Paper ID: 14724
oral
student

SPACE LIFE SCIENCES SYMPOSIUM (A1)
Biology in Space (7)

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HYPERGRAVITY EFFECTS ON PROLIFERATION AND DIFFERENTIATION OF PC12 NEURON-LIKE CELLS

Abstract

Purpose This study aims at investigating the effect of hypergravity on neuron-like PC12 cell behavior. Recent findings in the literature indeed show that hypergravity may enhance cell differentiation. Derived from a transplantable rat pheochromocytoma, PC12 cells mimic central dopaminergic neurons, and express a sympathetic neuronal phenotype upon administration of nerve growth factor (NGF). These features make PC12 cells ideal candidates for the investigation of cell behavior during both proliferation and differentiation, and pave the way for a possible therapeutic use of PC12 cellular constructs for different pathological conditions. **Methodology** PC12 cells were seeded at a density of 20,000 cells/cm2 on glass coverslips, which were previously incubated with a 100 μg/ml collagen solution to promote cell adhesion. For proliferation experiments, cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 5% horse serum (HS), 100 IU/ml penicillin and 100 μg/ml streptomycin. For differentiation experiments, cells were administered DMEM, additioned with 1% FBS and 50 ng/ml NGF, after 6 h from seeding. After 24 h of culture under standard conditions (temperature 37C; 5% CO2 saturated humidity atmosphere), cells underwent hypergravity stimulation. Hypergravity was applied either continuously (1 h at 10 g) or discontinuously (6 periods of 10 min exposure to 10 g, spaced by 10 min recovery periods at 1 g) in a thermostated centrifuge. Control cultures were kept at 1 g. Two days after stimulation, proliferating cells underwent DNA quantification and fluorescent staining of f-actin, whereas differentiated cells underwent fluorescent staining of β3-tubulin, the mRNA expression of which was also assessed by qRT-PCR. **Results** Preliminary investigations showed that cell proliferation was not affected by the different hypergravity protocols, being DNA concentrations in treated samples comparable to that one observed in control cultures. In proliferating cultures, cytoskeletal actin staining evidenced the different effect of hypergravity on PC12 cell clustering, which was lower after discontinuous hypergravity stimulation with respect to controls, and comparable to controls after continuous stimulation. Cell differentiation was instead enhanced by the two hypergravity protocols, with cell exhibiting

longer neurites after stimulation with respect to control cultures. This result was found coherent to the β3-tubulin mRNA quantification, which, in both stimulation cases, was twice that of control cultures. **Conclusions** Hypergravity was found to affect PC12 cell clustering during proliferation and to enhance cell differentiation, thus encouraging further investigations of altered gravity effects on neuronal cells.