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Selective stimulatory action of olfactory ensheathing glia-conditioned medium on oligodendroglial differentiation, with additional reference to signaling mechanisms

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ABSTRACT

We examined the effects of conditioned medium from olfactory ensheathing glia (OEGCM) on the differentiation of oligodendrocytes in mixed cultures of early postnatal hippocampi. Differentiation was judged from the numerical density (ND) of cells immunoreactive to 2′3′ cyclic nucleotide 3′phosphodiesterase (CNPase) and O4 antibodies. NDs increased according to inverted-U dose–response curves, particularly for CNPase+ cells (9-fold at optimal dilution) and these changes were blocked by inhibitors of ERK1, p38-MAPK, and PI3K. Our results raise the possibility that OEG secreted factor(s) may counteract demyelination induced by trauma, neurodegenerative diseases, and advanced age, and should stimulate novel methods to deliver these factors and/or potentiating chemicals.

1. Introduction

Olfactory axons may regenerate after sectioning of the fila olfactoria or within the olfactory fiber layer of the olfactory bulb [1,2]. Numerous studies on the potential of the olfactory ensheathing glia (OEG) for the promotion of axonal growth and/or myelination have been reported [3,4]. Although studies have provided evidence for and against the ability of the OEG themselves to myelinate central axons [5–9], few attempts have been made to test OEG as promoters of the differentiation and eventual myelination of oligodendroglial progenitor cells (OPC) (but see [10] for negative results).

The signaling pathways regulating OEG-neuronal and OEG-glial interactions are virtually unknown. This may be partially due to our poor understanding of OEG neurotrophins and other extracellular signals required for survival of neurons and/or oligodendrocytes [11,12]. Primary OEG are known to produce several trophic factors [13, 14, reviewed in 15], but only nerve growth factor (NGF), neurotrophin-4/5 (NT4/5) and neuregulin are considered to be secreted [13,16]. There is also some uncertainty regarding brain-derived neurotrophic factor (BDNF), which is thought to be produced in very small amounts [13,14] but seems to contribute significantly to axonal regeneration of cultured adult CNS neurons [17] and to be involved in OEG-enhanced axon regeneration, even on an unfavorable substrate such as myelin [18].

BDNF has also been described as involved in myelination by a direct action on oligodendrocytes [19]. Indeed, BDNF and its receptor, tropomyosin-related kinase B (TrkB), trigger several well-defined signaling cascades, including the Extracellular Signal-Regulated Kinase 1 (ERK1/MEK1) and the phosphatidylinositol-3 kinase (PI3K) [20,21]. There is also considerable work on p38 mitogen-activated protein kinases (p38MAPK) regulation of oligodendrocyte differentiation under the influence of insulin-like growth factor (IGF-1), or fibroblast growth factor 2 (FGF-2), but not BDNF [22,23].

In this study, we used a medium conditioned by cultured adult OEG (OEGCM) at different dilutions to investigate whether there is a monotonous (increasing or decreasing) change in the numerical density (ND) of cultured perinatal hippocampal cells expressing

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either of two markers of oligodendroglial differentiation: (a) 2′-3′ cyclic nucleotide 3′-phosphodiesterase, a protein mediating process outgrowth in OPCs [24,25], and (b) O4 antibody, a marker of gangliosides appearing at the pro-oligodendroblast stage as well as of the late-appearing glycolipids sulfatide and seminolipid [26,27]. To study signaling by OEG soluble factors, we used inhibitors for the ERK1, p38MAPK and PI3K signaling pathways.

2. Material and methods

2.1. Animals

Animal care was according with recommendations of the Sociedade Brasileira de Neurobiologia e Comportamento (Brazilian Society for Neurobiology and Behavior), and was approved by the Committee for the Use of Experimental Animals of our institution (CEUA IBCCF Protocol number 020).

2.2. Isolated cultures of olfactory ensheathing cells

OECs were collected and purified following our modification of a protocol previously described by [28]. Briefly, the olfactory nerve layer was removed and dissociated with a solution of 0.01% ethylene diamine tetraacetic acid (EDTA; Invitrogen, USA) [29,30]. After the removal of fibroblasts, astrocytes and oligodendrocytes by adhesion as in [28], the cells were plated onto laminin-coated (40 μg/mL, Sigma) 24-well cell plates in complete DMEM/F12 medium. The cells were maintained in 5% CO_2 at 37 °C, and the medium was changed every second day. After the cell culture had reached about 75% confluence (three weeks), the conditioned medium (OEGCM) was collected, filtered through a 0.2 mm membrane pore, aliquoted and stored at −70 °C until use.

2.3. Hippocampus mixed cell cultures

Hippocampus cell cultures were prepared following modifications of a protocol previously described by [31]. Briefly, four 0 to 2-day-old (postnatal; P0–P2) Wistar rats were decapitated, their brains removed and the hippocampus dissected. Dissociated filtered cells were plated on poly-L-lysine-coated (PLL, 100 μg/ml) glass coverslips at a final density of 10^4 cells/coverslip. The cells were maintained in complete NB27 – Neurobasal medium A (Life Technologies) containing 2% B27 supplement (Life Biotechnology), termed (NB27, positive control) plus 2 mM L-glutamine, penicillin (50 mg/ml) and streptomycin (50 U/ml), or under either of the following conditions: DMEM/F12 + 10% FBS only (negative control) or NB27 containing OEGCM dilutions (1:1; 1:3; 1:5; 1:10, and 1:20), in a 5% CO_2 atmosphere at 37 °C for 72 h. After this period, the cultures were fixed with 4% PF, washed with PBS, and stored in this solution at 4 °C until processing for immunofluorescence.

2.4. Inhibition of survival signaling pathways in hippocampal cells

Once the optimal dilution of OEGCM (1:5) for maintenance of hippocampal cells was established, three well-known inhibitors

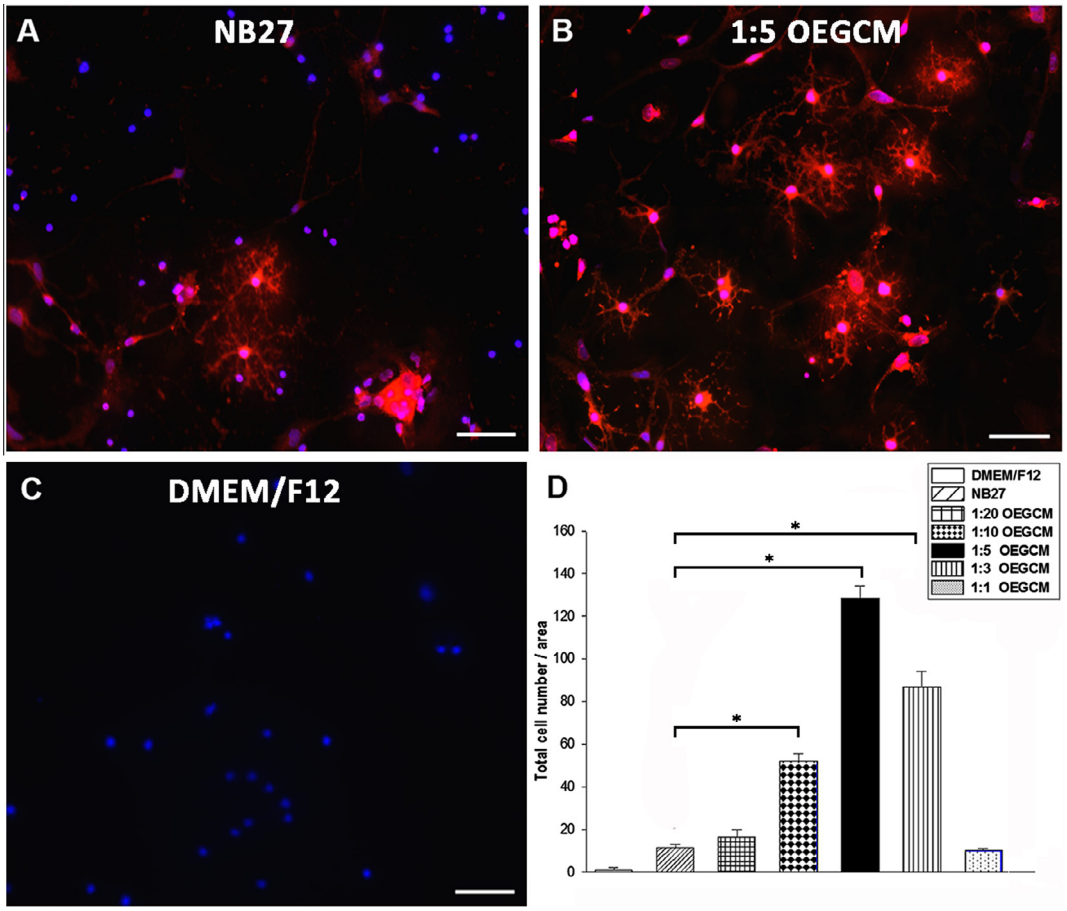

Fig. 1. OEG conditioned medium (OEGCM) increases the ND of CNPase-positive oligodendroglia in early postnatal hippocampal cultures, after 3 days of treatment. (A) Culture maintained in NB27; (B) treatment with 1:5 OEGCM; (C) culture in DMEM/F12; (D) quantification in all groups. Secondary antibody labeled with Alexa 594 (also in Fig. 2), nuclei stained with DAPI (all figures). Observe the inverted-U dose–response pattern, with maximal mean response (9× baseline) at 1:5 OEGCM. One-way ANOVA, Bonferroni test (treatment vs. culture in NB27), $^*p < 0.05$. ND = numerical density. Scale bar = 50 μm.