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Galanin inhibits the proliferation of glial olfactory ensheathing cells ☆

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Abstract

The effect of galanin (GAL) on neural proliferation was studied in this article using olfactory ensheathing cells (OECs). OECs were isolated from newborn rat olfactory bulb and cultured in vitro. RT-PCR was used to determine the expression of GAL and its receptors in these cells. MTT analysis and LDH assay were used to detect the effects of GAL and the agonist, antagonist of GAL receptors on the proliferation of OECs. Results show that OECs express mRNAs for GAL and GAL receptor2 (GalR2) but not for the two other GAL receptors, GalR1 and GalR3. In addition, GAL and two receptor agonists, GAL1-11 and GAL2-11, can inhibit the proliferation of OECs significantly, but cause no cytotoxicity in the OECs population. Moreover, the influence can be blocked by M35, a nonspecific antagonist of GAL receptors. It is suggested that GAL is an inhibitory factor in regulating OECs proliferation.

Keywords: Olfactory ensheathing cells; Galanin; Galanin receptors; Cell proliferation

1. Introduction

Olfactory ensheathing cells (OECs) are located exclusively in the olfactory bulb, olfactory epithelium, and olfactory nerve (Cuschieri and Bannister, 1975). They are a specialized glial cells which bears some phenotypic characteristics with both the Schwann cells of the peripheral nervous system (PNS) and the astrocytes of the central nervous system (CNS) (Barber and Lindsay, 1982; Norgren et al., 1992). Olfactory neurons are generated from basal cells throughout life, these newly formed neurons grow axons and are able to make the right synaptic connection with their target neurons in the olfactory bulb (OB). The permissibility of the adult OB to the growth of axons persists after transection of olfactory nerves (Doucette et al., 1983; Graziadei and Monti-Graziadei, 1980) or lesioning of the olfactory nerve layer of the OB (Doucette et al., 1983). The unique ability has been attributed to the permissive environment formed by OECs (Doucette, 1989, 1990). These data have been suggested that OECs may be more appropriate than Schwann cells for CNS repair (Doucette, 1995; Franklin and Barnett, 1997). Recently, transplantation of OECs into the injured spinal cord has been used as an experimental strategy to promote the regeneration of injured axons. Indeed, OECs injected into the injured spinal cord have resulted in a remarkable degree of axonal regeneration and functional recovery (Imaizumi et al., 1998; Nash et al., 2002; Ramon-Cueto et al., 1998), including remyelination of demyelinated axons (Imaizumi et al., 1998; Li et al., 1997). Perhaps most remarkable is the restoration of climbing ability of rats whose spinal cords have undergone complete transection (Ramon-Cueto et al., 2000). It suggests that OECs can be

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used as a perfect cell pool for the research of neural regeneration and the therapy of CNS diseases.

The neuropeptide galanin (GAL) is a 29- to 30-amino acid peptide initially isolated from porcine intestine (Tatemoto et al., 1983). In mammals, GAL is widely distributed throughout the central and peripheral nervous systems. In the CNS of mammals, high levels of GAL are observed in the ventral forebrain, amygdala, hypothalamus, brainstem and spinal cord, mostly in nerve terminals (Cheung et al., 2001; Kordower et al., 1992; Melander et al., 1986; Perez et al., 2001; Rökaeus et al., 1984; Skofitsch and Jacobowitz, 1985; Skofitsch and Jacobowitz, 1986). Additional novel findings in normal adult brain were the detection of GAL mRNA and immunoreactivity in immature progenitor cells in the subventricular zone and rostral migratory stream (Shen et al., 2003, 2005). In the injured neural area, the expression level of GAL increases significantly (Chang et al., 1985; Holmes and Crawley, 1996; Schreiber et al., 1994). Consistent with its widespread distribution, it has a wide range of effects, including neuromodulatory, reproductive, and endocrine functions (Bartfai et al., 1993; Iismaa and Shine, 1999; Kordower et al., 1992). The actions of GAL are mediated through interaction with at least three specific receptor subtypes that are members of the G protein-coupled receptor superfamily, named GalR1, GalR2, and GalR3 (Branchek et al., 2000).

The high expression level of GAL and GAL receptors in neurogenetic zone or neuroregenerative areas suggest that GAL maybe plays an important role in neurogenesis. In this study, OECs were used to determine the effects of GAL and agonist (GAL1-11, a non-selective GAL receptor agonist; GAL2-11, a selective GalR2 agonist), antagonist (M35, a non-selective GAL receptor antagonist) on cells proliferation.

2. Materials and methods

2.1. Olfactory ensheathing cell culture

Highly enriched cultures of OECs were prepared from neonatal (1- to 3-day-old) olfactory bulbs of Sprague–Dawley rats according to the method described by Chuah and Teague (1999). Ten neonatal rats were used to produce each batch of OECs. In summary, the olfactory nerve layer was peeled away from the rest of the bulb and digested with 0.125% trypsin for 20 min at 37 °C to dissociate the cells. This incubation was replaced with culture medium (CM) composed of DMEM (Gibco, USA) supplemented with 10% fetal calf serum and centrifuged for 5 min at 800*g*. Resuspended cells were plated in CM. After 24 h, the culture was treated with 2×10^{-5} M cytosine arabinoside (AraC; Sigma, USA) for 48 h to eliminate the contaminating cells such as fibroblasts. OECs were then enriched by addition of bovine pituitary extract (BPE) (12 μg/ml) (Sigma, USA) to the CM. The cells normally reached confluence in 2–3 days and the cultures were passaged to remove the few remaining fibroblasts. Once these cultures attained confluence again, the cells were trypsinized and used for proliferation assay, RT-PCR or immunofluorescent staining.

2.2. Immunofluorescence staining and determination of the purity of OECs

According to Chuah and Teague (1999) and Woodhall et al. (2001), anti-S-100 antibody was used to identify OECs. Double staining was used with anti-S-100 antibody (1:100, Sigma, USA) and 4′,6-diamidino-2-phenylindole (DAPI) (Sigma, USA). OECs-covered coverslips were fixed with 4% paraformaldehyde for 20 min at room temperature and processed for standard indirect immunofluorescence staining. Fixed coverslips were washed in phosphate-buffered saline (PBS) and incubated with rabbit anti-S-100 at 4 °C overnight. After washing in PBS three times, the coverslips were treated with goat anti-rabbit antibody conjugated with fluorescein isothiocyanate (FITC) (1:200, CHEMICON International, Inc, USA) for 0.5 h. Following repeated washing in PBS, coverslips were incubated with DAPI for 15 min to visualize nuclei and to determine total cell numbers. No non-specific staining of OECs was observed in controls in which the primary antibody was omitted. Following staining, random areas of cells plated on coverslips were photographed with the Olympus BX51 photomicrographic system. The number of double labeled cells identified as OECs was counted in 10 random fields (from five coverslips of different batches of culture). This was then expressed as a percentage of the total number of cells.

2.3. Proliferation assay

Before running assays to test the effect of GAL and agonist, antagonist of GAL receptors, some assays were performed using a range of known densities of OECs (Fig. 2) . These served to produce a standard curve for estimating the final number of OECs.

Cells were harvested by trypsinization, counted and plated into 96-well plates. In the proliferation assays 6000 cells were seeded into each well. After 24 h incubation in CM to allow the cells to attach, the cells were treated for 72 h with various concentration of GAL, GAL1-11, GAL2-11 and M35 that were added once a day in the settled time, In the fourth day, 20 μl MTT (Sabc, China) was added to each well and the plate was incubated for 4 h at 37 °C in a humidified 5% CO_2 atmosphere. Then, 150 μl SDS (10% in 0.01 M HCl) was added and the plate was kept in the same situation overnight. The absorbance at 595 nm was read by microplate spectrophotometer (Power Wave XS. Bio-Tek).