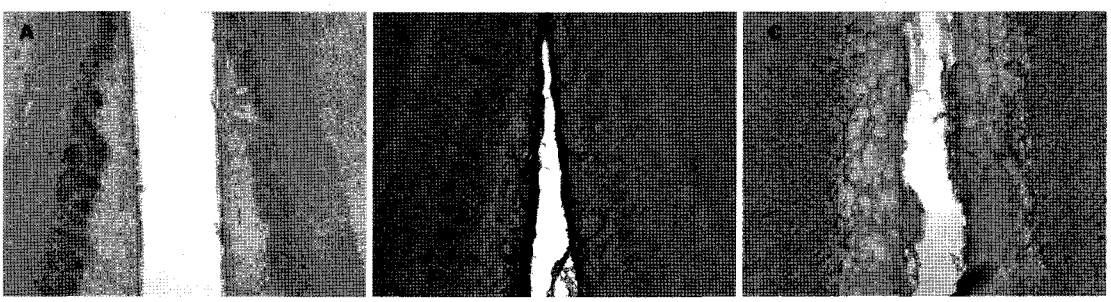

Fig. 1. PTEN expression was observed at 7th day after naris closure in P5 rat pups. One pair (contra lateral-left and ipsilateral-R) of olfactory bulb was stained with tyrosine hydroxylase (TH-A), phasphastase and tensin homolog (PTEN-B), and glial fibrillary acidic protein (GFAP-C). Complete and successful closure was confirmed with TH staining (A), showing down regulation of TH in ipsilateral olfactory bulb. PTEN was also down-regulated in glomerulus (B). Scales: 20x magnification.

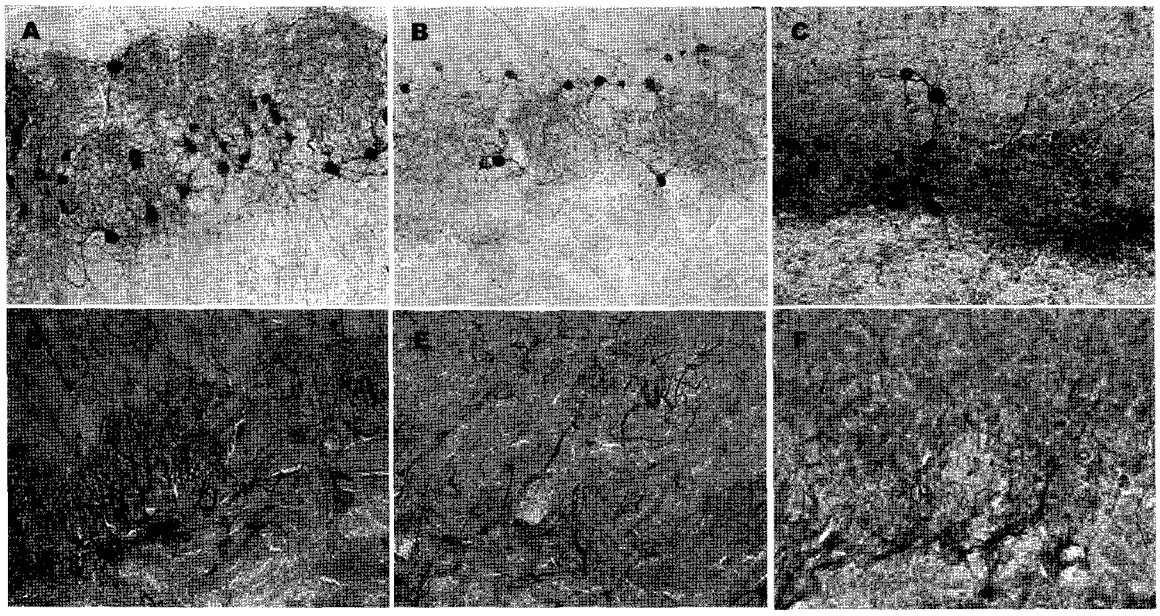

Fig. 2. Immunohistochemistry of TH (A-C) and PTEN (D-F). A-C: TH imunostainings, contralateral-A, ipsilateral-B, counterstained with cresyl violet to show the distribution of TH neurons-C. D-F: PTEN immunostainings, contralateral-D, ipsilateral-E and counterstained with cresyl violet-F. Scales: 40x magnification.

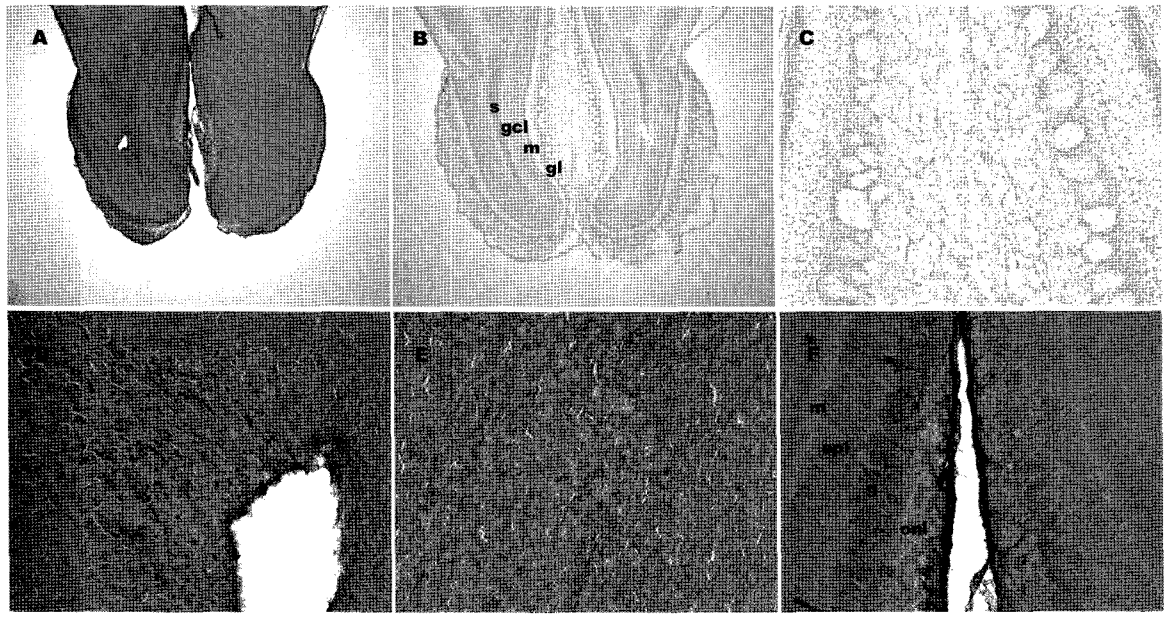

Fig. 3. Immunohistochemistry of PTEN. Low magnification of PTEN in olfactory bulb (A). Cresyl violet staining of olfactory bulb (B and C). Comparison of PTEN immunoreactivity between contralateral and ipsilateral olfactory bulb after naris closure (D-F). Immunoreactivity of PTEN in subependymal zone in contralateral (D) and ipsilateral side of olfactory bulb (E), showing that PTEN expression was decreased in ipsilateral olfactory bulb. PTEN immunoreactivity in other areas (glomerular, external plexiform area and mitral cell layers) was also down-regulated (F). s: subependymal zone, gcl: granule cell layer, m: mitral cell layer g: glomerular cell layer, onl: olfactory nerve layer. Scales: A and B, 10x magnification; C-F, 20x magnification.

(Stambolic et al, 2001). Here, we report that odor deprivation by naris closure down-regulated expression of PTEN in glomerulus, external plexiform, mitral and subependymal cell layers. To the best of our knowledge, this is the first report to demonstrate that PTEN expression is regulated by sensory deprivation signals.

PTEN is localized in several layers of olfactory bulb which suggest that it plays certain roles in the olfactory system

where continuous neurogenesis occurs through the receptor replacement. Loss of PTEN function results in an increased concentration of PIP-3 and in Akt hyperactivation, leading to protection from various apoptotic stimuli (Stambolic et al, 1998). Overexpression of PTEN in glioma and breast cancer cells results in the inactivation of Akt and the induction of anoikis, a specific apoptotic pathway initiated by cell detachment from the extracellular matrix (Tamura et al, 1999). Thus, PTEN controls cell death upon loss of contact with the extracellular matrix by inhibiting PI3-kinase-dependent survival signals (Zhang et al, 2003). PTEN inactivation might also result in increased cell cycle progression through the Akt-dependent phosphorylation and inactivation of glycogen synthase kinase-3 (GSK-3), which, in turn, leads to cyclin D1 stabilization.

The level of sensory activity within the olfactory system is an important regulator of tissue organization. Odor receptor vacancy by naris closure may have interfered local excitatory and global inhibitory processes that balance the control of the chemoreception.

Naris closure for the first 10 postnatal days leads to decreased cell survival within the deprived olfactory bulb (Cummings & Brunjes, 1997). The density of reciprocal synapses between mitral cell somata and granule cell dendrites is reduced in the mitral cell layer of the deprived bulb, and synaptic reorganization occurs (Benson et al, 1984; Johnson et al, 1996). However, we investigated 7 days after naris closure, and this is not enough time to see the reduction of bulb size and cell death. This may be a time when most of the fate determination occur.

Although not well been studied in the olfactory system, PTEN has been suggested to be a part of the regulatory circuits that maintain stem/precursor cells in the SVZ. A part of stem cells in SVZ migrates and forms new neurons in the outer layers of the olfactory bulbs. And, it has been reported that migration of the SVZ cells to the olfactory bulb was more rapid for PTEN +/− cells than for +/+ cells (Li et al, 2002), indicating that lower levels of PTEN accelerate migration of stem cells. Furthermore, there are decreased numbers of apoptotic cells for PTEN +/− cells in the SVZ (Li et al, 2002; Li et al, 2003). Taken together, down-regulation of PTEN may have a role in synaptic reorganization, olfactory receptor proliferation and apoptosis, when sensory signals were lost by naris closure.

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